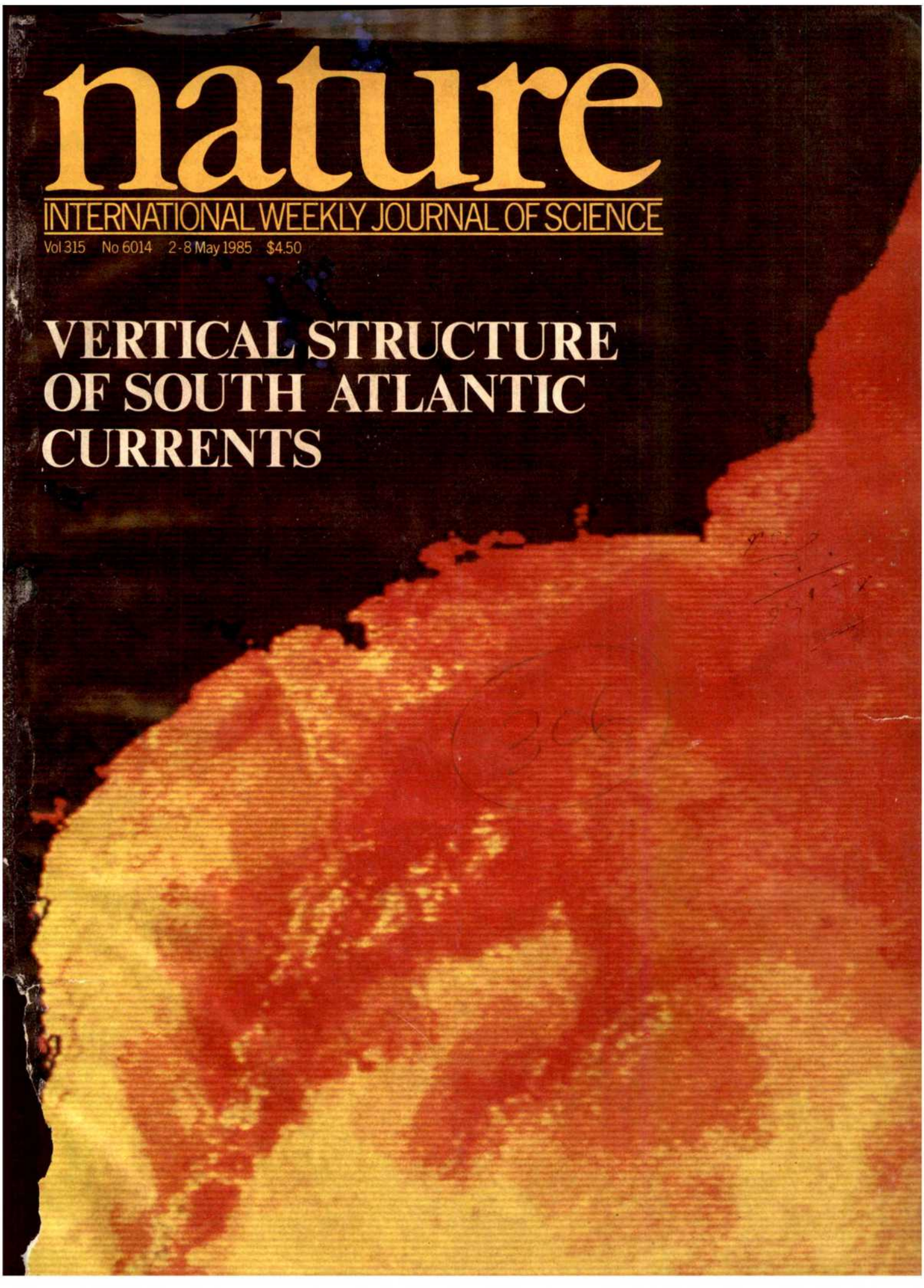
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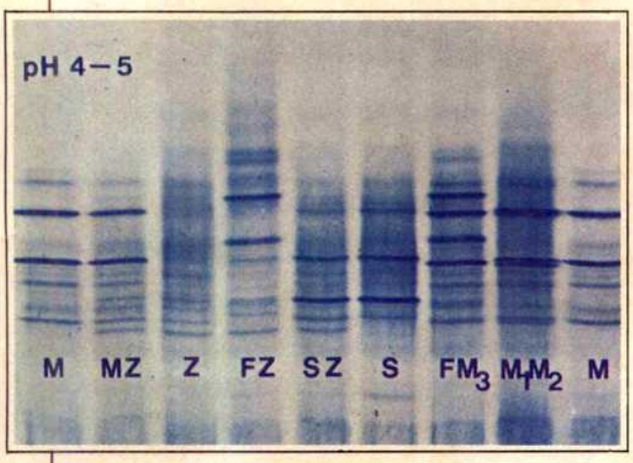
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READY-TO-USE GELS FOR MAXIMUM CONVENIENCE

All the owner of the LKB Horizontal Electrophoresis System needs to cover a wide range of electrofocusing applications is a supply of the popular and successful LKB Ampholine PAGplates. These allow the researcher to avoid the time and trouble involved in casting gels in the laboratory, while guaranteeing both high resolution and excellent reproducibility. As many as 96 samples can be separated simultaneously on a single PAGplate, enabling users with high workloads to spend their time screening samples instead of casting gels.

Eight phenotypes of α_1-antitrypsin are clearly identified using one of the convenient LKB Ampholine PAGplates ▼



ULTRATHIN GELS FOR MAXIMUM ECONOMY

Anticipating the need for less expensive electrofocusing gels, LKB developed the new UltroMould: a simple device designed to cast bubble-free gels as thin as 0.1mm in less than 30 seconds. Based on a patented sliding plate method, UltroMould is easy to use, producing perfectly accurate and uniform gels and ensuring a high standard of reproducibility. Adding UltroMould to the LKB Horizontal Electrophoresis System means that significantly less gel solution and smaller sample volumes are required, resulting in far more economical electrofocusing.

LKB UltroMould casts bubble-free ultrathin gels in only 30 seconds, making gel casting quicker, easier and more certain ▶



THICK GELS FOR MAXIMUM CAPACITY

Original electrofocusing techniques used thicker gels, and even today you may still wish to work this way in order to achieve higher capacity. The LKB Horizontal Electrofocusing System lets you easily cast and run Ampholine gels up to 2 mm thick, allowing much greater sample volumes to be analyzed successfully. The 2 mm thick gels are also ideal if you are new to electrofocusing and are following a procedure described in the literature.

After the thick gel has been cast, the sample is introduced with the help of the sample application foil ▶

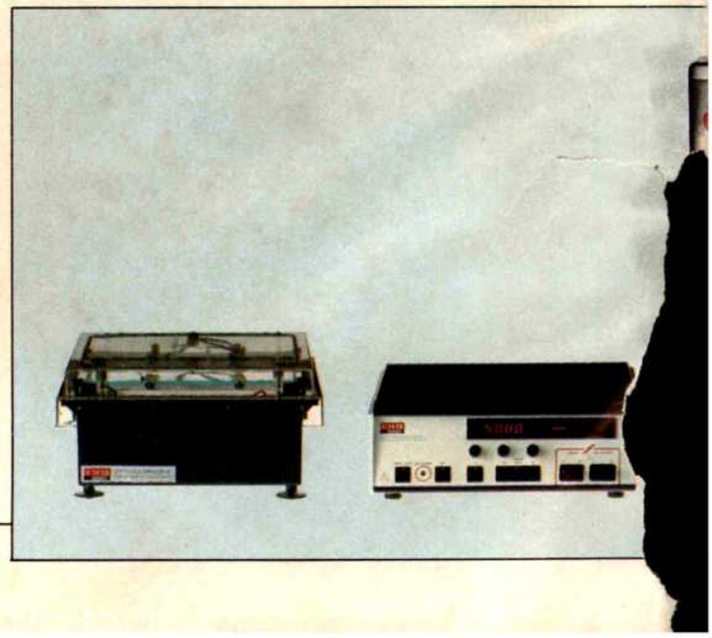


This is one in a series of advertisements describing the unrivalled versatility of the LKB Horizontal Electrophoresis System. For further information about analytical electrofocusing, and the many other applications of the system, send today for our brochure.

THE LKB SYSTEM

Only LKB can offer the research scientist a complete, integrated system in which each component works with and complements all the others. Based on the new and improved Multiphor II, the system also includes a 5KV power supply, thermostatic circulator, laser densitometer and data evaluation software.
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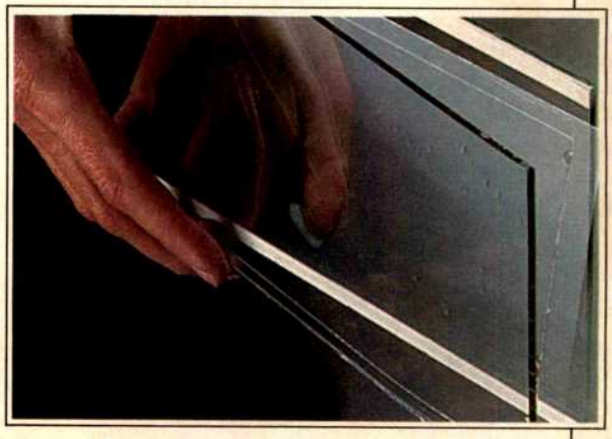
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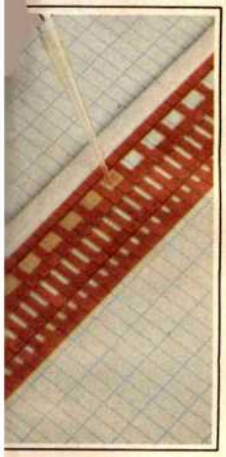


AGAROSE GELS FOR MAXIMUM POROSITY

Another of the many options available to the user of the LKB Horizontal Electrophoresis System is the application of Agarose electrofocusing gels. LKB supplies extremely pure agaroses, carefully developed for electrofocusing, and the original capillary casting method simplifies the task of producing 0.5 mm gels on GelBond Film backing sheets. Agarose is non-toxic and easy to handle and the entire analysis procedure, including casting and running, is very rapid.

Separating the GelBond Film from the glass plate gives a 0.5 mm agarose gel, firmly attached to its support and ready to use ▶

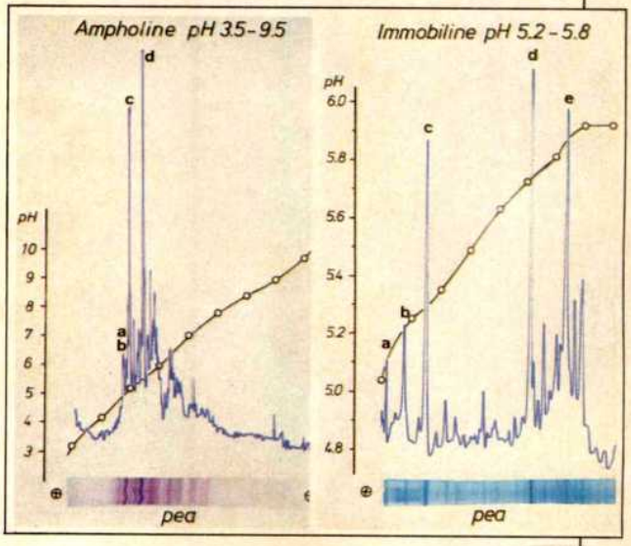


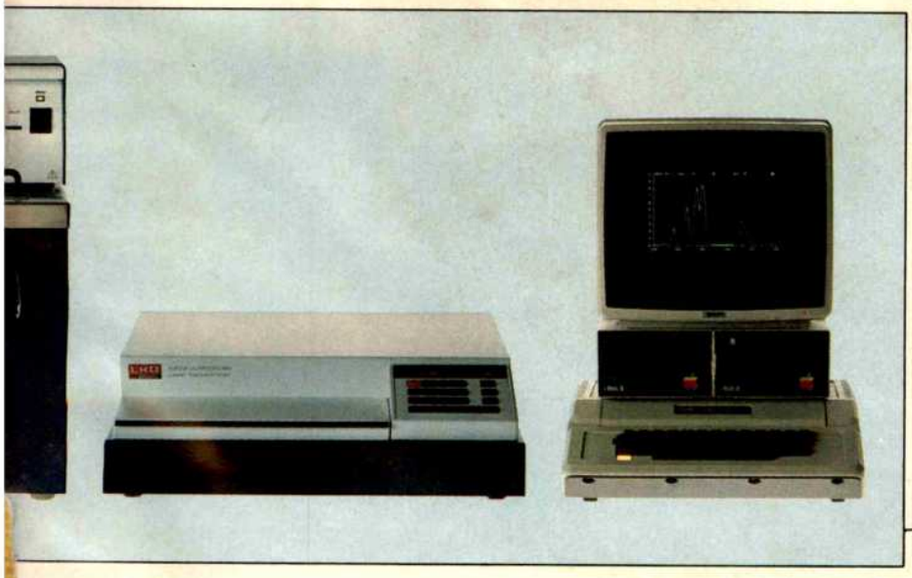


IMMOBILINE GELS FOR MAXIMUM RESOLUTION

The unique LKB Immobiline System is significantly different to traditional gels in that its pH gradient is an integral part of the gel. The system is designed to allow the user to select a pH gradient as wide as 3.0 pH units, or as narrow as 0.1 pH units, anywhere in the range 3.6 to 9.5. This tailoring of the gradient to suit specific applications permits extremely high resolution to be achieved with the LKB Horizontal Electrophoresis System. Stable and reproducible linear gradients having a slope as low as 0.01 pH units/cm are possible, allowing you to separate proteins whose pI values differ by as little as 0.001 pH units.

Comparison of traditional electrofocusing and LKB Immobiline System (courtesy of Dr A Görg et al, Technische Universität, München) ▶



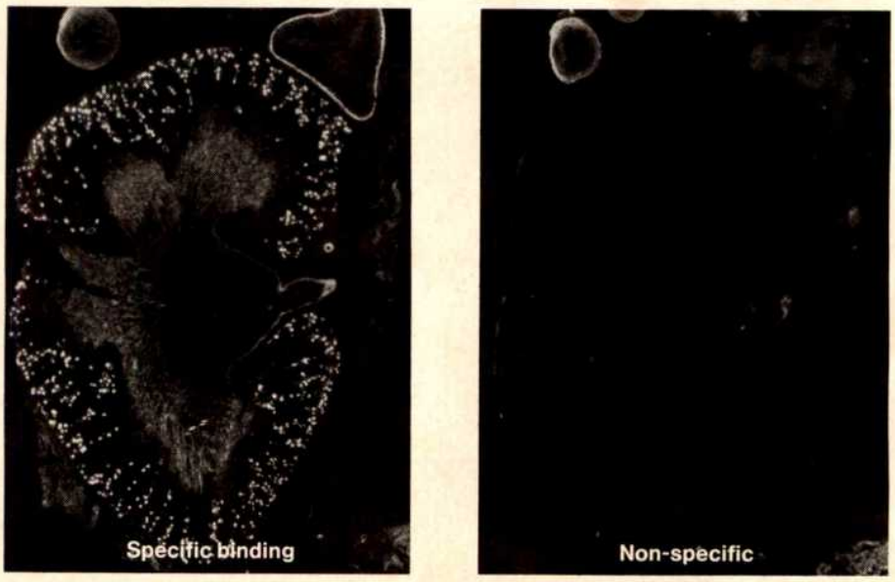


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H[illegible] blood pressure research

^{125}I-labelled atrial peptides



Amersham's ^{125}I-labelled rat ANP (28-residue-sequence) in tissue autoradiography of guinea pig kidney and adrenal. Photographs kindly supplied by Dr P W Mantyh, Center for Ulcer Research and Education, UCLA, USA.

Labelled peptides

- (3-[^{125}I]*iodotyrosyl*^{28})**Rat atrial natriuretic peptide** (28-residue sequence) IM.186
- (3-[^{125}I]*iodotyrosyl*^{28})**Human α-atrial natriuretic peptide** IM.187

These ligands are supplied at a specific activity of ~2000Ci/mmol, ~74TBq/mmol.

Tracer and antiserum for RIA

- This reagent pack, IM.1871, contains a rabbit antiserum to human α-ANP which cross-reacts totally with the corresponding rat sequence and [^{125}I]human ANP. Sufficient for 250 assay tubes using the protocol provided.

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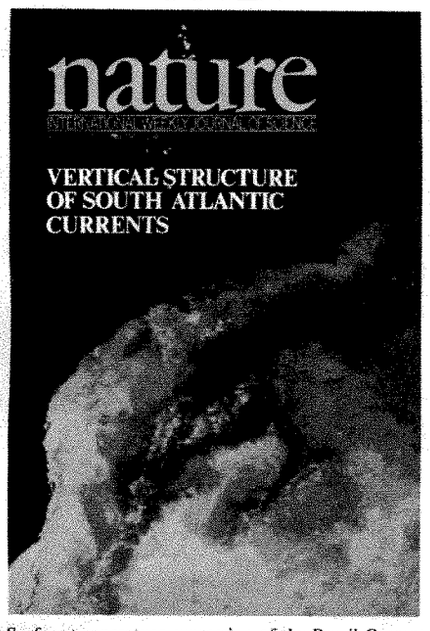
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COVER



Surface temperature expression of the Brazil Current between 20 and 30°S. The images are a digital composite from four passes of the NOAA-7 satellite on 3/4 October 1984. Red is warm and yellow is cooler. The Brazil Current is the red band parallel to the coast. The vertical structure of the current (the S. Atlantic equivalent of the Gulf Stream) is discussed on page 48. (Photo: INPE/Brazilian Space Agency/Univ. Rhode Island/O. Brown, R. Evans, J. Brown & A. Li, Univ. Miami.)

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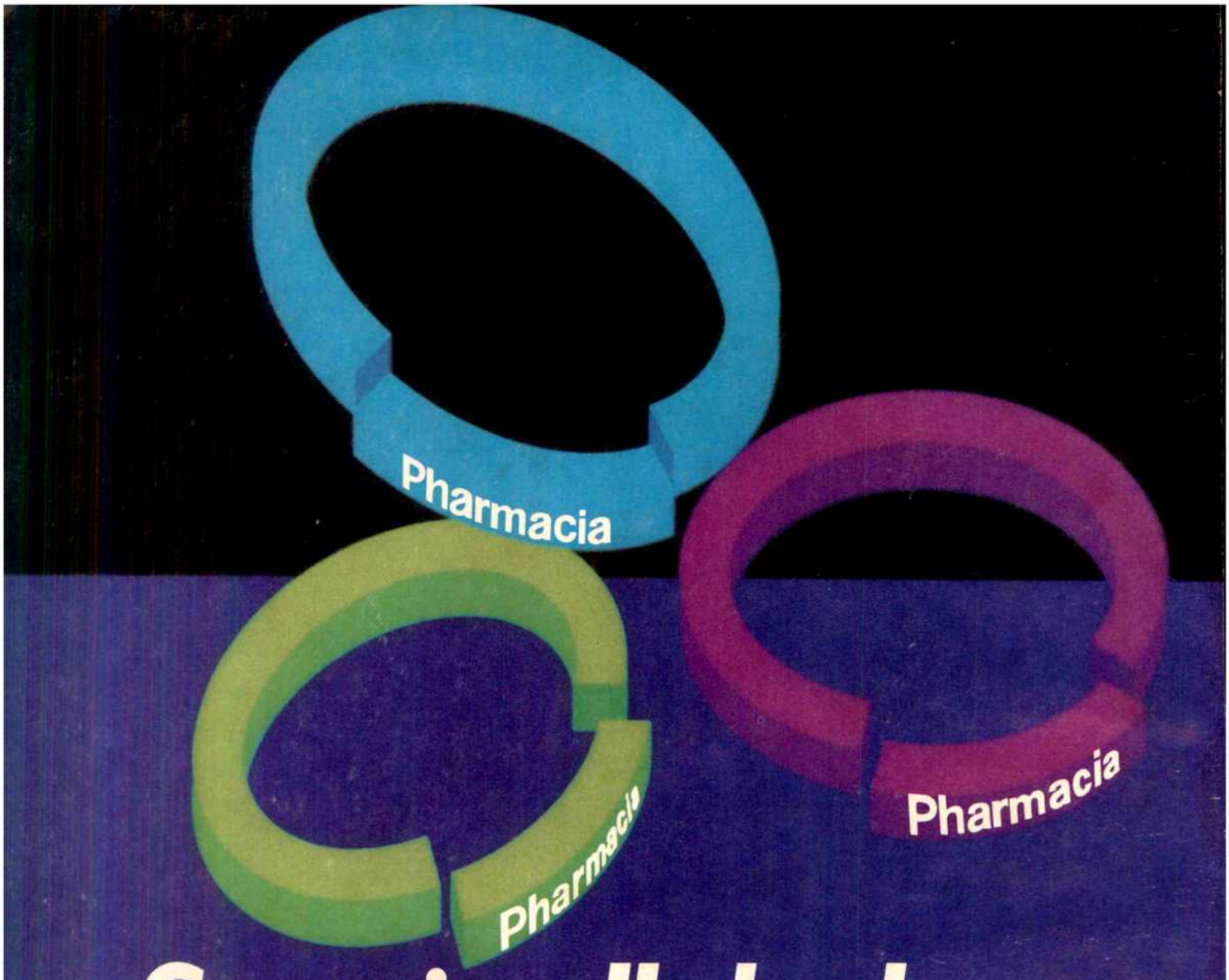
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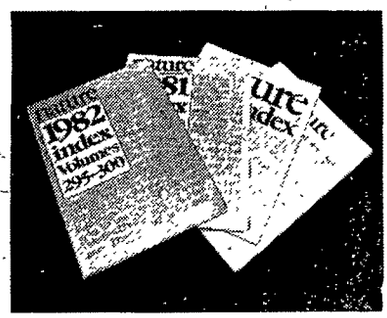
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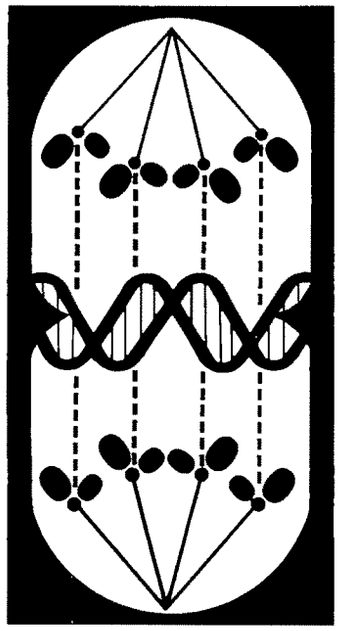
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For three days, you will meet and listen to preeminent scientists, physicians and biotechnologists describe their research and first-hand involvement in experimental approaches involving the application of molecular biology to systems and organisms. Topics will include the diagnosis and therapy of genetic defects, homoeotic genes, yeast development, development of the immune system—including T-cell receptors—and the use of new biological materials in medicine and industry. Roundtable discussions will be held each afternoon to give delegates ample opportunity to mix with and question the speakers. Participants will have an opportunity to take part in detailed discussions and present their own ideas. Poster presentations are also being invited.

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A major exhibition of scientific products and services will be held in conjunction with the Conference.

PROGRAM SCHEDULE*

DAY 1: MONDAY, OCTOBER 7

SYSTEMS

Speakers	*Topics*
F. Bloom (Research Institute of Scripps Clinic)	Vertebrate neural systems
J. Gerhart (U. of California at Berkeley)	Early development—Xenopus
C. Nüsslein-Volhard (Tübingen)	Early development—*Drosophila*
C. Goodman (Stanford)	Development of invertebrate neural systems
H.R. Horvitz (MIT)	Caenorhabditis

DAY 2: TUESDAY, OCTOBER 8

CELLS

Speakers	*Topics*
M. Davis (Stanford)	T-cell receptors
U. Storb (U. of Washington at Seattle)	B cells
J.M. Bishop (U. of California at San Francisco)	Oncogenes
M. Ptashne (Harvard)	Regulatory proteins
I. Herskowitz (U. of California at San Francisco)	Yeast development
E. Jaworski Monsanto	Plant transformation

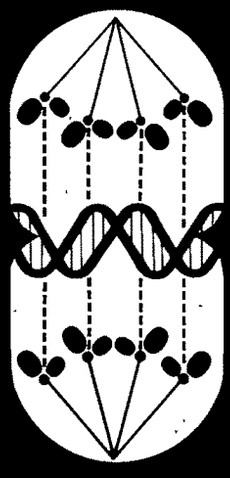
DAY 3: WEDNESDAY, OCTOBER 9

GENES

Speakers	*Topics*
R. Palmiter (U. of Washington at Seattle)	Transgenic mice
D. Hogness (Stanford)	*Drosophila* homoeotic genes
W. McGinnis (Yale)	Mammalian homoeotic genes
T. Friedmann (U. of California at San Diego	Human therapy

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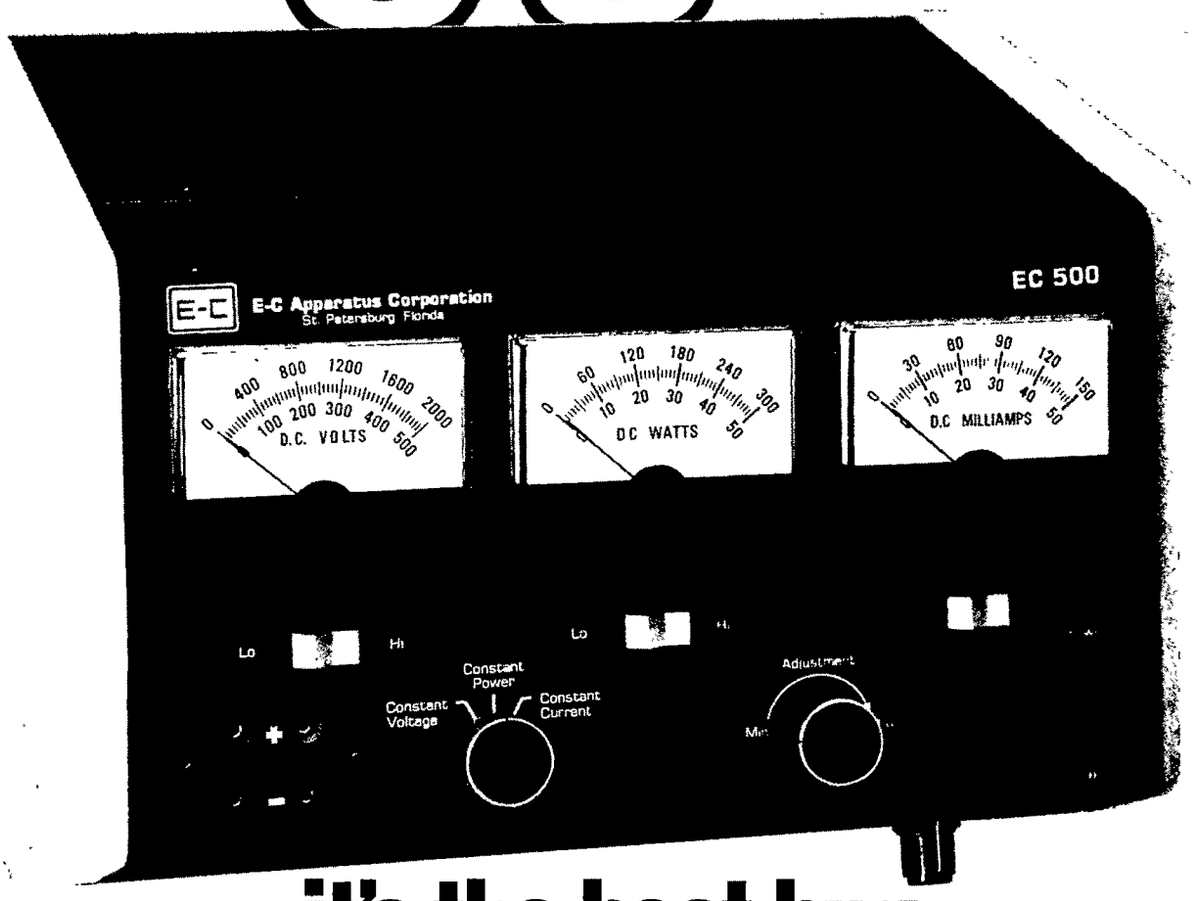
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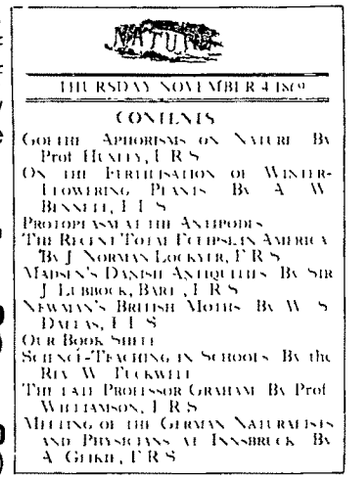
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Star wars threat to ABM treaty

The Pentagon recipe for testing star wars technology without technically violating the anti-ballistic missile treaty is an artificial exercise likely to do more harm than good.

THE Jesuits earned themselves a bad name in the sixteenth century not only for their enthusiastic conduct of the inquisition but for their casuistry, their willingness to bend general rules to the circumstances of particular cases. The Jesuits are now much reformed, but the US Department of Defense is well on the way to succeeding to their odious reputation. The Pentagon's publication last week of its recipe for testing the viability of its star wars plans without violating the Anti-Ballistic Missile (ABM) Treaty with the Soviet Union (see p.3) is as fine an example of the jesuitical arts as there has been for years. In defence of its reputation, the department may say that it would never have dreamed of producing a document certain to give as many hostages to fortune if Congress had not required it to do so. But the Congress, whose love for star wars has yet to blossom, intended to offer Mr Caspar Weinberger, the US Secretary of Defense, a poisoned chalice; it would have been possible and prudent for him to have replied in terms less obviously calculated to undermine the ABM treaty.

What the Pentagon has done is to exploit the verbal inconsistencies of the treaty, as drawn in 1972 when the two signatories had no immediate cause to contemplate anti-ballistic missiles of other than the then conventional kinds. The chief interdiction (Article 1) is that neither side should deploy an ABM system except at the sites agreed (originally two, reduced to one in 1974). An ABM system is defined (Article 2) as one "currently consisting" (in 1972) of interceptor missiles, launchers for them and radars designed for use against the ballistic missiles rather than, for example, in air defence. The treaty says that none of these components should be deployed (except as specified) and makes plain that the interdiction applies not only to operational components but to components "undergoing testing". Elsewhere (Article 5), the treaty goes on to say that the signatories shall not "develop, test or deploy" ABM systems or their components which are based in space, in the atmosphere, on the sea or which are even movable on dry land — a point emphasized by the way in which the US delegation to the negotiations asked for, and got (in January 1972), an assurance that it would be a violation of the treaty if either party built a radar or a launcher for an intercepting vehicle that was not fixed on the surface of the Earth.

Violation

The bearing of these provisions on the way in which two parties to the treaty conduct themselves ten years later is ambiguous only to those who wish that it should be so. Most people will, for example, understand the string of verbs "develop, test or deploy" to indicate that development is the stage in a technical process that precedes testing, which is in turn a preliminary to deployment. But on that reading, the Strategic Defense Initiative will violate the ABM treaty once the feasibility of space-based missile defences has been investigated (by research) and the development of hardware has begun. The distinction between research and development is notoriously arbitrary, in civil as well as military industry, but because the star wars project will be increasingly concerned with demonstration projects (aimed at convincing the military, members of the Congress and, no doubt, US taxpayers) that the game is worth the candle, it is inevitable that the United States will soon be accused of having broken the treaty. "But it's only research", will be the cry, which will be defensible only on the hollow grounds that the US Congress has not by then agreed to provide funds for more than the research programme as now defined.

Sham

The notion that it will be possible to test the efficacy of novel kinds of missile interceptors without violating the treaty by pointing them at satellites rather than at ballistic missiles, or that tracking radars will be permitted if operated at reduced power, is similarly a dangerous sham. Although a successful demonstration that this or that new kind of ray gun can destroy a satellite will not, for example, also test the effectiveness of the target-tracking systems that would be needed for an operating defence against ballistic missiles, a few experiments like these could provide the knowledge on which full-scale missile interceptors based in space may be confidently designed. It should be even easier to extrapolate from observations with low-power radars or infrared sensors to the components needed for a defensive system. So there is every reason why the course charted in last week's document will provoke the Soviet Union to fierce protests. The Pentagon should also worry about the extent to which this advance advertisement of how it will break the spirit if not the letter of the treaty will generally undermine even the support of those who accept that the case for a programme of research on anti-ballistic missile defence can be sustained.

But how else to arrange that the feasibility of star wars can be tested? As it happens, the ABM treaty provides for a mechanism for doing just this — the creation of a Standing Consultative Commission to consider questions arising under or about the treaty, including (Article 13(d)) "possible changes in the strategic situation which have a bearing on the provisions of this treaty". By agreement, the commission was lumped together with that required by the first SALT agreement. This, no doubt, is the body to which the United States will turn if and when the time comes to raise with the Soviet Union the case for deploying some kind of system based on star wars. (This is also the forum in which the United States should have raised its proper doubts about the function of the Soviet radar, now being built near Krasnoyarsk in central Siberia, which is of a type whose siting there is forbidden by the ABM treaty.) It would be better to bite that bullet sooner rather than later, if only to keep some kind of relic from the days when arms control agreements seemed desirable and feasible.

But why bother? Nobody forgets that part of President Reagan's objective in embarking on star wars was his wish to make arms control agreements unnecessary. If the two superpowers (or even just one of them) could shelter behind solid missile defences, those endless visits to Geneva might be dispensed with. This argument (some say wish-fulfilment) is often heard in the United States; much less commonly in Western Europe. There are two difficulties. First, the United States is already engaged in arms control negotiations at Geneva (adjourned until later in the month). Second, the arguments that made the ABM treaty seem worthwhile in the 1970s are still valid even in the changed circumstances of the 1980s. The objective then was to protect the strategic retaliatory forces of each side from being rendered ineffectual. The proliferation of warheads and their increased accuracy has since made counter-force strategies

credible, but only just. But there is no reason to believe that a working star wars strategy is more easily realized. In short, and for as long as star wars remains a hypothesis to be tested, the need for arms control is as pervasive and as irresistible as President Reagan found during the first few months of his first term, in 1980.

But what is to be done about Soviet duplicity in this and other connections? At times, the US administration sounds a little like the lawyer whose defence of his client against a charge of murder takes the form of asserting that the client did not kill the victim and, then, that he did so in self-defence. The Krasnoyarsk radar is a sign (in the absence of argument to the contrary) that the Soviet Union has not been resting on its laurels, the anti-ballistic missile system around Moscow. It may also be the case that the Soviet Union is already hard at work on a research programme not very different in character from star wars itself, as the US administration claims, but that is irrelevant. The lesson that needs now to be learned is that, for as long as these schemes remain dreams (not necessarily illusions) they should not be allowed to affect policy. And if they are found feasible, their deployment will have to be discussed between the superpowers. Why not accept that now, not later? □

Fun made dull?

British school mathematics may be made worse by the government's recipe for reform.

WHAT should a young person know of mathematics? And how should mathematics be taught at school? From time to time, these questions recur almost everywhere, as some mathematicians would say. Under the idiosyncratic guidance of Sir Keith Joseph, Secretary of State for Education and Science, the British government has just embarked on yet another attempt to breathe new life into a jaded curriculum. Things may seem to have changed a lot (but not for the worse) since earlier decades, when British governments behaved as if they had no right to tell the local education authorities, which administer British schools, what should be taught, but only a kind of admonitory right to complain if teaching and (more often) other aspects of school life were hopelessly awry. But on this occasion, the government has decided to stiffen its exhortation to reform by the appointment of more than 300 full-time teachers whose sole responsibility will be to ensure that the pace of reform of mathematics teaching does not flag. Those with long memories should be forgiven for reminiscing about the early 1960s, when the government encouraged renewal of the mathematics curriculum, and when local education authorities showed willing by appointing people called "advisers" to help teachers change their teaching habits.

The objective now is also much what it was two decades ago, when the general opinion was that mathematics should be made to seem more like fun to students, that it should seem more "relevant" and that teaching should concentrate more on conceptual matters than on the learning of manipulative skills. (In Britain in the 1960s, although concepts such as that of sets made their way into the curriculum even of younger children, the "new math." never made quite the headway that terrified parents and some younger people in the United States.)

The occasion for the British government's new venture is a booklet by Her Majesty's Inspectors (of schools), called *Mathematics from 7 to 16* (HMSO, £2.00), which argues much the 1960s case for reform, but in a way that has predictably excited the British popular press. The most inflammatory passage is that which argues cogently that young people should be helped to use calculators as a means of carrying out calculations, not simply be allowed to use them for confirming the results of calculations done by other means, and which goes on to say that "long division.. should no longer be generally taught" and that "logarithms, as aids to calculation, are obviously redundant". The rumble of conservative protest has been palpable, but even radicals have been taken aback.

Conservatives should not be afraid that the end of their world has come. The new document is a recipe not so much for upheaval as for consolidation, even for consolidation about modest goals. This is not surprising, since the school inspectors have themselves relied on a study of school mathematics carried out by a government committee under Dr (now Sir) William Cockcroft and published three years ago. The inspectors echo that earlier plea that the mathematics taught in schools should consist of a central core of knowledge and understanding determined by the needs, interests and abilities of most students. On this view, mathematics at school is largely a utilitarian study. Even the section in the manifesto on "conceptual structures" is largely devoted to a sensible discussion of the reasons why students should appreciate the importance of estimating the results of calculations and approximating to them (or even to the insoluble problems of the real world). The document repeatedly urges that teachers should engage their students in practical work, but it is partly redeemed by its advocacy of open-ended questions in mathematics examinations.

The more serious objection to what is now proposed is its dullness, or its potential dullness in the hands and heads of many teachers. While the language of sets may, in the 1960s, have seemed a licence to some teachers to make algebraists of five-year-olds, the repeated exhortation to teachers now to bear in mind the practical needs of their students, especially to find jobs, will give many teachers the idea that mathematics should be turned into sophisticated mensuration. And while the inspectors' document repeats in humdrum language the belief that mathematics is more a part of modern life than of that of any previous age, it does very little to suggest to teachers that they should help their students understand how mathematics has become so powerful. Thus, absurdly, in spite of the inspectors' advocacy of computers as a means of making calculations, nothing is said about the benefits of understanding that even the simplest techniques for arithmetical manipulation (not to mention long division) involve the use of algorithms of a kind that also underlie computer programs. Fortunately, most teachers and many of their students already know better.

All is not lost, however, for the inspectors' prospectus is described as a discussion document, on which opinions will be welcome. Here are some complaints that the inspectors (and their minister) might usefully accommodate. First, because mathematics is as important as the document says, students of all ages and ability should be helped to understand how these circumstances have arisen (which argues for a little history) and why mathematics is so powerful (which argues for school-leavers knowing something of what can be accomplished by the calculus even if they have not learned to differentiate a simple function). Second, and consistently with the inspectors' utilitarian cast of mind, the curriculum should give students a general understanding of how problems of the real work are turned into problems in mathematics which, in practice, are soluble only exceptionally. Third, students need to have their spirits lifted by repeated demonstrations of the sheer neatness of much of mathematics, whence much of its power. But they need also to appreciate that, like other parts of science, mathematics remains unfinished business, as can be told at the earliest ages from the lack of an analytical formula specifying the prime numbers.

Can Sir Keith Joseph's band of advisers hope to accomplish all this? With luck, they will do much good, but there are two respects in which they will be handicapped from the outset. First, the mathematics teachers in British schools are too few and are also, too often, as unconfident of the material they hope to teach as are their students. The immediate remedy is for more in-service training, as the government agrees. The long-term solution may be unattainable, given the demand for people who can count in the economy at large. Second, a point which the government does not so far concede, there is a need for yet another deliberate attempt to redesign the mathematics curriculum by practical work by teachers working in the schools, not by edict from on high. Plainly, as things have turned out, what British school mathematics most needs is a flood of protest to the Senior Chief Inspector, Mr E. J. Bolton. □

Star wars

DoD makes a monkey of anti-missile treaty

Washington

THE Pentagon's Strategic Defence Initiative (star wars) office has produced the first detailed account of how it intends to carry out field tests of new ballistic-missile-defence hardware over the next five years without violating the 1972 Anti-Ballistic Missile (ABM) treaty. The explanation, contained in a report ordered by Congress when it approved the star wars budget last year, is likely only to inflame criticism that the Reagan administration is playing fast and loose with the treaty requirements.

Most of the field tests would be justified on the grounds that they do not involve "ABM components" or "prototypes of ABM components". The treaty does not prohibit the development of weapons to shoot down satellites, cruise missiles or tactical ballistic missiles. So when railguns and rocket-propelled projectiles are tested in Earth orbit in the early 1990s, their targets will be satellites, not missiles. The Department of Defense (DoD) report says that such tests will demonstrate the technology needed to defend space-based ABM battle-stations against Soviet anti-satellite weapons. But these technologies are also candidates for ABM systems proper, although DoD says only that the tests will "permit a decision to be made on the applicability of more advanced technology for ABM purposes".

Tests of lasers and space-based tracking systems, on the other hand, would be justified on the grounds that they will have only "limited capability". DoD says because the hardware to be tested is not advanced enough to operate in a fully fledged ABM system, it is not a "prototype". At least some of the limitations may be artificial. Thus a system to track Inter-Continental Ballistic Missiles (ICBMs) will not qualify as an ABM component prototype because it "may measure the signature of booster plumes, but not in real time".

Congressional critics of Star Wars were quick to point out, after the report was issued last week, that the Pentagon's reliance on the anti-satellite-weapon loophole is further evidence of the Reagan administration's opposition to any progress on a treaty limiting testing of anti-satellite weapons. Representative George Brown (Democrat, California) has called for a renewal of a congressional ban on further anti-satellite testing. He also called plans to test star wars hardware against satellites a "circumvention of the spirit of the ABM treaty".

An analysis by legal scholar Abram Chayes of Harvard University reaches a similar conclusion. He says that attempts to develop star wars technology under the label of anti-satellite or anti-tactical-ballistic-missile programmes are "legally disingenuous" as well as technically costly. Chayes points out that the course charted by the Reagan administration will inevitably generate disputes over its legality under the treaty and argues that, instead of being simply a "research" programme that will leave open a decision to renounce the treaty in the mid-1990s and proceed with deployment, star wars as now conceived may make that decision a foregone conclusion.

The DoD report suggests two other ways to get around the provisions of the ABM treaty. The treaty specifically allows field testing of fixed land-based ABM systems or components. This was the administration's justification for the experiment last year in which an interceptor launched from Kwajalein Missile Range in the Pacific hit an ICBM launched from White Sands Missile Range in California. Several further tests of both radars and interceptors is planned under this provision.

The report also hints at a more drastic way to evade the treaty's constraints. Noting alleged Soviet violations of the treaty, the report says "we do reserve the right to respond to those violations in appropriate ways, some of which may eventually bear on the Treaty constraints as they apply to the United States".

Stephen Budiansky

Star wars

Academics sign on for research

Washington

WHATEVER their first reservations, US universities seem to be having no scruples about cashing in on President Reagan's star wars programme of ballistic missile defence, known formally as the Strategic Defense Initiative (SDI). The SDI organization's innovative science and technology office, which will spend 5 per cent of the star wars budget, has started to award multi-million dollar contracts to university/industry consortia for basic research with possible applications to missile defences. The office, established only last November, will soon have committed over $50 million for basic research. More than 50 US institutions will be working on basic star wars research within the next few months.

The three consortia already named by the innovative science office will be working on non-nuclear power sources suitable for space-based systems ($19 million over four years), optical signal processing ($9 million over three years) and advanced composite materials ($15 million over three years).

Smaller institutions are not being left out. Besides such well-known research establishments as the California Institute of Technology and Massachusetts Institute of Technology, the consortia include, for example, the University of Texas at Arlington and the University of Alabama at Huntsville.

Applied (as distinct from basic) research, which accounts for the greater part of the SDI budget, is being carried out in-house in government laboratories or in industrial companies; several hundred SDI research contracts have already been negotiated. SDI has a total budget of $26,000 million over the five years from 1 October 1984.

The director of the innovative science and technology office, James Ionson, says he has no illusions about the magnitude of the technical problems confronting SDI, but he is convinced that solutions to many of them will eventually emerge from speculative basic research of the sort supported by his office. According to Dr Ionson, many ideas that at first "seemed crazy" have now demonstrated their potential and will be included in the SDI effort.

All the basic research supported at present through Ionson's office is unclassified, but the classification requirements for SDI research are under scrutiny by the Department of Defense (DoD). Ionson is confident, however, that DoD's policy of relying on classification alone to control on-campus basic research will be maintained.

The recent disruption caused by DoD at a meeting of the Society of Photo-optical Instrumentation Engineers (see *Nature* 18 April, p.569) raised the possibility that DoD might in future make more use of Export Administration Regulations to restrict the flow of sensitive technology abroad. According to Dr Ionson, use of such regulations is most likely when commercial interests become involved but each case will be considered on its own merits.

Export regulations will undoubtedly be the subject of politically-delicate negotiations if overseas institutions participate in star wars research, because foreign governments will want to ensure that they receive the benefits of technology spin-off. But Defense Secretary Caspar Weinberger has still not given the formal go-ahead for foreign collaboration.

Among the major research threads supported through Dr Ionson's office is optical systolic computing, which could bring within reach the enormous computing power that would be needed for a star wars defence. Ionson is also looking at the possibilities for molecularly engineered dielectrics for capacitors, plasmoid weapons and the protection of satellites in radioactive environments by pumping out the Van Allen radiation belt.

Tim Beardsley

Japanese Science Council

Elected body goes out fighting

Tokyo

THE Science Council of Japan (JSC)'s final meeting as a directly elected body ended last week in the liberal tradition it has long upheld. One of its final actions was to call on the government to improve the status of female scientists.

Since 1948, Japan's unique "Parliament of Scientists" has provided a means for Japanese research workers to express their opinions to government. Every three years, 210 representatives from every branch of science have been elected to the council by a direct vote of more than 200,000 of Japan's scientists. Committees organized by the council have been instrumental in shaping the grants policy of the Ministry of Education, Culture and Science (MESC), in pressing for the establishment of new institutes in areas where they were thought most needed and in upholding scientists' rights. The president of JSC is automatically a member of the Council for Science and Technology, the highest science policy-making body in the land, and thus has the ear of the Prime Minister.

In recent years, however, things have not always gone smoothly between the government and JSC. As MESC relied more and more on advice from its own internal science council, JSC came to see its influence on grant policy decline. In turn, voters became apathetic and, as the government sees it, the council came to be influenced more by those with a "political" axe to grind than by those concerned with purely scientific matters. Apparent opposition to nuclear power by JSC particularly irritated the government.

In 1982, the government, which pays all JSC's expenses, finally demanded reform, and when it proved hard to agree, reform was forced on JSC. The result is that from now on, members will no longer be directly elected but will instead be appointed through the scientific societies (see *Nature* **305**, 361; 1983).

The call to improve the lot of women scientists stems from surveys carried out by JSC over the past few years of the special difficulties encountered by women scientists. It was plain that few women were reaching the top posts: there are no female full professors in the science faculties of the principal universities, few women among the laboratory chiefs of government research institutes and a relatively small percentage of women entrants to science courses in top universities.

These observations mirror problems for women in Japanese society as a whole. The average salaries of women are barely half those of men, not so much because of different rates of pay for the same job but because women generally occupy lower positions. Surveys show that three-quarters of companies hiring university graduates (who will eventually go into management positions) hire only men. Of those that do hire women, half will not allow them any possibility of promotion and two-thirds will not give them access to training or education programmes. "Retirement" at marriage is a not unusual condition of work.

JSC's subcommittee on the status of female researchers carried out its own survey on the position of women scientists after an earlier plea, in 1977, failed to persuade the government to conduct a proper survey. Some 2,000 women scientists and 1,400 male scientists were asked to complete questionnaires, and the results confirm women's poor promotion prospects.

Taking into account academic record, the chances of a woman becoming a full professor appear much lower than those for a man. Most women who successfully complete their doctorates cannot hope to gain a post higher than assistant lecturer and the higher they go, the more difficult further promotion becomes. Professor Katsuko Saruhashi, one of the few women to reach laboratory director level (within the government's Meteorological Research Institute) and a veteran campaigner for the rights of women scientists, says it is bad for the nation not to make use of women's abilities and that what is now being demanded is a proper government survey that could be used to make the reforms that would improve the position of women.

On the government's recent record, however, it is unlikely that her wishes will be fulfilled. The Diet will vote next month on an equal opportunities bill intended to give the government an opportunity to ratify the United Nations Convention on the Elimination of All Forms of Discrimination Against Women. The bill has, however, been thoroughly emasculated by employers' representatives, and apart from abolishing some old restrictions, such as those that prevent women working late at night, it provides little in the way of new rights. There are so many loopholes in the bill that it would be almost impossible for legal action to be taken on its provisions. Although it calls for women to be given equal treatment to men in job recruitment, allocations and promotion, there are no substantial penalities for non-compliance.

Alun Anderson

Romania

Nuclear power in the distance

PRESIDENT Nicolae Ceaucescu of Romania last month cut short an official visit to Canada after he was told that he could not visit the Pickering nuclear reactor plant because of a labour dispute. He did, however, visit Hydro-Quebec's nuclear power station at Becancour, which uses Candu reactors, similar to those the official handouts somewhat misleadingly referred to the "two plants now under construction in Romania".

Romania signed contracts for the purchase of two Candu reactors in 1978 and 1981, and has options on up to eight more. But the deal has been beset by difficulties; firstly by Romania's lack of hard currency, which makes necessary a countertrade (barter) arrangement, and then because the Romanians insisted on dealing with all the Canadian subcontractors separately, instead of negotiating with Atomic Energy of Canada Ltd (AEC). As a result, although the proposed site at Cernavoda in Romania is being prepared to receive five reactors, hardly any equipment has yet arrived from Canada. The 4,500 Romanian on-site workers are simply carrying out preliminary construction work, including the containment buildings for the firt two Candus. (Containment buildings are a rarity in East European power stations; Soviet doctrine considers them a Western device to raise costs, although the Hungarians insisted on having them for their Soviet-produced reactors at Paks.)

Mr Ceaucescu is clearly anxious to get the Candus installed as soon as possible. Although Romania is an oil-producing country, heavy investment in the petrochemical industry during the 1970s has turned it into a net energy importer. By the early 1980s there was an energy crisis, and the severe winter of 1984–85 meant prolonged power cuts for both domestic and industrial users and a ban on all private motoring. Not surprisingly, the Party daily, *Scintea*, on the eve of the President's visit to Canada, hailed the Candu as the answer to Romania's energy problems.

The project is, of course, badly behind schedule. The first two Cernavoda Candus were meant to be operational by 1985; instead, the first major equipment will not be delivered until the autumn, and the primary cooling pumps, from Bingham Willamette of British Columbia, will not be delivered for two or three years.

But Canada too needs the Romanian contract to save jobs in its nuclear industry. Although the existing deals provide for Romania gradually to absorb Canadian technology and eventually to build its own Candu reactors (paying a royalty on the first fifteen), the Canadian content of the third and fourth Cernavoda reactors, though less than the first two, would still be significant. Furthermore, after prolonged negotiations, the two sides have signed a third-market contract, by which (subject to Canadian requirements on non-proliferation), Romania would cooperate with Canada in selling Candu reactors to other countries, particularly in the socialist and non-aligned blocs where Romanian contacts could be particularly useful.

Vera Rich

European Communities

More attention for basic research?

Brussels

Karl-Heinz Narjes, research commissioner of the European Economic Community (EEC), seems to be edging towards a more balanced programme of Community research. At an informal meeting of research ministers in Rome last week, he urged that industrial competitiveness should dominate the programme to the exclusion of research in fields with fewer technological benefits.

As things are, the EEC programme is dominated by schemes for improving European competitiveness vis-à-vis the United States and Japan (notably the ESPRIT and BRITE programmes). But Narjes stressed last week that there are "yawning gaps" in agricultural research, for example, especially the reduction of surpluses, and the improvement of food quality and of animal production. There is no research at all in fisheries and aquaculture, he said.

The interim report on the current framework programme, which will run until 1987, also talks of a serious imbalance between nuclear and non-nuclear objectives, and says that schemes for stimulating cooperation and interchange have been more "gradual" than expected.

The aim of the presentation was to start EEC research ministers thinking about their priorities for future Community research on the eve of the review of the framework programme, planned for the second half of 1985. That debate will be preceded by a comparison of national and Community scientific policies by the COPOL (Coordination of National Policies) group meeting in June 1985.

The Commission's own proposals for future areas of research include health care and technology, the management of energy resources, training, European cooperation, the mobility of researchers (inside and outside the Community) and the more effective use of scientific equipment. Moreover, the Forecasting and Assessment in Science and Technology Group, whose preliminary conclusions are due in mid-1985, wants more research of an economic character into companies and services.

Whether ministers will accept these proposals will depend not least on what they are prepared to spend on research out of the Community budget and their own resources. By March 1985, the activities of the current programme had cost 2,685 million European Currency Units (ECU, 1 ECU = £0.60) out of a total of 3,750 million ECU budgeted until 1987 — three per cent of the total Community budget. In 1984, ministers committed themselves to increase research funding. Both Jacques Delors, the president of the Commission, and Mr Narjes would like to see that figure doubled for the second framework programme covering 1988-1991/92.

The meeting in Rome also gave ministers a foretaste of the agenda for the formal EEC research council scheduled for 4 June in Luxembourg.

- **Telecommunications research.** The European Commission is keen to get a speedy decision on its proposals for research in advanced communications in Europe (RJCE, see *Nature* **314**, 209; 1985) but the United Kingdom, France and West Germany are sceptical, asking for a clear definition before embaking on research.
- **Tritium-handling laboratory.** To support the thermonuclear fusion programme, EEC has agreed to build a laboratory at the Joint Research Centre at Ispra in Italy, but Britain and France continue to doubt its siting and timeliness, given that the Next European Torus (NET), for which the tritium would be needed, will not be ready until the 1990s, and that the site for that machine has not yet been chosen.
- **European Synchrotron Facility.** In exchange for its agreement to the proposed site at Grenoble, Denmark is seeking a marine research institute in its own country.
- **Initiative for Research in Informatics applied to Society (IRIS).** This proposal, put forward by the Italians in Rome, would encourage companies to develop new products and social services in fields such as medicine and agriculture. The proposal has been greeted with scepticism by the United Kingdom, but the Commission has agreed to a preliminary study.

Ministers also discussed proposals by the European Commission for greater control of state aid in the member states. Such control over national research programmes would be particularly unwelcome in countries such as the United Kingdom, France, West Germany and Italy.

Anna Lubinska

French research

CNRS to concentrate resources

THE French national research council (CNRS) is to devote 40 per cent of its resources to twenty newly defined "strategic objctives", according to the CNRS 5-7 year plan published last week. The twenty disciplines include many fundamental subjects, such as nuclear and particle physics, but these are sometimes combined in significant new associations. Thus, the "sciences of the Universe" combine in one objective with particle and nuclear physics. Other objectives specify boundary-breaking explicitly, such as that named as "the interaction between and chemistry biology". It has long been a passion of the CNRS director Pierre Papon to forge unity among the highly divided disciplines in France; the CNRS council seems now to have given him the hammer and anvil he needs.

The new objectives also stress long-range applied science, such as Earth observation from satellites (in anticipation of the launch of the French SPOT satellite later this year), process engineering, biotechnology and so on, but Papon said last week that their selection is not a threat to the basic sciences. Many of them, he said, are in any case basic science and "it is not a major problem to defend basic science in France".

According to Papon, the real objective of the CNRS "list of 20" is "to unify science". He says he was struck, during a European tour last year, by the many links between chemistry and biology departments in Britain, whereas there is just one such department in France. Unification of disciplines is also more advanced in Sweden and Switzerland. Research in France is more fractured than elsewhere, though many modern scientific problems are multidisciplinary.

Mobility, both among disciplines and geographically, is poor, but Papon says CNRS is working with the ministry of research and technology to solve many purely administrative and practical obstacles, such as finding a job for a spouse. Last year there was a growth of some 15 per cent in the number of CNRS researchers moving into industry for a spell of three years or more. This might be "slow progress", but there is a "psychological problem" said Papon. Last year, one in two hundred CNRS staff made a move to industry. "It would be nice to double the figure."

Robert Walgate

Areas of research

THE research areas that CNRS will now emphasize are:

- **The universe, atomic nucleus and elementary particles (some of the costs of these disciplines are borne outside CNRS).**
- **Mathematics.**
- **Optics.**
- **Materials sciences.**
- ***Filière électronique* (science applied to the microelectronics industry).**
- **Plasma physics and nuclear fusion.**
- **Energy and thermodynamics.**
- **Process engineering.**
- **New methods in earth sciences.**
- **Dynamics of the ocean systems.**
- **Remote sensing.**
- **Continental water.**
- **Understanding chemical reactions.**
- **Chemistry-biology interaction.**
- **Biotechnology.**
- **Neurosciences.**
- **Sciences of evolution.**
- **Sciences of communication.**
- **Employment, work and technology.**
- **Urbanism, architecture and society.**
- **Large scientific equipment.**

UNIDO biotechnology

Twinned-centre jumps legal gun

New Delhi

DESPITE legal and other hurdles, the United Nations Industrial Development Organization (UNIDO) is trying hard to put its International Centre for Genetic Engineering and Biotechnology (ICGEB) on an operational footing this year. The sixth meeting of the ICGEB preparatory committee held last month in New Delhi has decided to launch the centre's scientific activities without waiting for the ratification of the ICGEB statutes by member countries

So far, 36 nations have agreed to be members of the first international biotechnology centre, but only Iraq has ratified the statutes. Mr Adolfo Teylhardat of Venezuela, chairman of the preparatory committee, says the statutes must be ratified by at least 24 countries before ICGEB becomes a legal entity

UNIDO mooted the idea of the centre in 1981 with the aim of bringing the benefits of biotechnology to bear on problems unique to the developing world. By a decision in 1984, ICGEB is to have two components, one located in New Delhi and the other in Trieste, Italy. The host countries have yet to ratify the statutes and finalize the agreements with ICGEB.

These legal hurdles are not expected to hold up the activities of the centre. The New Delhi meeting, attended by 25 countries, decided formally to begin the centre's work with a workshop on plant biotechnology in New Delhi in September. This will be followed by another workshop at Trieste on a topic yet to be decided.

In establishing ICGEB, UNIDO is being guided by an panel of eminent scientific advisers (PSA). One of the panel's suggestions, based on the experience of the Salk Institute in the United States, was to hire between five and ten distinguished scientists to serve as non-resident members of the faculty of the centre This suggestion, though on the agenda, was not followed up at the New Delhi meeting. Nor was any decision taken on the selection of a director for the centre. Taylhardat said that ICGEB is looking for a single director for its two components who would be "a scientist of world repute with managerial experience" Selection has been deferred until the next meeting of the preparatory committee in Havana in November.

A major recommendation of PSA is that excellence and not nationality should be the basis for selection of the director and other senior staff, who need not necesarily be from member countries. Except for Greece, Italy and Spain, most of the ICGEB members are developing countries with little infrastructure or trained manpower for genetic engineering research. For this reason, most of the staff is initially expected to be from developed nations.

The ICGEB statutes provide certain immunity and privileges to internationally recruited staff that will not be available to the locally recruited scientists. This, together with the proposed discriminatory salary packages ($50,000 and above for international staff) may create problems, Indian scientists fear. India has made it clear that its contributions to the centre in New Delhi would not cover the salaries of such international staff members, the cost of which will be met with funds from Italy.

Although no countries except the hosts have pledged funds, UNIDO says the operation of ICGEB for its first 30 months is assured by offers of a total $24 million by India and Italy. ICGEB's operational cost for the first five years is estimated at $47.8 million. It is hoped that the balance will be covered by initial and yearly contributions from members and others. Laboratory facilities are yet to be set up in Trieste or New Delhi. India has allocated $4.3 million for 1985-86 for permanent facilities on 25 acres of land and temporary laboratory space in the Jawaharlal Nehru University.

Under existing arrangements, the New Delhi component of ICGEB will conduct research on biotechnology applied to agriculture, human and animal health whereas the Trieste half will focus on industrial microbiology — the conversion of biomass into fuel, bio-recovery of hydrocarbons and protein engineering. Trieste will have full facilities for developing industrially viable technologies based on genetically manufactured organisms, for scaling up technologies developed in New Delhi and a central computer facility.

By the time the centre reaches full operating capacity, each site will have a team of 30 scientists, 20 postdoctoral fellows, 30 technicians and 30 trainees from developing countries. According to Taylhardat, it will serve as an international channel for the flow of information on biotechnology from industrialized to developing nations. A gene bank containing genetic stocks and information is also planned.

Taylhardat said that ICGEB will also be the centre of a network of affiliated regional and national research institutes for biotechnology research. The New Delhi meeting considered requests for affiliation status from ten countries, including Egypt and China **K.S. Jayaraman**

US science budget

Deficit-cutting means freeze

Washington

RESEARCH is unlikely to emerge unscathed from Congress's efforts to reduce the federal budget deficit despite its relatively privileged position in the Reagan administration's list of priorities. The Senate is considering proposals to hold spending on various scientific and space programmes at 1985 levels. The agencies affected include the National Science Foundation (NSF), the National Aeronautics and Space Administration (NASA) and the Department of Energy. The House of Representatives, for its part, has refused to authorize the administration's proposed 4.4 per cent budget increase for NSF. Earlier this month, the House of Representatives similarly refused to authorize an increase for NASA (see *Nature* 11 April, p.488).

The proposal before the Senate to freeze spending on general scientific and space research, known as function 250, is part of the deficit-reduction package agreed last month between Senate Republican leaders and the White House, which was still being debated last week. Were the package to be adopted by the full Senate, it would become politically almost impossible for Senate committees to authorize budget increases for scientific agencies

The House action in freezing NSF's budget would keep the authorization at $1,501 8 million. The $67.6 million saved would come from research ($58.3 million) and from the US Antarctic programme ($9.3 million). The House Science and Technology Committee had earlier recommended going along with the President's proposed budget of $1,569.4 million, but during the debate in the House, committee members offered the cut, which was accepted by a large majority.

The House bill (HR 1210) would also transfer to the director of NSF the authority to appoint assistant directors. In the past, there have been long delays in filling these posts because of the requirements that assistant directors be appointed by the President and then confirmed by the Senate. The bill also enjoins the NSF to place "emphasis" on acid rain research. The Senate has not yet voted on the NSF budget authorization, but the Commerce Committee, one of two Senate committees vying for jurisdiction over the foundation, last week recommended a budget increase broadly in line with the administration's request. The Labor and Human Resources Committee will tackle the NSF budget later this month, after which the Senate will vote; a conference committee to resolve differences with the House is likely.

Arguments in the authorization committee are partly political posturing, as it is the appropriations committees that finally decide what money goes where. The Senate has been unable to pass an NSF authorization bill for the past four years, and few have missed it; if, however, such a bill were passed by both Houses that implied a budget freeze, it would be technically difficult and politically risky for the appropriations committees to fund a budget increase. **Tim Beardsley**

Australian science

Plans laid for new lobby

Canberra

AUSTRALIAN scientists and technologists have taken the first step towards the formation of their own national lobby group, to take its place alongside the others crowding the political landscape. Almost every other interest group seems to have a lobbying arm. But Australian scientists, although producing two per cent of the world's published scientific literature from a population base comprising less than one-half of one per cent of the total, have only now realized that a performance-enhanced mousetrap, say, will not automatically be taken up by industry, or even recognized by the media, let alone by politicians. This is why, with a few notable exceptions, scientists are a fugitive breed in Australia, misunderstood when not actually invisible. Last week's nucleation at a meeting in the Australian Academy of Science of a body to lobby government and spread the good word generally may help to rectify the situation.

The spur has been the August 1984 federal budget, in which publicly supported science was cut back by five per cent. With recruiting among academics and in the Commonwealth Scientific and Industrial Research Organization (CSIRO) already at a standstill, it began to look as if even scientists holding permanent jobs would have their research hindered. An *ad hoc* group of eight scientific societies expressed disquiet about the cuts. The Academy of Science established a 16-member National Committee for the Promotion of Science and Technology under the chairmanship of Professor Max Bennett, a neurobiologist from the University of Sydney.

The immediate outcome was the National Meeting of Concern on Science and Technology at which the elected leaders of 68 scientific and industrial societies and two academies representing about 80,000 members voted to elect an interim federation committee. That committee's first task is to explore the setting up of two secretariats, one of which will be a small professional lobbying group based in Canberra to facilitate communication between government and the scientific community, especially at budget time.

The other committee (probably based in Sydney), will provide a science and technology information service modelled on the Media Resource Service of the US Scientists Institute for Public Information. Through it, people in the news media, education and politics throughout Australia would be put in touch with experts for comment on scientific matters. The twelve-member interim committee, under the chairmanship of Professor Fred Smith, a physicist at Monash University, is due to report in six months.

A mood of optimism pervaded the meeting, but there were objections that political consensus may be difficult to achieve between academic, CSIRO and industrial scientists, particularly because the specialist societies (some with their own lobbying apparatus) differ greatly in size.

The federal Minister for Industry, Technology and Commerce, Senator John Button, said that, although he welcomed the possibility of discussions with such widely representative groups, not all basic research could be convincingly sold as seed corn for the future. In the interests of industrial restructuring, he said, public financing "may not be just where you are used to it being".

The Minister for Science, Mr Barry Jones, was absent from Australia during the meeting, but in February this year something was salvaged from the 1984 budget wreck in the form of one of his favourite projects — the Commission for the Future.

The aim of the new body is also to raise public awareness and debate about science and technology, especially about the ways in which technological change will affect the lives of ordinary people, and the choices they must make in education and employment. The commission is headed by Mr Philip Adams, an advertising executive and all-round pundit, who is an old friend of Mr Jones.

The scene is set, then, for the emergence of science (and some scientists) from the shadows where they have languished politically for so long. Noting how badly the job of advocacy has been done in the past, Professor Smith says that, realistilly, a major impact may not be felt for five years. **Jeffrey Sellar**

Famine

Remedy not by bread alone

Anaheim, California

THE more equitable distribution of available food supplies is not, by itself, the best way of avoiding famine. This is what a gathering of biologists was repeatedly told last week. And so it is a matter of urgency that biologists should pay more attention to the prediction, the causes of onset and the prevention of famine.

Organized by Dr Barry Bloom of the Albert Einstein College of Medicine, the symposium was belatedly and exceptionally tagged onto the 300 or so sessions already jamming the programme of this year's meeting in Anaheim of the Federation of American Societies for Experimental Biology (FASEB). Bloom, an immunologist with a special interest in leprosy, was prompted to organize the symposium by the present African famines. "We have learned so few lessons", he says, "that each new catastrophe tends to repeat some of the classic errors from the past."

One priority in research should be to develop criteria for predicting famines in time to prevent them. "In most circumstances, one is likely to have one group of people politically or emotionally interested in asserting that the situation is worse than it is and another arguing the opposite", says Bloom.

That theme was taken up by John Mellor of the International Food Policy Research Institute in Washington, DC, who argued that if more famine-prone countries had democratic governments and a free press, we might learn of problems in time to prevent them. Foreign aid must be channelled into transportation systems so that food ca be distributed, he said, and into the training of an elite of agricultural personnel. The priority for scientific research should be the mounting of a green revolution in Africa.

Although confident that scientists could produce the means for such a revolution, Dr Robert Goldman, of the plant biotechnology company Calgene, wondered if it would be put into effect. The science that fuelled the green revolution that saved India and Mexico from famine was developed in the public sector and applied without profit. Now that the impetus for plant improvement has passed to the private sector, particularly agricultural biotechnology companies, the developing countries may be less able to win benefits.

Famine is, in any case, the end result of a chronic condition of malnutrition. Eight hundred million people worldwide are calory-deficient, and "it seems likely that whole societies have been forced to adapt to this", says Dr Neville Scrimshaw of the Massachusetts Institute of Technology. Adaptation means reducing physical activity, which can have severe social consequences. Less obvious deficiences of iron, vitamin A and iodine are widespread, debilitating and make people more prone to infections which, in turn, can exacerbate malnutrition.

The downward spiral that ends in famine is best broken by immunology, in the opinion of Stephen Joseph of UNICEF. Calling for more research into both vaccine development and the inter-relationship between immunity, nutrition, infection and growth, he said that scientific research must go hand in hand with improved management of preventive programmes and better education of those who should benefit.

After the symposium, Dr Bloom complained that no more than 200 people had attended. Up to 15,000 people were expected to register for FASEB, and although the famine symposium was held the evening before the full meeting got under way, 2,000 people turned up next day at a lecture on the molecules involved in lymphocyte activation. **Peter Newmark**

AIDS

Undesirable import to Japan

Tokyo

WITH Japan under pressure to increase imports of US goods, it is ironical that one product Japan imports freely from the United States may have brought acquired immune deficiency syndrome (AIDS). According to last month's *Taisha*, the Japanese medical journal, two now-dead Japanese haemophiliacs were killed by AIDS, probably by infected blood plasma, almost certainly imported from the United States.

One of the victims, aged 48, died in July 1983 and the other in November last year, aged 62. The victims, the first from AIDS in Japan, showed an extraordinary loss of immunity, and the presence of antibodies to the virus HTLV-III has been confirmed.

No cases of AIDS have yet been reported among Japan's homosexual community, but a confirmed Japanese homosexual now living in the United States who returned to Japan in mid-January was diagnosed as an AIDS sufferer. The AIDS Research and Study Committee of the Health and Welfare Ministry says there is little chance that the man infected others in Japan.

The emergence of AIDS cases among Japanese haemophiliacs seems to be attributable to Japan's almost insatiable demand for US blood plasma. Despite one of the highest rates of blood donation in the world, Japan depends on the United States for nearly 90 per cent of the plasma it consumes, amounting to 300,000 litres a year. Demand for plasma-protein fractions and blood-component preparations is rising, for the latter by 15 per cent a year.

So why is Japan so dependent on blood plasma imports when there were more than eight million blood donations last year? Officials at the Health and Welfare Ministry explain that donors in Japan give only 200 ml of blood on each occasion, compared with about 400 ml in other countries. But a Japan Red Cross official says that in the United States in 1982, just under six million donations of about 450 ml were made, suggesting that *per capita* blood donation in the two countries is similar.

Dependency on blood imports is in part a legacy of the system before 1982. Blood donations were then saved almost exclusively for the use of the donor himself, who would resist the idea of volunteering blood for others, probably for the reasons that also make Japanese reluctant to donate organs for transplant (see *Nature* **313**, 338; 1985).

The Health and Welfare Ministry's planned solution of this vexing problem is threefold. First, Japanese donors will be asked to donate 400 ml instead of the present 200 ml. Second, plasmaphoresis, which separates the plasma and returns red cells to the donor, will be more widely used. Third, doctors will be asked to avoid unnecessary wastage of plasma. In the meantime, however, imports will continue and there are no plans to screen imported blood for AIDS.

All of this must provide small comfort for Japan's haemophiliacs. The article in *Taisha* even reports that the blood of 23 out of a random sample of 50 Japanese haemophiliacs contained antibodies to the virus HTLV-III, which suggests that a large proportion of Japan's 5,000 haemophiliacs may have already been exposed.

Even if imports stopped, there would be another danger. Drs Kazuo Ohkouchi and Hiroyuki Sato of Kyushu University in southern Japan have now shown that the virus HTLV-I, which causes adult T-cell leukaemia (ATL), can be transmitted by blood transfusion. ATL is endemic in southern Japan, where approximately 20,000 carriers are found among blood donors each year. Dr R.C. Gallo, chief of the tumour cell laboratory at the US National Cancer Institute, has even suggested that an AIDS-like epidemic of ATL may be imminent. Clearly, the management of blood supplies in Japan is a matter that in future will come under increasing scrutiny by the eyes of the world.

David Swinbanks

ESA, Japan agree on participation in planned US space station

EUROPE formally agreed last week to join the United States in the development of a space station, but details such as who spends how much on what remain to be decided. The European Space Agency (ESA) will now sign a memorandum of understanding with the National Aeronautics and Space Administration (NASA) which requires that the design of the space station, with costs and commitments, must be ready by the end of 1986. This phase of the work will cost ESA $65 million.

NASA has already signed a similar agreement with Canada, which aims to provide robot-servicing equipment at a cost of some hundreds of millions of dollars, and is due to sign another with Japan this month (see above).

The European contribution may finally be some $2,000 million, which should buy a pressurized module that could be used as a manned laboratory (called Columbus), a number of free-flying payload carriers for both low-inclination and polar orbits, a servicing vehicle and a resources module that could provide both the pressurized module and the payload carriers with electric power, cooling and stabilizing systems.

These housekeeping elements have been included in ESA's plan at French insistence, and would enable the European modules to fly separately from the US space station if necessary, although (for the moment) Europe would still have to rely on the US space shuttle to put its astronauts into orbit. France, however, has plans for a mini-shuttle (Hermes) and in Britain, British Aerospace has described the concept of a horizontal take-off and landing spacecraft (HOTOL). Either of these could ultimately provide people with independent access to space.

There are more immediate issues still to be resolved. US fears that technologies transferred to Europe would leak to the Soviet Union engender corresponding European fears about access to US science, technology and data. There are also the issues of who pays the still unknown maintenance and running costs of the station, the legal framework for deciding patent rights arising from work on the station and the management and decision-making structure of the enterprise.

Robert Walgate

Tokyo

JAPAN has decided to take part in the US project to build a manned space station. James Beggs, head of the US National Aeronautics Space Administration (NASA), who has made earlier trips to Japan to promote the space station, will return to Tokyo for the signing on 9 May of an agreement between NASA and Japan's Science and Technology Agency (STA).

The initial agreement will cover a two-year period of design work and will provide Japan with technical information on the space station. STA is refusing to release details of the budget until after the signing, but it is known that some 1,400 million yen ($5.6 million) has been earmarked for the project in this year's budget.

Informally, a consensus seems to have been reached that Japan should take the opportunity to master new technology and build one whole experimental module of the space station. A subcommittee of STA's Space Development Committee has recommended that it consist of three parts: a pressurized cabin ten metres long and four metres in diameter for use in experiments on new materials and new pharmaceuticals more easily produced in gravity-free conditions, a service platform for testing of telecommunications equipment in outer space and a storage cabin. Total design and construction expenses are likely to exceed Y200-300 thousand million million ($800 million).

Industry has already expressed considerable interest in the space station and several large corporations, including Mitsubishi, Mitsui, Sumitomo and Fuyo, are now merging their respective study groups to find the best way to win commercial advantage from the station. **Alun Anderson**

Popularization

Royal Society asks for more

THE Royal Society, the premier British scientific society, is to be given a hefty nudge towards the popularization of science, to judge by a meeting on the "public understanding of science" held under the auspices of the British Association for the Advancement of Science in London on Monday.

The meeting was convened by Sir Hans Kornberg, British Association president, to provide a debating forum for the Royal Society *ad hoc* committee on public understanding. And while many old chestnuts were brought out on Monday and re-roasted, Dr Walter Bodmer, chairman of the Royal Society committee, used the occasion to demonstrate his committee's determination that its work would not gather dust on any shelf.

The need is certainly great. In Britain, on the whole, there is little sign of a scientific lobby, nor of any determined effort by the universities or research councils to educate or even entertain the public in science. Bodmer and his committee wish this situation to change, and by starting at the Royal Society, traditionally a somewhat austere institution whose motto is "*nullus in verba*" (nothing in words) they are certainly taking the bull by the horns. Dr Bodmer hoped the Royal could provide a lead which others could follow.

During Monday's meeting, Dr Bodmer, director of the Imperial Cancer Research Fund, received support from many quarters both in the media and in government. Sir Robin Nicholson, chief scientist in the Cabinet Office, claimed it is now almost impossible to find a political issue without scientific or technical content, and that "90 per cent of his time" is used in advising government on these matters (and only 10 per cent on science itself). Science and technology are now central to many foreign policy issues, said Sir Robin, in the way that international trade took centre stage 25 years ago. But "the attention span grows shorter as one gets older" so now cabinet ministers can only cope with five-minute briefings or three pages of A4. The same is true of leaders of industry. But scientists, for good reasons, are trained to write *in extenso* and giving equal weight to many ideas. This is completely at odds with what is needed in government, or in the press.

Dr Bodmer was keen to affirm that he would like the Royal to play an active role in getting scientists to approach the media. A member of the science advisory panel to the British Broadcasting Corporation (which, incidentally, reckons it shows four times as many science hours in proportion to output as does US television), Bodmer is also taking seriously advice to expand the Royal Society's programme of science briefings, to institute rapid press conferences on scientific issues behind and in the news, to run deeper, background seminars on scientific matters, and to produce detailed condensed briefing documents for journalists.

On a warning from Sir James Gowans, chairman of the Medical Research Council, that such exercises cost money and a lot of time, Dr Bodmer said he hoped the Royal Society would be able to find backing "if not from government" from industry — which should benefit in the long run from better public knowledge. His committee's report is due in a few months' time. **Robert Walgate**

Animal experimentation

Alternatives neglected

Washington

A STUDY of animal experimentation by the National Academy of Sciences* suggests that institutional barriers within the biomedical community in general and the National Institutes of Health (NIH) in particular may discourage researchers from developing models of human disease and physiology based on non-mammalian organisms. The study, which was commissioned by NIH, does not directly criticize NIH's funding patterns; it does, however, recommend that the agency should support good research "without taxonomic or phylogenetic bias" and consider directing funds specifically to the development of promising model systems. Harold Morowitz of Yale University, chairman of the study, said there is "anecdotal evidence that it is difficult for people working on unconventional models to get funding". The natural bias has been in favour of using organisms closest to man, he said; unconventional proposals submitted to NIH for support do not usually fit into the mandate of any of the standard study sections, and are reviewed by *ad hoc* committees that are not always sympathetic.

Morowitz said that researchers working in unconventional systems often report that they managed to get started only by "bootlegging" funds from other more conventional research projects.

The study notes that as biological understanding shifts more and more to the molecular level, increasingly the best model for human disease and physiology is not a closely related whole organism that displays a similar macroscopic feature; rather it is often many organisms, each an analogue of a fundamental biological principle involved. One example cited in the study is research on the human disease myasthenia gravis, a neuromuscular disorder. Six different animal models have been used to discover its cause: an autoimmune attack on acetylcholine receptors at neuromuscular junctions. Studies on chick muscle cells in culture showed that a snake venom binds specifically to these receptors; the venom was then used to demonstrate that patients had a striking shortage of receptors. A cobra neurotoxin that blocks the receptors was administered to rats to see if a shortage of receptors could explain the observed disease symptoms. And to see if an autoimmune response could be involved, rabbits were immunized wth receptor cells isolated from invertebrate species.

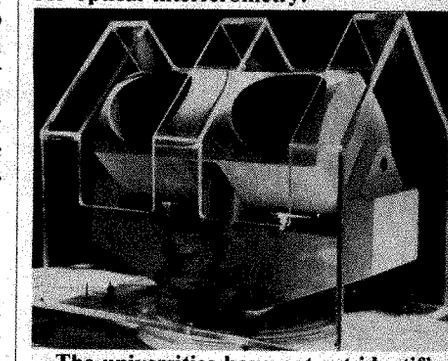
Stephen Budiansky

**Models for Biomedical Research*, National Academy Press, Washington, DC, 1985 $18.50.

Big telescope race

Washington

OHIO State University and the University of Arizona have decided to raise a total of $25–30 million for an 8-metre monolithic mirror instrument to be built on Mount Graham in southeastern Arizona. If others can be persuaded to join the project, the telescope may even be equipped with two 8-metre mirrors, which could be combined for optical interferometry.



The universities have not yet identified the private sponsors who they hope will supply most of the capital cost of the instrument. Both universities have, however, recently launched large fund-raising efforts, with a target of $100 million at Arizona and $250 million at Ohio. Astronomers at both universities are confident that the money will be found.

The telescope, which would also operate at infrared wavelengths, will have computer links allowing it to be operated remotely from both Arizona and Ohio. The miror or mirrors will be made using a technique developed by Roger Angel at Arizona (see *Nature* 295, 651; 1982) in which mirrors of honeycomb structure are spun-cast into a parabolic shape. A new computer-actuated lap allows very deep dishes to be polished with a focal ratio of *f*1, so that telescopes can be built that are much smaller and cheaper than hitherto. A 1.8-metre mirror made recently using the new technique "came out beautifully", according to Dr Angel. Preliminary design work on the new telescope is due to start later this year; construction might take a further four years.

Tim Beardsley

Changing the objectives

SIR — Your leading article of 7 March (p.1) deals with policies for UK science, including astronomy, in the context of fixed or declining resources. You say: ". . . one, or the other, or perhaps both of the observatories must go . . .". You suggest that the "good work these laboratories do . . . could be transferred intact to universities". Another problem is that experimental facilities have become "institutionalized".

I suggest that a contract-based laboratory might be set up in the general subject area of physical technology, of which astronomical technology would be part. It would absorb some staff and property of the Royal Greenwich Observatory and the Royal Observatory, Edinburgh. It should probably not absorb the Nautical Almanac Office and historical collections of the observatories, which need limited resources and could continue independently.

This suggestion is intended to be more far-reaching than simply merging the observatories. The organization is not going to be a literal observatory because the relevant instruments are overseas. It is more of a research and development organization, a design office and a workshop. Although such a new laboratory would start by working on instruments for astronomy, that need be only one of its jobs. There seems little to be gained from total specialization. The aim would be to add versatility and university contacts and to give the establishment responsibility for its own fate. Versatility in the face of change is relevant to present circumstances. There could be many other gains. Developments in one area of technology such as astronomical instrumentation need inputs from other subjects and in turn ought to be generally disseminated — that process would also be helped. A vigorous working environment is foreseeable.

The question must be asked: would such a laboratory take money or talent needed elsewhere? The answer must lie in the way it is run. Its income would not be unfair if it was gained by offering a service that could be refused. Such operations would be based on contracts.

For a new laboratory, the foundation itself could take the form of a contract or agreement between the Department of Education and Science and the management authority, which could be a university. A notable example is the vast Jet Propulsion Laboratory in Pasadena, which is managed by the California Institute of Technology on a contract. To maintain the service principle, the foundation agreement itself should not provide much of the running expenses for technology. The foundation could reasonably support some scientists. The research councils should probably not be legally involved in the foundation as the laboratory would be one of their contractors.

It should be appreciated that the observatories' research astronomers are few in number and their continued support is not a problem that can relevantly be compared with that of providing and operating the larger research facilities which are basically for universities. The practice of astronomy has been the attractive feature of the observatories for many staff who have eventually worked in technology. The total contribution of the observatories' astronomers to the provision of facilities, as well as to the provision of fundamental data and to research programmes, is such that they can surely be included in the framework proposed. Some posts would be part of the foundation agreement, some would be Science and Engineering Research Council (SERC) fellowships applied for in the usual way, some posts including direct responsibility for instrumentation would be supported by contracts. It would be open to the organization to use any net income to employ extra scientists.

Such a laboratory would be clearly distinguishable from others. The differences would be in the combination of the university connection, contract operation, flexibility in the type of work, the laboratory and the university together seeking work and the origins in astronomical technology (mainly optics, detectors, computing and mechanical engineering).

The operation of existing research facilities and building of new ones would be on the basis of a number of separate long or short-term contracts. Scientists requiring such work would probably act through SERC, using either a grant or a direct contract from the council for common facilities. Ultimately, these contracts could be placed or not as required and could be refused by the laboratory: cancellation would be regulated by the contract.

The laboratory would therefore have the need and the opportunity to support itself (as distinct from subsidizing the facilities). This it would do by seeking government and commercial work which should not be circumscribed. The laboratory could sell its services, software and the rights to its inventions.

From the scientists' point of view, much would depend on the continuing goodwill of the managing university. Success would depend on the local management and staff, and sanctions would be available through the contracts.

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Plight of refusniks

SIR — Ten years ago, on 12 March 1975, my family applied for exit visas from the Soviet Union for the first time. I was immediately removed from my position in the mathemetical department of the Institute for Chemical Physics of the Academy of Sciences of the USSR. At the same time, a total boycott was organized against my family in the scientific centre of the academy in Chernogolovka.

For ten years I have been prohibited from attending seminars, conferences and symposia. Correspondence from abroad is not delivered to me. I do not receive scientific journals, including those of the American Physical Society and the American Mathematical Society. The Academy of Sciences presses my foreign colleagues to abandon attempts to meet me and then to take part in the scientific boycott against me.

For ten years we have been stubbornly making efforts to get exit visas. In 1979, representatives of the academy, academician N. Emanuel, Academician N. Semenov and others, officially stated that the academy had no objections to my departure, and we were informed that the Soviet leaders had made an official decision to give our family exit visas. But this decision has not been implemented.

My numerous meetings with the general secretary of the academy, Academician G. Scrjabin, showed that the academy blocked the fulfilment of this decision.

The situation has abruptly become worse after negotiations between the Academy of Sciences of the USSR and the National Academy of Sciences of the United States. Academician Scrjabin and Academician Yu. Ovchinnikov, a vice president of the academy, immediately refused to discuss my problem with me. And Academician E. Velikhov, another vice president of the academy, refused even to speak with me.

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Abstract policy

SIR — In regard to the previous correspondence on the abstract policy of the Biosciences Information Service (BIOSIS) (H. E. Kennedy, *Nature* **310**, 96; 1984), I would agree with those favouring their inclusion. I would point out, however, that the only thing worse than no abstract is a reference to one when there is no obvious way of obtaining a copy. BIOSIS apparently does not intend to offer OATS (original article tear-sheet service) as does the Chemical Abtracts Service.

Would it be too much to ask that some record be provided as to which libraries hold these abstract volumes in a manner similar to CASSI (Chemical Abstracts Service Source Index) or that they be deposited in the British Library Lending Division or a library which reports its holdings to the Online Computer Library Center?

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Industrial collaboration on show

Professional people do not spend time as salesmen at trade fairs each year. But there is much to be learned from what went on at Hannover last week.

Hannover

ALMOST as a matter of principle of general applicability, most people agree that it is wrong to throw out the baby with the bathwater, an image that no doubt derives from the time when baths were movable and plumbing for carrying away waste water had not been developed. The difficulty in most interesting cases is to know which is which. That may be one of the reasons why British universities have for so long felt so threatened by the British government's mixture of exhortation and incentive intended to make them contribute more directly to national prosperity. More applied research means less fundamental research, as in any zero-sum game. The huge annual trade fair that ended here last week provided ample illustrations that this calculation is far too simple.

One of the most unexpected features of this trade fair is that universities take their place alongside industrial companies as exhibitors, sometimes under their own steam, sometimes under the wing of the governments that support them, the *Länder* in West Germany. This year, one of the most important shows of this kind was that provided by the universities of North-Rhine Westphalia, the regional government whose policy on higher education was the most expansionist of all when the whole of higher education in West Germany was growing rapidly fifteen or twenty years ago. Lower Saxony, the local *Land*, was also represented, as was Berlin, universities such as Heidelberg as well as a host of publicly supported laboratories whose work is, in part at least, academic in character. At a guess, there would have been more than 200 people with academic positions or pretensions manning stands at all times during the eight days of the fair.

Why were they there? There is no secret. As with the strictly commercial exhibitors, money is the objective, either an agreement with an industrial company for the commercial development of a device or such a powerful expression of industrial interest that further funds for a research project can be had from one of the public sources of research support, the *Deutsche Forschungsgemeinschaft* (an all-embracing research council), the ministry of research and technology (BMFT) or, more often than one would guess, some other ministry.

The ingenuity and diversity of what these academic people have had to show has been compelling, which is attested by the way in which the university exhibits have generally been occupied with visitors engaged on deep discussions. (Quite often there is also a certain amount of socializing, as when people from industry try to discover what their ex-teachers are doing now.) Machines and devices predominate, computer programs and applications come next, but there is also a cement of biological research, and of electronics applied to medicine, to give the show coherence.

Diversity is also represented by the variety of institutions with things to show. Some of the great universities are there, but so too are the technical colleges of West Germany (*Fachhochschülen*) and the more recent and controversial creations, the comprehensive universities of the 1960s (*Gesamthochschülen*). In interest and ingenuity, there has been little to distinguish between them. One feature of this unusual academic show is that the chief salesman (and inventor) has been a full-throated enthusiast for what he has to show who can be diverted only with difficulty into talking about more general questions, how his university is faring, or how he balances research and/or development with teaching. (The personal pronoun "he" seems generally applicable.)

The question where the idea came from in the first place seems to yield a great variety of answers. One academic inventor says he had returned from a previous Hannover fair astonished that the great engineering companies had not thought of a way of solving a simple technical problem. Many say their work has its roots in the immediate needs of a local hospital or industrial company.

Showing off the product is not exhibitionism, but an attempt to put the development concerned on a sounder footing. The academic exhibitors also agree that success will bring them kudos back home, perhaps inversely to the standing of their institution. Although the exhibitors are necessarily a self-selected group, they make it seem entirely natural that showing a device should be a part of normal academic life.

Like the academically inclined researchers from the West German national laboratories, they no doubt sense pressure from above to be doing something useful. Indeed, for the national laboratories, technology transfer has been this year's theme for the fair. Inevitably, many of their projects are on the grander scale. The surprise is that many of them are just as particular. Techniques for bonding metal and ceramic may as well be used in useful domestic gadgets as, for example, in thermonuclear machines. But for the people from the national laboratories, where government edict may matter more directly, there is less enthusiasm for the incidental applications than for the real purpose.

The texture of the exhibition of research is the biggest surprise. It is natural that inventors should be enthusiasts with, occasionally, an air of zealotry. Predictably, the things on show range from particular devices to generally applicable techniques. The theme that holds them all together is the belief that there may be profit in what they have to show.

Everybody acknowledges that this is just a chance, but they are keen to be on show as if that were sufficient in itself; at least they will be able to compare their work with that of others, or learn something useful from their visitors.

The morals in this experience for academic communities other than West Germany are obvious and widely understood. Indeed, in places such as Britain, everybody pays lip-service to them. First, there is no necessary contradiction between membership of an academic faculty and a research interest which is better called development.

Second, there is no way in which the professional people manning these stands can be distinguished from those encountered at quite different gatherings, conferences for example; perhaps this is something distinctive about West Germany, but the machine builders seemed refreshingly interested in what, say, the particle accelerator people are up to. (To university administrators, in any case, the only achievement that counts is success.) Third, even in publicly supported systems of higher education (and the West German system institutionalizes government support for universities to a degree no longer allowed in Britain), extra funds without strings attached are translated into ordinary currency at a higher rate.

That industrial collaboration can be rewarding and respectable is understood elsewhere in Western Europe and North America. (Japan is different; universities deal privately with industry at the outset and exhibit only their successes at even grander fairs.) But while understood, there is a sense in which it is not believed. Academics who dispute that will be more convincing when they put themselves on show, at Hannover or elsewhere.

John Maddox

Population biology

Evolution of pesticide resistance

from Robert M. May

ALL natural populations contain genetic variability. The selective forces resulting from continued application of pesticides (or herbicides or antibiotics) will therefore lead to the target population eventually becoming resistant. There are now several examples of evolution at work in this way that have been studied in at least as much detail as industrial melanism in the peppered moth, the standard example of microevolution in elementary biology texts. Over 400 species of agricultural pests are now resistant to one or more pesticides, and this erosion of the early successes of chemical pesticides is reflected in the fact that in the 1940s around 7 per cent of all US agricultural crops were lost to insects, whereas the corresponding figure is now 13 per cent. Although the ultimate appearance of population-level resistance is usually inevitable, a variety of measures may be adopted to retard its appearance in particular cases.

For an enormous range of different pesticides and target organisms, the number of generations taken for a significant degree of resistance to appear exhibits a surprisingly small range of variation, typically being around 5-50 generations, despite great variation in the underlying genetic mechanisms and in the intensity and frequency of pesticide application. Conventional population genetics can shed light on this observation. Suppose, to begin with, that resistance involves only one locus with two alleles: *SS* genotypes are susceptible to the pesticide, *RR* genotypes are resistant, and *RS* heterozygotes have some intermediate degree of resistance (that is, $w_{RR} > w_{RS} > w_{SS}$, where w_{ij} is the fitness of the genotype ij in the presence of the pesticide; if R is completely recessive, $w_{RS} = w_{SS}$; if R is completely dominant, $w_{RS} = w_{RR}$). The resistance allele R will usually be at very low frequency (p_o) in the pristine population, and under pesticide application its frequency will at first tend to increase in the ratio w_{RS}/w_{SS} from generation to generation[1]. The number of generations, n, that elapse before the R gene attains a frequency sufficiently large to produce noticeable resistance in the population as a whole (p_f) may thus be estimated by compounding this fitness ratio until $(w_{RS}/w_{SS})^n \sim (p_f/p_o)$.

From this very crude approximation, we can see that the number of generations required for the population to evolve resistance depends only logarithmically on fitness ratios and on p_o. In other words, the characteristic time to evolve resistance indeed depends on such quantities as p_o (estimates of which range from 10^{-2} to 10^{-13} in various circumstances according to R.T. Roush of Mississippi State University), the degree of dominance of the R allele (usually intermediate between the extremes of recessive and dominant), the strength of selection (which ranges over several orders of magnitude, depending on the management of the particular pest/pesticide system), and other factors. When logarithms are taken, however, these enormous ranges collapse to a factor of around ten separating the further extremes, making the roughly 5-50 - generation 'rule' a good deal less surprising.

Discussion at a recent symposium* revolved around the interesting details and exceptions that make the preceding crude generalizations inapplicable to specific cases. For example, is an insect pest that has evolved resistance to one pesticide likely to be faster in evolving resistance to another pesticide? May mixtures of pesticides work better than the same pesticides used in sequence? Better understanding of the molecular biology underlying the genetics of resistance can help here. Faster resistance to new pesticides is clearly likely if they are chemically similar to old ones. Less obviously, as emphasized by M. Whitten (CSIRO, Canberra) and others, the first pesticide could promote selection for mutator genes, thus speeding the evolution of subsequent resistance even to chemically dissimilar pesticides.

Although much of the discussion centred around the molecular biology of resistance, more attention to the population biology of resistance is also needed. G.P. Georghiou (University of California, Riverside), Roush and others emphasized that migration from untreated to treated regions by susceptible individuals represents flow of susceptible genes that works against selection for resistance. This invokes echoes of the grander theme of whether geographical isolation is necessary for speciation[2,3]; that is, under what circumstances will gene flow (migration) wash out the selective forces (pesticide application) that are tending to adapt an organism to its local environment (evolving resistance)? H.N. Comins and others have shown that the answers depend both on the magnitude of migration in relation to the strength of selection, and on the type of density dependence exhibited by the pest population[4-7].

In deliberately oversimplified terms, a population is said to exhibit 'perfect' density dependence if, following any disturbance, the population tends to return to some constant value in the next generation. In contrast, if an 'overcompensating' population is perturbed below its long-term average value in one generation (for example, by pesticide application), it will tend to bounce back above this value in the next generation. A population with 'undercompensating' density dependence will tend to recover steadily and monotically following disturbance; if depressed to low values by pesticide application in one generation, such populations will tend still to be lower than usual in the next generation. When these considerations of population dynamics are added to selection and gene flow, it is concluded that — other things being equal — pest populations with overcompensating density dependence will tend to evolve pesticide resistance faster than ones with 'perfect' density dependence, because the migration of susceptible genes into the overcompensated population that follows pesticide application has less effect than would be the case under 'perfect' density dependence[4]. Conversely, if the pest population exhibits undercompensating density dependence, the resistance-slowing effects of migration from untreated regions will be relatively more significant[4].

In practice, the weather and other environmental factors often superimpose density-independent fluctuations that mask the density-dependent effects, at least in the short run; for this and other reasons, analyses that characterize the degree of overcompensation or undercompensation of particular insect populations are more easily carried out for laboratory than for field populations. However, whereas most field populations for which good data are available appear to exhibit undercompensating density dependence (among the exceptions, the most pronounced is the Colorado potato beetle, which is notorious for the speed with which it has developed resistance to a wide range of pesticides), the majority of laboratory populations show marked overcompensation[8-10]. The difference, which probably derives from the many natural mortality factors that are seldom present in the laboratory, underlines the need for caution in extrapolating laboratory studies of the evolution of resistance into a field setting.

The propensity for pest species to evolve resistance more quickly than do their natural enemies has often been remarked, and creates obvious problems. Building on earlier work by Croft and others[11,12], B.E. Tabashnik (University of Hawaii) surveyed two possible explanations. The first is that the co-evolution between plants and phytophagous insects has preadapted the insects to the rapid evolution of detoxifying mechanisms, whereas their natural enemies have had less opportunity to preadapt. But laboratory studies show that there are no simple general patterns of this kind and that under controlled conditions the rate of evolution of resistance in prey and predator populations depends on the detailed molecular mechanisms underlying detoxification[13,14]. This, in turn, has prompted a search for pesticides that may be less lethal for natural enemies than for

*National Academy of Sciences/National Research Council International Symposium on Pesticide Resistance Management, 27-29 November 1984, Washington D.C.

pests[15-17], or even for the release of natural enemies that have been artificially selected for resistance to specific pesticides[18].

The second possible explanation lies in the dynamics of prey/predator associations. The essentials of Tabashnik's detailed numerical simulations[11] can be appreciated from the qualitative arguments given above. Suppose application of a pesticide kills a large fraction of both prey and predators in the treated region: for the surviving prey, life is now relatively good (relatively free from predators), and the population is likely to increase rapidly — exhibiting overcompensating density dependence; for the surviving predators, life is relatively bad (food is harder to find), and their population will tend to recover slowly — exhibiting undercompensating density dependence. But we have just seen how the interplay between selection and gene flow is such that overcompensation tends to speed the evolution of resistance, and undercompensation to retard it. Thus, on dynamical grounds alone, we may expect that pests evolve resistance faster than do their natural enemies.

Although many well-understood resistance systems involve one locus with two alleles, with heterozygotes of intermediate fitness, other systems are possible and can pose different kinds of management questions. For instance, *RS* heterozygotes may be the most fit in the presence of the pesticide. Resistance of the Norway rat to warfarin is the clearest example, and anyone who is tired of presenting sickle-cell anaemia to classes as the canonical example of heterzygous advantage may care to note the fitness ratios of the Norway rat in Britain in the absence of warfarin are $w_{RR} : w_{RS} : w_{SS} = 0.46 : 0.77 : 1.00$, whereas in the presence of the poison they are 0.37 : 1.00 : 0.68 (J. Greaves, MAFF, South Tolworth, Surrey). But there may also be examples of heterozygous superiority among insects, as in the resistance of the spotted root-maggot *Euxesta notada* to DDT or dieldrin[19]. Heterozygous superiority of this kind undercuts the simple analyses presented above[20].

Resistance systems that are sufficiently polygenic to warrant treatment by quantitative genetics, rather than being inherited in discrete, diallelic fashion, present interesting possibilities (S. Via, University of Iowa). For example, resistance to two different pesticides can be negatively correlated, enabling the evolution of resistance to be retarded by alternating the pesticides. Conversely, if the correlation were to be positive it would result in swift appearance of resistance to the second pesticide, following sustained use of the first. M. Uyenoyama (Duke University) discussed possible complications arising from modifier genes in two-locus systems of resistance. One possibility is the removal of fitness disadvantages in resistant genotypes in the absence of pesticide usage, if ever the resistance gene attained high frequency; if this happened, one could not rely on 'back-selection' to purge resistance genes from the population after application of the pesticide was discontinued.

If dosage levels, migration, refugia, natural enemies and other factors are to be managed so as to slow down the evolution of pesticide resistance, coordination of efforts over large regions will be required. Some crops lend themselves to this, and some do not. Often the best interests of individuals will differ from those of groups, leading to problems that are social and political rather than purely biological. Beyond this, even with good biological understanding and coherent planning of group actions, I think it will often be that different sectors — pesticide manufacturers, farmers, and the planners responsible for feeding people — have different aims, stemming from different rates of discounting the future and the absence of a truly common coinage. Population biology can make these tensions clearer, but it cannot resolve them. □

Most ancient aggregation of serpents



THE oldest record of serpent aggregation is preserved in this rare fossil from the Oligocene White River Formation of Converse County, Wyoming. The assemblage consists of three virtually complete 32 million-year-old snakes passively coiled in life-like positions. As described by Brent H. Breithaupt and David Duvall of the University of Wyoming, USA (in a paper in the press in *Rates of Behavioral Evolution*, ed. Bocout, A.J., Plenum, New York; and in a manuscript under review) the largest snake (435 mm long) belongs to the genus *Ogmophis*, whereas the smaller pair belong to the genus *Calamagras*. Both genera are common middle Tertiary forms of the subfamily Erycinae, family Boidea, and are distantly related to the North American rubber boa (*Charina*) and rosey boa (*Lichanura*). This specimen represents the most complete material known for these two fossil genera and is the first serpent cranial material recovered from Oligocene strata of North America. It is also the only known specimen in the world in which more than one complete snake is preserved and it suggests that aggregation, a behaviour that is extremely common in living snakes, was already a feature of serpent social behaviour at least 32 million years ago.

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Growth factors

Neuropeptides as mitogens

from Michael R. Hanley

THE current enthusiasm for the study of proliferative growth factors is mostly based on the conviction that they are mitogens, endogenous regulators of cell division. A favourite model for mitogenic signalling is experimentally induced cell division, where both quiescent and normally dividing cell populations are stimulated to divide rapidly and replace damaged tissue. The stimuli for tissue repair are unknown but most speculation has focused on tissue-derived or blood-born mitogens such as platelet-derived growth factor. On page 61 of this issue, however, Nilsson *et al.* report that two related neuropeptides contained in sensory neurones, substance P and substance K, can act as mitogens for connective tissue[1]. This discovery is particularly significant because both peptides may also participate in other aspects of the reaction to injury: sensory signalling and neurogenic inflammation[2]. The discovery has important implications for other peptides found in peripheral nerves and also suggests that neural factors have a previously unsuspected role in the coordination of cell proliferation.

The authors use the standard strategy for identifying potential mitogens; the target cells are isolated in primary culture and the onset of cell division monitored autoradiographically by measuring the incorporation of ^{3}H-thymidine into nuclei after stimulation with the potential mitogen. This permits quantification of responses in a morphologically defined cell population and provides a selective measure of a rapid initial event in mitosis, entry into DNA synthesis. In two connective tissue populations, skin fibroblasts and vascular smooth muscle cells, Nilsson *et al.* show a potent, dose-dependent stimulation of DNA synthesis in the presence of the tachykinin neuropeptides substance P or substance K, but not to another neuropeptide, bombesin.

The actions of substance P and substance K seem to be mediated by an authentic tachykinin receptor because their effects are reduced by a specific tachykinin antagonist. Recently, the existence of multiple tachykinin receptors, which may respond to different endogenous peptides, has been suggested[3]. Substance K has a greater potency then substance P, suggesting that a specific subtype of tachykinin receptor[3] is involved in the mitogenic responses, which poses the interesting possibility that the mitogenic effects of tachykinins may be associated uniquely with this class of receptors, and that other cell populations bearing these receptors may also have proliferative responses to tachykinins.

The work of Nilsson *et al.*[1] is the latest contribution to the accumulating evidence that neuropeptides can act as mitogens. For some time, Rozengurt and his colleagues have explored interactions between mitogenic signals in a cell-line model of quiescent cells, serum-starved 3T3 cells. In this system, they have correlated mitogenic activity with the peptides vasopressin[4] and bombesin[5]. Unlike substance P and substance K, however, these peptides have a complicated, possibly synergistic, interaction with other mitogens in 3T3 cells. This difference reflects fundamental differences in the responses of a growth-arrested permanent cell line and a normally quiescent primary culture cell. Nevertheless, the 3T3 model provides a simple prediction that vasopressin and bombesin may be mitogenic for normal cells. Vasopressin has been reported to stimulate DNA synthesis in chondrocytes[6] and bone marrow cells[7]; bombesin, or its mammalian relative gastrin-releasing peptide, can be a growth factor for bronchial epithelial cells[8]. As with the tachykinins, the possibility that these peptides may be endogenous mitogens gains added interest because related peptides occur in mammalian peripheral nerves[9,10], providing ideal local sources.

The tachykinin-stimulated DNA synthesis is neither additive nor synergistic with that elicited by platelet-derived growth factor. In other systems, this observation has been taken as evidence that a common effector mechanism is involved, but it is pertinent to consider whether this explanation is plausible. Elsewhere, the inositol phospholipid pathway, recently delineated in some detail, has been proposed as a common route in the actions of all mitogens[11]. Significantly, the tachykinins, vasopressin and bombesin all stimulate the inositol phospholipid pathway and mobilize cellular calcium, possibly as a direct result of producing the second messenger inositol 1,4,5-trisphosphate, which releases calcium from a non-mitochondrial intracellular pool[11]. Consequently, any receptor acting on the inositol phospholipid pathway may ultimately stimulate cell division. Whether this is a useful generalization for peptides awaits a detailed evaluation of the inositol phospholipid-regulating peptides for their mitogenic activity on target cells. If, as already seems likely, only a subset of such peptide receptors has mitogenic effects, it will be a major challenge to identify any unique aspects of the biochemistry of lipids and second messengers that may correlate with the stimulation of DNA synthesis.

The next logical step will be to evaluate the mitogenic potential of the tachykinins *in vivo*. There are already some preliminary pieces of evidence that strongly support the idea that sensory neurones containing these peptides participate in both normal and stimulated cell growth. A single neonatal dose of capsaicin, the pungent agent of capsicum peppers, permanently destroys a population of sensory neurones that are involved with pain perception and nerve-mediated inflammation[12]. Although this population is chemically heterogeneous, it includes the tachykinin-containing neurones, so neonatal capsaicin treatment provides a method of deleting the cellular influence of tachykinin neurones. Intriguingly, lesions in skin[12] and corneal epithelium[13] frequently result from this treatment. The mechanism of the lesion formation has not been examined in detail but morphological studies suggest that in the cornea the proliferating basal layer of epithelial cells and the normal geometry of cell renewal are severely impaired[12]. Clearly, a specific role for the tachykinins cannot be inferred, but this evidence suggests that a factor from sensory neurones is essential for normal cell turnover in the cornea and, possibly, in the skin.

If corneal damage were to result from the loss of a mitogenic peptide input, could there be a corresponding disorder arising from the local overproduction of a mitogenic peptide? Although skin keratinocytes have not been shown to be targets for tachykinins, there is a large density of tachykinin nerves, presumably of sensory origin, in the basal layers of the epidermis[14], which contains actively mitotic cells that divide to replace the outer layers of skin. Local factors are presumed to regulate keratinocyte proliferation, but little is known about such factors. Psoriasis is a skin disorder in which normal control is lost and the cells proliferate at a greatly increased rate. An expanation for the condition may be that the sensory tachykinins exert a tonic control over normal basal layer cell division, and that, in psoriasis, there is an excessive mitogenic stimulation of keratinocytes. This hypothesis could be conveniently evaluated by the topical use of tachykinin antagonists.



100 Years Ago

REPORTS from Japan state that grave fears were entertained of an outbreak of the long quiescent volcano Fujiyama, and that officials had been sent to investigate the matter. The people living in the neighbourhood believed an eruption to be imminent, because, while the snow on the mountain had begun to melt two months before the usual time, all the wells at the fort became dry, and difficulty was experienced in procuring water. The phenomenon is considered the more remarkable from the fact that the winter has been unusually cold, and that the surface of the snow remains hard, the part nearest the ground being the first to give way. Intelligence has also been received from Java of the eruption of the Semiroo mountain, the most active Javanese volcano.
From *Nature* **31** 610, 30 April 1885.

Strictly, the conclusions of Nilsson *et al.* apply only to peptides found in sensory neurones. However, the results with bombesin and vasopressin[4,5], which have not so far been found in sensory neurones, strongly suggest that other parts of the peripheral nervous system may also exert proliferative influences on normal, stimulated or developing cell populations. One intriguing consequence of these investigations may be to reveal for the first time a defined cellular origin for an identified substance with mitogenic action. □

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Protein structure

Hands on the calcium switch

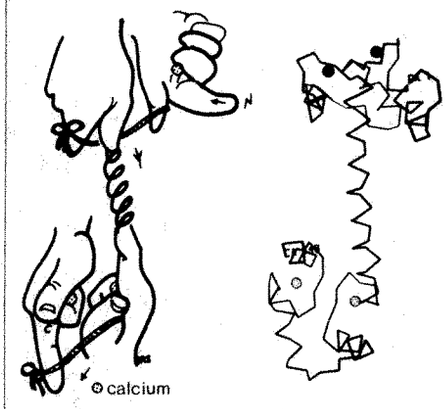
from C. Schutt

THERE is no such thing as a boring protein; newly solved protein structures always contain surprises. Yet the calmodulin structure published on page 37 of this issue[1], and the closely similar troponin-C molecule[2,3], turn out to be even more interesting than usual. The simple 'dumbbell'-shaped molecules, with their easily grasped polypeptide fold, invite close scrutiny of the fundamental mechanism of molecular switching.

These proteins sense intracellular calcium signals and trigger, through changes in conformation, appropriate responses in associated proteins. Calmodulin is a ubiquitious, highly conserved, eukaryotic protein implicated in many coordinated, calcium-dependent cellular responses[4], whereas troponin-C is part of a protein complex bound to actin filaments in vertebrate muscle that 'switches on' contraction when calcium is released from the sarcoplasmic reticulum[5].

The three-dimensional organization of calmodulin is easy to visualize: knobs at both ends of a long exposed helix, with each knob having paired, back-to-back, calcium-binding subdomains (EF-hands) of the type first described for parvalbumin[6]. An EF-hand is a structural motif (see figure) with a 12-residue calcium-binding loop, pictured as the middle finger of the right hand, flanked by two nearly perpendicular helices, represented by thumb and forefinger. In the crystal structure, the concluding helix of the second calcium-binding subdomain runs without interruption into the starting helix of the third binding subdomain, forming the long central helix. It is the splendid isolation of this helix that is unexpected — the tertiary fold of the knobs had been predicted.

In a pioneering use of sequence homology to predict protein structure, troponin-C was determined to have four calcium-binding subdomains[7-9]. Using the criterion of compactness and structural homology with the parvalbumin molecule, Kretsinger and Barry produced a pleasing model of troponin-C with four EF-hands symmetrically packed around a hydrophobic core[10]. This model has recently been challenged by Tsalkova and Privalov[11], who argue from thermal denaturation results that the protein melts as if comprising three independently folded 'cooperative units'. They conclude that the middle part of the sequence, now



known to form the central helix, somehow prevents the other two units from interacting — a remarkable prediction in view of the three-dimensional structures just published. It is also known that limited proteolysis can split these molecules into two intact but non-associating calcium-binding domains[12]. Would this information have helped to get the prediction right? Perhaps such zero-dimensional experiments will become a more important component in future attempts to predict protein structure from sequence homology.

How might the binding of calcium to such a structure act as a conformational switch? In the case of troponin-C, two of the calcium-binding loops (in the amino-terminal lobe) lack calcium in the crystal and, significantly, have altered inter-helix angles from the standard EF-hand, the thumb and forefinger being nearly parallel. These differences are large enough to be recognized by other proteins, especially if there is a relative rotation of the two lobes about the axis of the central helix. A more radical (but not unreasonable) suggestion by Herzberg and James[2] is that a glycine residue in the middle of the long central helix serves as a pivot about which the two halves of the dumbbell can freely rotate, perhaps even coming into contact. Such a dramatic change could certainly serve as a switch, possibly initiated by ligand-induced changes in the calcium-binding subdomain at the amino-terminus of the helix.

The question, then, is whether the secret of this molecular switch lies with the instability of the long bridging helix? Proteolytic cleavage studies show that this region does have an altered conformation in the absence of calcium. Furthermore, thermal denaturation studies[11] identify the helix as the least stable part of the structure. Perhaps an exposed helix — a rare feature in protein structures — tottering on the edge of conformational stability has exactly the required sensitivity to respond to energies produced by calcium binding. One is reminded of glucagon, a small protein of borderline stability in solution, which only reveals its helical conformation in the crystalline state[13]. Such a delicate balance between order and disorder could be the basis of switching in all these molecules.

It is tempting to carry these speculations too far. Calmodulin and troponin-C are only functional while interacting with other proteins and the central helix could well be buried in these situations, or more subtle aspects of protein-protein recognition might predominate. Nevertheless, particularly by the use of genetically engineered mutants, these proteins may yield deep insights into the relationship between the signalling and stability properties of proteins. Finally, we might ask why such a simple structure was not anticipated, when most of its subdomains had been correctly predicted? My guess is that someone, somewhere, did get it right — but who would want to go down as the first to predict a dumbbell when, if the prediction was wrong, their name might so easily become attached to it? □

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Environmental science

Acidification and decline of Central European forests

from Nico van Breemen

WHAT is probably the most complete set of data available on the effects of atmospheric sulphur and nitrogen on the weathering of soil and rock, and on the composition of drainage water in forested and agricultural areas, is reported on page 31 of this issue by Thomas Paces[1]. Of particular interest is that one of the areas (all of which are in Czechoslavakia) is characterized by extremely high sulphur inputs and a complete dieback of spruce forest, a situation that may be typical of several industrial regions in eastern Europe. Paces concludes that the forest in the industrial region is strongly acidified. But is acidification responsible for the dieback?

Dry deposition of sulphur dioxide is responsible for 75 per cent of the acidification in the industrial region whereas wet deposition (acid rain in the strict sense) contributes only 15 per cent to the total acid load of the system studied. In relatively remote forests, the acid load is only about a third of that in the industrial region, and the contributions of wet and dry deposition to the total acid load are about equal. Soil acidification is considerable in such areas but the drainage water is almost neutral. Agricultural activities (fertilizer application and harvesting) contribute considerably to the acid load of soil and rock, resulting in increased mineral weathering.

Changes in the chemical composition of water of the Elbe river over the past 80 years indicate that these conclusions, although based on data drawn from four small representative watersheds, are valid for large areas. All the four have similar bedrocks, soils and climates but differ in their exposure to atmospheric pollution and land use. Paces combines detailed hydrological measurements and estimates of chemical input and output (including records of fertilization and harvesting) over five to seven hydrological years with a recently developed steady-state model of weathering, to quantify acid production and consumption by known geochemical and biological processes. Inputs of sulphur and nitrogen by dry deposition were estimated from measured concentrations and published values of deposition velocities.

Although some authors still contest that atmospheric deposition of anthropogenic sulphur and nitrogen seriously contribute to acidification of soils[2,3], Paces's data are in agreement with several independent lines of evidence for increased environmental acidification as a result of atmospheric deposition. The first direct evidence for the strong soil acidification in Central Europe of the past decades came from *p*H measurements of old and new soil samples by Butzke[4]. At a workshop at Uppsala last September, C.O. Tamm from the University of Uppsala reported a small drop in the *p*H of forest soils over the past 50 years in northern Sweden but a much larger acidification in the southern part of the country. Tamm attributes the small *p*H decrease in the north mainly to biological processes and the large decrease in the south to atmospheric inputs. Other input-output budgets[5], geochemical studies of soil weathering profiles[6] and simulation models of soil acidification[7] also lead to the same inescapable conclusion: the rate of acidification of forest soils in a large part of Europe (and north-eastern America) is greatly accelerated by atmospheric pollution. Although this acidification has markedly depressed the *p*H of sensitive forest soils in Central Europe, forest soils in the United States and Canada seem still to be able to buffer the *p*H in a range that can be considered normal.

Although there is general agreement that the acidification of lakes and streams is detrimental to fish and other biota[8], the relation between soil acidification and the widespread decline in forests of Central Europe is still controversial; the link between the acidification of soil and death of spruce forests indicated by Paces is not proven. Usually, direct effects of atmospheric gases, including photo-oxidants, may be more important than soil acidification in damaging trees. Nevertheless, the removal of base cations and release of potentially toxic aluminum by acidification must be detrimental to trees and other forest plants. In the long run, soil acidification may be a bigger problem than the direct effects of gases on vegetation: although a reduction in pollutant emission would have an almost instantaneous effect on air quality, recovery of acidified soils is presumably very slow and is only possible if sufficient quantities of weatherable silicate minerals are still present.

In that respect, the acidification of agricultural soils (also demonstrated by Paces) is a minor problem. The acidifying effects of harvesting and fertilizer application are normally counteracted by liming. Additional effects of atmospheric acids can easily be undone in the same way.

Liming, however, probably offers no practical solution for forests. Apart from the fact that acid deposition rates are higher in forests than on land[9] and the problem of how lime could be applied in forest areas, forest liming could result in undesirable side effects from increased decomposition of organic matter, nutrient imbalances, increased susceptibility to pests and diseases and changes in the species composition of the ground vegetation. Both the direct effects of atmospheric pollution and the long-term problems associated with acidification of the environment can only be solved by a reduction in the emissions of gaseous sulphur, nitrogen and hydrocarbons. □

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Primate cognition

Can apes learn to count?

from Brendan McGonigle

DANZIGER once described mathematics as the language of size, natural language as the language of sort. Are these languages confined to the human brain? Although research (and controversy) continues on whether apes share important features of our natural language system, attention is now turning to the question of whether they might also possess the rudiments of a number system. Thus, on page 57 of this issue, Tetsuro Matsuzawa reports on the 'use of numbers' by a chimpanzee[1].

Matsuzawa claims that the ape successfully identified six arabic numbers by selecting from a keyboard the numeral that was appropriate to the number of objects displayed. In addition, it provided the appropriate colour name and category label for some 300 sample types, stringing these into, for example, '3 red pens'.

The study is perhaps unique in requiring a level of description of an array of objects in terms of both numerosity and the type of object displayed. These findings are surely provocative, the fairgound appeal of an ape that selects numerals felicitously apart. Do they, however, imply that the chimpanzee is endowed with the rudiments of a system of calculation which finds a refined and rarified expression in the axiomatic systems devised by man? Only a convergent programme of comparative developmental research into the natural or intuitive calculus of animals and humans

will answer this question. Meanwhile, it is instructive to evaluate these and related findings in the light of current advances in child development, which already implicate some basic psychological mechanisms underlying mathematical understanding, particularly the conception of number.

Subitizing — the simultaneous apprehension of up to four elements through direct pattern recognition — is one rudimentary counting mechanism that has been identified in humans and is, perhaps, implied in the chimpanzee by Matsuzawa's study. The process is revealed by the measurement of the time it takes subjects to enumerate small numbers of items, such as dots, on a visual display. Increasing the number of items up to four has relatively little effect, adding around 200 milliseconds per extra dot with child subjects; above that number, however, each additional dot increases the enumeration time by over 1000 milliseconds. This 'break' in the range of times is consistent with the notion that subitizing, or simultaneous number perception, applies for up to four items, beyond which counting, a more costly procedure, begins.

It is impossible to evalutate the role of subitizing in Matsuzawa's study as identification measures are not reported. It is, however, interesting that the chimpanzee made by far the greatest number of mistakes (over 63 per cent) in the discrimination between numbers five and six, just outside the subitizing range for humans. It may be that the span of apprehension for apes is different from that of man. This needs to be determined empirically, and the research could also clarify the contentious relationship between subitizing and counting in the genesis of the number system[2].

Subitizing apart, developmentalists are agreed that even 'true' counting begins with small numbers. Many six-year-olds cannot count quantities above ten. Initially, there is an apparent need to use '1' as an anchor; asked to begin counting from a higher number, performance falters. And forward counting begins before counting backwards, which indicates that there is a preliminary temporal or serial-order basis for the number system. (It is interesting to note that temporal succession was suggested by both the philosopher Kant and the mathematician Hamilton as an intuitive basis for number.) The subsequent development of successor links in the counting chain seems to depend largely on a mental representation with (spatial) analogue properties[3]. This is inferred from the inverse relationship between the time taken to identify the greater of two numbers and their ordinal separation (for example, it takes longer to decide that 5>4 than 7>4) and suggests that there is a 'mental line' or scale on which numbers are mapped.

Such phenomena are seen to reflect important stages in the development of informal arithmetic skills, so their investigation in animals would help inform us whether a rudimentary calculus is shared with other species. Already, we have reported evidence for a representational device analogous to the 'mental line' (also based on decision-time data) for squirrel monkeys tested on a related symbolic (logic) task[4]. The expectation is therefore provoked that comparative research into number representation could reveal the communality of at least some counting mechanisms in humans and other primates.

The use of number, however, implies more than mere counting skill *per se*. Children count before applying their knowledge in situations where it would be desirable to do so. Asked to determine which of two rows of objects has 'more', four-year-old children tend to base their judgments on the shape of the set, selecting the more extended row and thus appearing to treat discontinuous quantities as continuous ones[5]. Yet children who fail in these tests can count under other circumstances. Should true counting be found in non-human primates, therefore, it would be necessary to evaluate their spontaneous use of counting as a measuring device, especially under conditions which afford other (if less reliable) solutions. □

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Biopolymers

The spinning of silk

from Paul Calvert

SILK is stored as a concentrated aqueous solution in glands of the larval silkworm until it is spun as a dry fibre by a gentle waving of the head of the larva. The equivalent process for spinning a nylon fibre — the synthetic fibre that is closest to silk — consists in forcing the molten polymer out through a small jet, a spinneret, and cooling it. The resulting fibre is soft and not very strong, as it is not possible to run the polymer out through the small jet fast enough to align the molecules. Alignment is only achieved in a second process where the fibre is cold-drawn to about six times its original length, whereupon the molecules become oriented parallel to the fibre axis and the fibre becomes strong and stiff. So how does the silkworm manage to produce the same effect in a single step at room temperature? As silk fibres are insoluble in water, the solution stored in the silk gland must be metastable yet remain liquid until the mechanical process of spinning induces precipitation; no chemical change or drying seems to occur during spinning. But how does the apparently slow process of spinning a mobile liquid nonetheless result in a highly ordered fibre? And why does the production not become blocked up with the precipitating solid?

Most of the recent published work on the physiology of silk production has been carried out in Japan and much of it is summarized in a group of reviews in *Experientia* **39**, 441; 1983. Silkworm silk is secreted as a 15 per cent aqueous solution of fibroin in the narrow, convoluted posterior gland. From there it moves to the middle storage gland where the fibroin is enveloped in a solution of sericin that will ultimately become the gummy coating holding the fibres together in the coccoon. It is in the middle gland, where the concentration of silk rises to 30 per cent, that one might expect the polymer to be precipitated out. Akai (*Experientia* **39**, 443; 1983) describes the fibroin forming fibrous spherical masses of about 1 μm in diameter, but an electron microscope study of such a system is prone to artefacts and the conformation of the fibroin has been much debated.

In its most stable fibre form, silk has a β-sheet structure with chains extended and packed into layers. If the solution in the silk gland is removed and allowed to dry undisturbed, it forms silk I. Because this can only be obtained as crystal aggregates and not as oriented fibres (Lotz, B. & Colonna-Cesari, F. *Biochimie* **61**, 205; 1979), it does not lend itself to a complete X-ray structure determination. Nevertheless, a structure has been suggested in which the conformation of alanine and glycine residues are near the β-sheet and α-helix, respectively, and which is much more contracted than β-silk. But new data from magic-angle ^{13}C nuclear magnetic resonance instead suggest a loose 4-fold helix (Saito, H. *et al. Macromolecules* **17**, 1405; 1984).

In the liquid itself, the silk has been believed to be predominantly in a randomly coiled state with some α-helix, but another recent ^{13}C nuclear magnetic resonance study (Asakura T. *et al. Macromolecules* **17**, 1075; 1984) shows that it is randomly coiled in dilute aqueous solution and probably changes over to the loosely helical conformation of silk I as the concentration rises above five per cent, when the chains start to aggregate. This loose helix probably provides a conformational energy minimum that is readily reached and so prevents the chains from going over to the more stable β-sheet form and solidifying. The block copolymer structure of fibroin may help to stabilize the loose helix.

The concentrated liquid is certainly quite fluid: liquid readily flows out of the cut ends of glands of spinning silkworms (Magoshi, J., Magoshi, Y. & Nakamura, S. *Rep. Progr. Polymer Phys., Japan* **23**, 747; 1980). The precipitation step that occurs during spinning seems to depend on a critical shear rate being reached. *In vitro* measurements on solutions of less than five per cent show that precipitation occurs if liquid silk is sheared at rates greater than 50 s^{-1}. The silkworm spins the fibre at about 1 cm s^{-1} from the anterior gland, which narrows to 50 μm at the entrance. This corresponds to a shear rate of about 1 s^{-1} and causes precipitation of silk at the much higher concentrations found *in vivo* (Kataoka, K., & Uematsu, I. *Kobunshi Ronbunshu Engl. Ed.* **6**, 621; 1977). Magoshi *et al.* also report that the change to the β-sheet structures occurs at draw ratios of about four and that the silk can be induced to flow out of cut glands in a liquid crystal form.

From recent efforts to prepare high-strength synthetic fibres, it has become clear that to produce high levels of orientation the inter-chain forces must be controlled so as to permit slippage of the chains past one another while preventing rapid relaxation back to a randomly coiled state. Strong aromatic polyamide fibres such as Kelvar, are produced by spinning from liquid crystalline solutions in which the chains are locally aligned even before starting, thereby greatly increasing the orientation. Apparently silk production is similar, in that the aggregated state promotes local alignment of the chains while still keeping them in solution. They then easily spin to high orientations and to moduli that are comparable with the highest values achievable with nylons and about half those of Kelvar. The change from coiled to rigid conformation that happens with increasing silk concentration apparently corresponds to the 'induced rigidity', believed to occur in stiff polymers in solution (Matheson, R.R. *Mol. Cryst. Liq. Cryst.* **105**, 315; 1984). Again we have failed to recognize a natural process until rediscovering it the hard way. □

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Icthyology

The biology of coelacanths

from Jared M. Diamond

ON 22 December 1938, a taxi driver on the docks of East London, South Africa, was hailed by a lady with a very oily dead fish that proved to be the coelacanth *Latimeria chalumnae*, sole survivor of a group of fish that had been believed extinct for 70 million years and traditionally considered close to the ancestor of all land vertebrates. Since then, many other specimens have been captured and pickled or frozen but scientists have yet to observe a healthy live specimen. But much has been learned from preserved specimens about ecology, sensory biology, reproductive biology, physiology, and taxonomic affinities of this 'living fossil'[1-3].

As for *Latimeria*'s geographical distribution and habitat, all but the first specimen were caught by native fishermen off two of the Comoro islands, between Madagascar and the east coast of Africa. Most specimens have been taken at depths of 100-300 metres as an accidental by-product of night fishing for oilfish, and the apparent restriction of *Latimeria* to these two islands is probably an artefact of how socio-economic and oceanographic conditions constrain the search for oilfish. The South African specimen taken 2,000 miles from the Comoros is the only proof of a wider distribution, but coelacanths may eventually turn up at other seamounts and banks in the western Indian Ocean.

Most likely, coelacanths perch on a reef, lunge for prey and suck it in, rather like a grouper[2]. They feed on fish and cuttlefish, swallowed whole, that live on or near the bottom of reefs in deep water. *Latimeria*'s most distinctive anatomical feature relevant to feeding is a joint across the skull that permits rotation by up to 15 degrees and thereby widens the gape and increases the strength of the bite[4]. Such intracranial joints are known from some fossil fish but no other living fish. Sensory equipment for prey detection includes large, nearly colour-blind eyes that are adapted to low light levels, densely packed rods but very few cones, and a large cavity in the snout, the rostral organ, which has the structure of an electroreceptor[5].

One of the most surprising discoveries about *Latimeria* concerns its reproductive biology. Initially, the coelacanth was assumed to lay eggs for external fertilization, like most fish, because males lack a penis, pelvic claspers, modified anal fins or any other obvious copulatory organ. Then, dissection of a female revealed shell-less and very large eggs, 8.5-9 cm in diameter[6]. Finally, Smith *et al.* proved that the eggs are fertilized and hatch internally by finding five miniature coelacanths, 30-33 cm long, in the oviduct of another female[7]. Dates of capture suggest that the gestation period is around 13 months. How the male achieves internal fertilization remains a mystery; two pairs of erectile caruncles flanking the cloaca may play a part[8,9].

The other big surprise concerns the osmoregulation of *Latimeria*. Living marine animals osmoregulate in one of three ways: by being iso-osmotic and similar in ion concentrations to sea water (hagfish and marine invertebrates); by being iso-osmotic, despite having ion concentrations far below those in sea water, through retaining urea and trimethylamine oxide (sharks); or by having ion concentrations far below those in sea water and being hypotonic but drinking sea water and excreting salt to remain in water balance (modern bony fish). *Latimeria* proves to resemble sharks rather than modern bony fish[10,11]. With 197 mM Na^+ and 187 mM Cl^- in its blood, as in bony fish, and 377 mM urea and 122 mM trimethylamine oxide, both the blood and urine of the coelacanth are iso-osmotic to sea water.

The osmoregulatory resemblance of *Latimeria* to sharks begs the question of its taxonomic affinities. Traditionally, coelacanths have been considered the sister group of the rhipidistians, an extinct group of bony fish that gave rise to amphibians and thence to other land vertebrates. Lagios, however, considers coelacanths the sister group of chondrichthyans (sharks and rays) on the basis of similarities not only in urea retention but also in pituitary structure, pancreatic structure and the presence of a salt-secreting rectal gland[2,12]. Other authors argue that these similarities do not necessarily ally coelacanths with sharks[2,10,13]. For example, urea retention arose independently in lungfish, certain frogs and some primitive ray-finned fish and so may also have arisen independently in coelacanths and sharks.

Thus, the dogma on which the current generation of biologists was reared — that coelacanths are close to the ancestors of tetrapods — is under vigorous debate. But the outcome is unlikely to shake their image as a symbol of long preservation without change. Thus, Time Magazine once branded Richard Nixon as a "coelacanth of American anti-communism", and a British MP ridiculed a fellow MP as a 'coelacanth' on the grounds that from his long silence in that august assembly it was a surprise to find him still alive[2]. Whether these calumnies of coelacanths are taxonomically justified is a question that can be resolved only when political conditions allow fresh specimens to be obtained from the Comoro islands or neighbouring waters. □

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Destruction of fisheries in Africa's lakes

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The introduction of the Nile perch into Lake Victoria, East Africa, has had disastrous consequences. Fisheries have been not merely damaged but destroyed. Lake Malawi has the world's most species-rich fish fauna and the prospect of introduction of alien species could mean disaster.

LAKE Victoria in East Africa, with an area of some 68,635 km^2, has a naturally rich and diverse fish fauna and formerly supported important fisheries, based largely on herbivores and detritus feeders of the family Cichlidae[1-3]. Fisheries based on such short food chains are ecologically the most efficient. Although over-fishing in Lake Victoria has for some time been a cause for concern, fisheries technology having advanced more rapidly than the scientific knowledge needed to devise rational means of exploiting this complex multispecific assemblage, a further serious hazard now faces the fisheries. In the 1950s, a suggestion was made that the Nile perch, *Lates niloticus* (Fig.1), a piscivorous species that grows to a large size, should be introduced into the lake. Although it was pointed out that simple ecological principles show that such an introduction would be detrimental[4] (yields of predators can never be as great as those of their prey), the idea was pursued by its supporters and in 1960 the Nile perch found its way into the lake, apparently from ponds into which it had already been introduced. Subsequently this species was deliberately introduced into the lake.

Since then the Nile perch has spread rapidly in the northern parts of Lake Victoria, having reached Kenyan waters by the mid-1960s. It is now abundant in many areas and is spreading south. It has invaded the Tanzanian waters of the lake and, in the early eighties, increased rapidly in numbers in the vicinity of Mwanza, where there are important fisheries based on indigenous species[5].

Invasion

The consequences of this invasion and of the establishment of the Nile perch are serious. While some of them were predicted, not all could have been anticipated. For example, the Nyanza (formerly Kavirondo) Gulf was the site of important fisheries for an endemic tilapia, *Oreochromis esculentus*, and for other fishes including the abundant haplochromine cichlids. Large quantities of the former supplied Nairobi and other centres of population and the latter, easily preserved and transported, were sold over a wide area. Since the arrival of the Nile perch, almost all the indigenous fishes of commercial importance have not merely declined but have virtually disappeared[6,7]. These include not only the herbivores but carnivores such as certain catfishes.

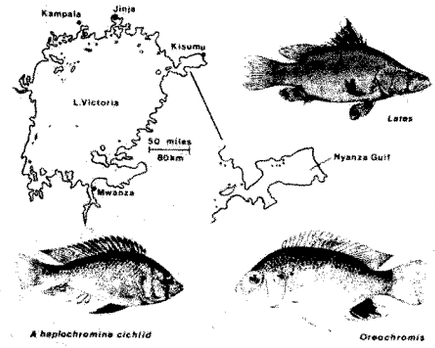


Fig.1 Lake Victoria and, inset, the Nyanza Gulf, the introduced Nile perch, *Lates niloticus*, and representatives of some of the cichlid fishes, formerly the basis of important fisheries in the Nyanza Gulf and elsewhere, that have recently declined catastrophically. Illustrations of fishes after Boulenger[20]. *Lates* reach up to 2 m long, *Oreochromis* up to at least 30 cm, and the many species of haplochromine cichlids are usually less than 20 cm in length.

Large numbers of *Lates* are being caught, however, reflecting the fact that they now make up well over 80 per cent of the fish biomass in the Nyanza Gulf[6], an atypical and clearly unsustainable role for a predator at the top of the food chain. When first introduced, *Lates* fed mostly on small haplochromines (Fig. 1), a group estimated formerly to comprise ~ 80% of the demersal fish stocks of the lake[8]. Having virtually eliminated this food source, small *Lates* now depend on the small prawn, *Caridina*, which is consumed by juveniles in several lakes. Recent observations show that ~ 80% of the diet of individuals less than 45 cm in length consists of these prawns and even fishes up to 100 cm in length are eating *Caridina*[6]. Large individuals are feeding predominantly on young of their own species.

Such a situation is inherently unstable and the ecology of the Nyanza Gulf has been irreparably upset. Furthermore, *Lates* is not liked by the local people, who constantly complain that they are unable to find their favourite food fishes. Prices are low: at the lakeside, a kilo of *Lates* sells for a shilling, but in areas where it is still available, a kilo of *Oreochromis* fetches 30 shillings. Moreover, because of its large size and oily nature, *Lates* cannot be sun-dried but has to be smoked, which is leading to the deforestation of a number of islands as trees are felled to produce firewood. The establishment of *Lates* has also had unfortunate sociological consequences. Fishermen who could formerly subsist on small-scale haplochromine and *Oreochromis* fisheries are being driven out of business and production is being concentrated in the hands of wealthier individuals who can afford the larger, stronger and more expensive nets required for *Lates*.

Fishery decline

Against this background, the conclusions of a symposium of the UN Food and Agriculture Organization (FAO)[9], held in October 1984, make curious reading. The symposium was convened under the auspices of the Committee for Inland Fisheries of Africa as the concern of its subcommittee for the development and management of the fisheries of Lake Victoria. Its purpose was to consider the status of the fish stocks of Lake Victoria, to suggest management measures, and to define priorities for research. Its report confirms that the fisheries, formerly based on a wide range of species or species groups, are now dominated by only four such groups, one of which, that comprising the haplochromine cichlids, "is itself threatened", and that fisheries for most species are in decline. The ill-advised trawl fishery for haplochromines was already in decline before the upsurge of the Nile perch and the report now admits that it is "almost certain" that haplochromine stocks cannot support the twin stresses of heavy predation by *Lates* and a fishery, and thinks it would be "extremely unwise" to invest further in this industrial fishery. In the Nyanza Gulf at least, the haplochromine fishery is already virtually nonexistent. It is admitted that, under what is seen as the best of two scenarios, production from Lake Victoria will settle down at a level "assumed to be some 80% less than the productivity achieved by the

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pre-Nile perch community due to energy losses by the predator". Alternatively, in what is described as a boom or bust situation — another guess — the disrupting influence of *Lates*, which is admitted, is likely to lead to even lower productivity. Despite this it is claimed that "the introduction of Nile perch to utilize the haplochromine cichlids of the lake may be regarded as successful in terms of its original objectives"! With such criteria for success all things are possible. It is also claimed that losses in productivity "may, however, be compensated for by the greater value and quality of the fish caught". Lakeshore prices cast serious doubts on this prediction and no improvement of quality could compensate for the enormous losses in both yields and diversity brought about by the introduction of the Nile perch.

The establishment of *Lates* is not only an economic and ecological tragedy but also an enormous loss to evolutionary biology. Lake Victoria is the site of a remarkable radiation of cichlid fishes which has given rise to several hundred endemic species[10,11]. As an example of explosive speciation this assemblage is of immense scientific interest[12] and the loss of these fishes, often before their biology has been elucidated, is incalculable.

Irreversibility

We also wish to stress the irreversibility of introductions into large lakes — removal of *Lates* from Lake Victoria is now impossible — and to emphasize that the introduction of this fish is seen as a failure, not merely by a small group of concerned scientists who think they know best and who find the endemic faunas of African lakes of academic interest, but by the people it was meant to benefit. This is no minor incident but a disaster whose magnitude is put into perspective by noting that Lake Victoria is much larger than Switzerland.

Great damage has already been done to Lake Victoria, so it is disturbing to learn that the introduction into Lake Kivu of *Boulengerochromis microlepis*, the largest piscivorous cichlid fish of Lake Tanganyika, is now contemplated. Even more disturbing is the suggestion that the endemic pelagic zooplankton-feeding clupeid fishes of Lake Tanganyika should be introduced into Lake Malawi (= Nyasa). One of these, *Limnothrissa miodon* (Fig.2), has been successfully transferred to, and established in, the man-made Lake Kariba, and into Lake Kivu, a lake of recent origin with an extremely depauperate indigenous fish fauna, and which drains into Lake Tanganyika. These situations are utterly different from those prevailing in Lake Malawi. Malawi is an ancient lake in which evolution has given rise to exceedingly rich assemblages of endemic fishes, whose differentiation has proceeded further than in Lake Victoria, and of which it has more than any other lake in the world. These include a group of zooplankton-feeding

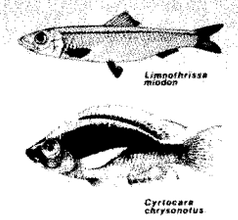


Fig.2 The zooplankton-feeding Tanganyikan clupeid *Limnothrissa miodon* (top, after Poll[21]) and one of the Utaka group of haplochromine cichlids (bottom, after Boulenger[20]) that occupy a similar niche in Lake Malawi.

haplochromines, the Utaka, that are caught for human consumption. Other zooplankton-feeders are also present, so the niche that would-be introducers wish to exploit is already filled. The great age of Lake Malawi has enabled its fishes to evolve into complex communities whose members interact in many subtle ways, and the effect of the introduction of alien species is impossible to predict. It is unlikely that a situation which has evolved over perhaps one to two million years can be improved by the introduction of an alien species whose impact can only be guessed at. The consequences could be disastrous and irreversible, as they have been in Lake Victoria, in the Laurentian Great Lakes of North America[13], Lake Titicaca (S. America)[14], Lake Lanao (Philippines)[15] and elsewhere. Similar concern has recently been expressed about introductions into Lake Kinneret, Israel[16]. The introduction of seemingly innocuous organisms, even those whose ecology has been much studied, can have unforeseen (and unforeseeable) consequences. Such have followed the introduction of the mysid crustacean *Mysis relicta* into certain lakes where it was hoped it would increase fish yields. For example, in one well-documented case it virtually eliminated planktonic cladocerans, an important food for fishes[17]. As a result a group of experts has unanimously approved a resolution that a complete moratorium be imposed on future introductions[18].

With one disaster accomplished in Africa, we urge those interested in improving the fisheries of Lake Malawi, whether in FAO or other bodies offering technical and scientific assistance, to concentrate on rational exploitation of the indigenous fish stocks, efficient distribution of catches and the reduction of unnecessary losses during preservation and storage. It is in addressing these questions, rather than in indulging in potentially dangerous introductions, that true progress can be made.

Lake Malawi is not only an important source of protein but is a site of international significance as a laboratory of evolution. Its future to the welfare of those who crop it, and to the international scientific community, is too important to be jeopardized by those who, with no means of predicting the consequences, would introduce alien fishes. We, of several nationalities, with experience of African lakes, therefore urge the governments of the states bordering Lake Malawi (one of which is Tanzania whose Lake Victoria fisheries are now threatened by the Nile perch) never to allow such introductions. The introduction of alien species could seriously jeopardize the food supplies and livelihoods of future generations. Such an action, the outcome of which is unpredictable, and scientifically indefensible, could prove disastrous to the continued existence of the viable Malawian fishery.

Conclusions

Fishes have often been moved from lake to lake, sometimes with the laudable goal of increasing the yield of human food. Except in fishless lakes and man-made reservoirs, such efforts have failed to achieve the desired objective, and sometimes the results have been disastrous. When large lakes whose fisheries are of immense importance as sources of human food are involved, especially in areas where other animal protein is scarce, much is at stake. A major scientific objection to such introductions is that the outcome is often unpredictable. Detrimental consequences can sometimes be predicted, as in the case reported here, but as controlled experiments cannot be carried out, and the reactions of the organisms involved cannot be determined in advance, a large element of guesswork is inevitable. The outcome is also often irreversible. An additional danger, not considered here, is that introductions may lead to the introgression of new genes. This can hamper taxonomic and evolutionary studies and hinder progress in aquaculture, as is happening among the economically important tilapiine cichlid fishes of Africa[19]. □

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Does the ocean–atmosphere system have more than one stable mode of operation?

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The climate record obtained from two long Greenland ice cores reveals several brief climate oscillations during glacial time. The most recent of these oscillations, also found in continental pollen records, has greatest impact in the area under the meteorological influence of the northern Atlantic, but none in the United States. This suggests that these oscillations are caused by fluctuations in the formation rate of deep water in the northern Atlantic. As the present production of deep water in this area is driven by an excess of evaporation over precipitation and continental runoff, atmospheric water transport may be an important element in climate change. Changes in the production rate of deep water in this sector of the ocean may push the climate system from one quasi-stable mode of operation to another.

MANY feedback loops that may amplify climatic change have been proposed, one of which has recently caught the imagination of palaeoclimatologists. Suggested by the atmospheric CO_2-content record recovered from the polar ice caps[1-3], this scenario involves interactions between climate and ocean circulation[4-7].

To appreciate the significance of the observations on polar ice, one must be aware of their context. Over the past several decades studies on deep-sea cores have provided a detailed history of climate during the past million years[8-10]. The first picture that emerged was that continental glaciers have waxed and waned on a timescale of 100 kyr[9]. These climate cycles are asymmetric, involving long periods of glacial buildup suddenly terminated with rapid warmings. Further work revealed that these long intervals of glacial buildup were modulated by cycles of ~20 and ~40 kyr duration[11,12], closely allied in timing and relative amplitude to variations in seasonal contrast produced by cyclic changes in the Earth's orbital elements[11,12]. Orbital forcing is accepted widely as the primary cause of glacial cycles (Fig. 1). Because the exact linkage between changes in seasonality and changes in climate has yet to be established, however, the issue remains open. Although it is plausible that nonlinearities in the response of snow and ice cover to seasonal variations drive glacial growth and retreat[13], it is not clear whether this mechanism alone is sufficient to do so.

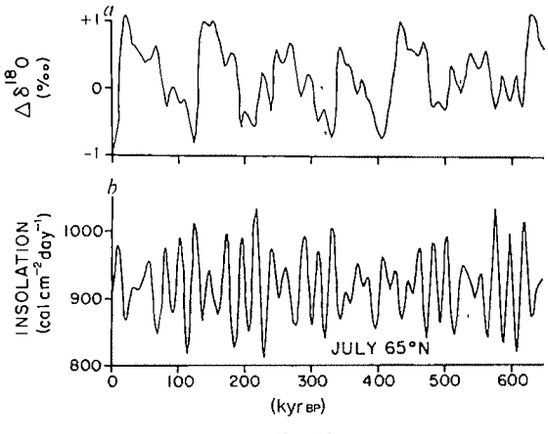


Fig. 1 *a*, Composite of the $^{18}O/^{16}O$ record for planktonic foraminifera from several deep-sea cores[12] This record primarily reflects changes in the amount of continental ice in the Northern Hemisphere The more positive the $\delta^{18}O$ value, the more ice. *b*, Variation with time of the July insolation at 65° N (ref 51) The rapid disappearances of the ice that terminate the episodes of very large ice cover correspond to prominent peaks in summer insolation for the latitudes at which the excess ice was located. In addition, there is a strong coherence between the ~20 and ~40 kyr spectral components of ice volume and insolation records Taken together these ties between insolation and ice volume have convinced most scientists concerned with palaeoclimates that the Earth's glacial cycles are driven by changes in seasonality brought about by cyclic changes in the Earth's orbital elements

Ice-core record

Scientists examining the oxygen isotope record preserved in ice cores from Greenland and Antarctica (see Fig. 2), checked whether the major features of this record matched those found in the deep sea. In one sense they did match. Glacial conditions prevailed from before 50 to ~10 kyr BP at which time a transition to interglacial conditions occurred. Once this transition was complete, climate remained remarkably uniform. No evidence for the 20 and 40 kyr cycles characteristic of orbital forcing appears in the ice-core record, in part because the record extends to only ~100 kyr BP and in part because of the exponential foreshortening with depth of the thickness of ice representing a single year. This flow-induced distortion produces a considerable uncertainty in the ice-core chronology before 10 kyr BP.

Although not showing the orbital periodicities, the ice-core record for glacial time does show many brief events during which climatic conditions returned about halfway to their interglacial state Because such events would be blurred totally by the stirring of worms in all deep-sea sediments with typical accumulation rates (that is, a few centimetres per kyr), this finding is not in conflict with the marine record. However, as

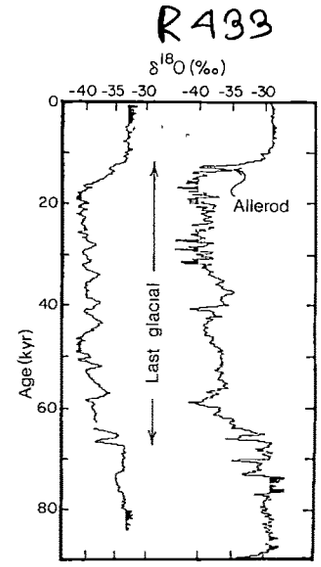


Fig. 2 Oxygen isotope records for ice cores[43] from Camp Century, Greenland (on the right) and Byrd Station, Antarctica (on the left) These records reflect mainly changes in air temperature over the ice cap. The more negative the $\delta^{18}O$ value, the colder the air temperature. The timescales are very approximate as there are as yet no direct radiometric ages for the glacial sections of the ice. Note that the glacial portion of the Greenland record shows a number of events of <1,000 yr duration. These events are muted (or absent) in the Antarctic record.

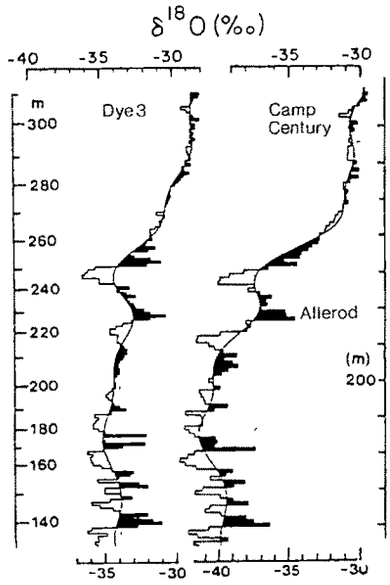


Fig. 3 Comparison between the $^{18}O/^{16}O$ records for the Camp Century and Dye 3 sites in Greenland for the time period ~40 to ~8 kyr BP. For each core a depth scale (metres above bedrock) is given After some adjustment of the Dye 3 depth scale a good match is achieved between the records in the two cores (Results obtained by Dansgaard *et al*[14])

these idiosyncrasies of the ice-core record were not seen in other records, the initial temptation was to pass them off as climate 'noise' without global significance. A rapid succession of findings has since changed this view of the noise, now the focus of much interest.

First, $^{18}O/^{16}O$ measurements by Dansgaard *et al.* on a second long core from Greenland[14] revealed the same events as the first (see Fig. 3), indicating that the events were both real, and occurred throughout Greenland Second, the events were recorded not only by $^{18}O/^{16}O$ ratio changes but also by dust[15], ^{10}Be (ref. 16), SO_4, NO_3 and Cl content[17] changes in the ice. The ice formed during the warm events is similar to interglacial ice with respect to these properties. Third, the $^{18}O/^{16}O$ pattern found for the most recent Greenland event was also recorded in $CaCO_3$ from lake sediments in Switzerland[18], which gave credence to a correlation made early on by Dansgaard *et al.*[19] (see Fig. 4). In interpreting their $^{18}O/^{16}O$ results for the Camp Century Greenland ice core, they suggested that the strong oscillation just before the onset of postglacial time was a record of the Alleröd-Younger Dryas oscillation seen in pollen records throughout Europe. During the Alleröd, trees abruptly replaced the grasses and shrubs that had characterized glacial time, only to be replaced by the cold flora characteristic of glacial time (see Fig. 4). This so called 'Younger Dryas' cold period lasted ~800 yr (that is, from ~11 to ~10.2 kyr BP) before Holocene forests appeared. However, these results did not influence broader thinking about glacial cycles and their causes.

A few years earlier Berner *et al* found that the CO_2 content of the air trapped in ice from the glacial sections of the Greenland and Antarctic cores was about two-thirds that for air trapped in ice from the interglacial sections[1]. Intrigued by the $^{18}O/^{16}O$ events found in the ice and the apparent correlation of the last of these with an oscillation in plant cover seen throughout Europe, Oeschger and co-workers found that there were CO_2 changes associated with these events[20,21]. In the Dye 3 core, oscillations (as recorded by the $^{18}O/^{16}O$ ratio) were accompanied by CO_2 oscillations of ~60 p.p.m (see Fig. 5). The CO_2 change was about two-thirds of the change that accompanied the major glacial–interglacial transition[1-3]. Although Fig. 5 shows only the CO_2 records for the oscillations within glacial time, a similar CO_2 change is found during the Alleröd-Younger Dryas interval at the end of glacial time[20].

Oeschger and co-workers are now searching for the CO_2 events in ice cores from the Antarctic. Whereas the other changes found in Greenland (that is, ^{18}O, ^{10}Be, dust, etc.) may have been restricted to the region under the influence of the northern Atlantic, the CO_2 change must, of course, be global Until confirmed in Antarctic ice, the possibility remains that the sharp changes in CO_2 content found in Greenland are artefacts of summer melting, which may have occurred during the warm events as it did at this low elevation site during much of the Holocene. We emphasize that although it was the discovery of CO_2 events that prompted serious consideration of possible ocean circulation–climate linkages, our speculations below about possible multiple climatic modes do not depend on the validity of the CO_2 changes.

Causes of atmospheric CO_2 change

The finding from ice-core studies that the CO_2 content of air extracted from glacial ice was lower than that extracted from postglacial ice[1-3] stimulated investigations as to the origin of natural CO_2 changes, which must be rooted in ocean chemistry[22,23]. The first scenario for the 90-μatm rise in CO_2 content at the close of glacial time called for the deposition of organic residues on the coastal shelves during the transgression of the sea that accompanied deglaciation. The subsequent finding of brief CO_2 events within glacial time, however, led to a re-examination of the question. If these CO_2 changes are typical of the atmosphere, then explanations involving the transfer of nutrient substances between the ocean and its shelf sediments must be abandoned. Such transfers could certainly not occur fast enough to produce 60-p.p.m. CO_2 changes in ~100 yr. To be viable, hypotheses will have to invoke redistributions of C, P and fixed N in the sea. Such redistributions are feasible if the ocean mixing and/or biological cycling change substantially[4-7,24]. This explains why CO_2 measurements on ice cores

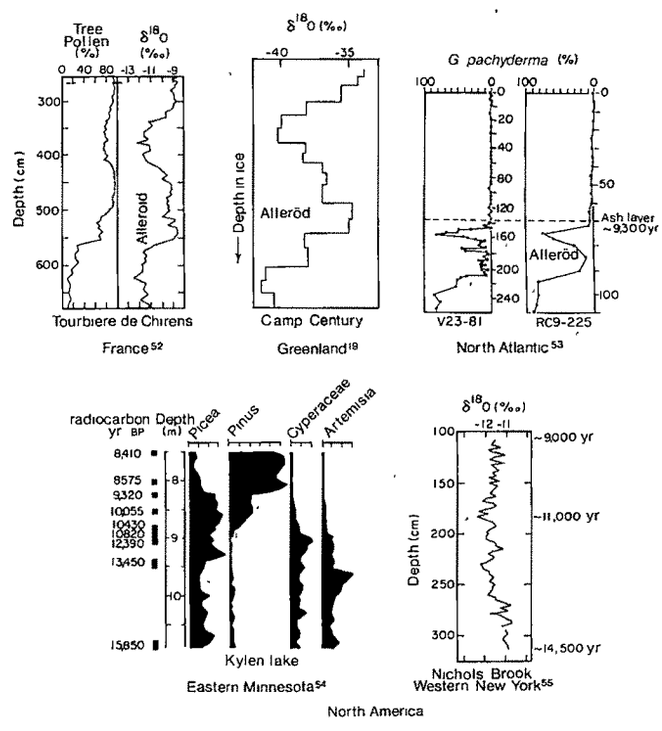


Fig. 4 Examples of records for the time period 13–9 kyr BP from Europe (pollen and $^{18}O/^{16}O$ in $CaCO_3$), Greenland ($^{18}O/^{16}O$ in ice) and the North Atlantic (% *G. pachyderma* in the planktonic foraminifera). All show the pronounced Alleröd-Younger Dryas oscillation By contrast no such oscillation is seen in either pollen diagrams or $^{18}O/^{16}O$ records from the United States

suggested links between climate and the joint operation of the atmosphere and ocean system.

Three quite different scenarios have been proposed. Broecker[24] pointed out that the evidence from deep-sea cores cited in support of his shelf-deposition hypothesis could also be explained by a change in the C to N and/or C to P ratio in the organic residues falling to the interior of the sea. A 30% decrease in this ratio was needed to explain the CO_2 drop at the end of glacial time.

Broecker and Takahashi[4] pointed out that a change in the transfer rate of water between the cold- and warm-water spheres of the ocean would produce a redistribution of ΣCO_2 between these reservoirs and a consequent change in atmospheric CO_2 content. The reason is that the biological 'pumping' of carbon by organisms from the surface ocean to the deep ocean is compensated partly by a flow of CO_2 through the atmosphere from the cold- to the warm-water sphere. Hence, a change in the rate of transfer of water between these spheres alters the balance between these two competing effects. To explain the lower CO_2 content during glacial time, Broecker and Takahashi invoked a several-fold higher rate of transfer of water between these spheres.

Three groups[5-7] have arrived independently at a different ocean scenario. Having pointed out that a reduction in the NO_3 and PO_4 contents of polar surface waters (that is, more effective biological use) would produce the desired glacial CO_2 reduction, all three groups demonstrated that a decrease in the rate of exchange between polar surface waters and the rest of the ocean may result in reduced nutrient content of polar surface waters and, in turn, of the CO_2 content of the atmosphere.

As the Broecker–Takahashi (B–T) ocean mixing scenario requires enhanced mixing during glacial time whereas the Princeton–Bern–Harvard (P–B–H) scenario requires reduced mixing, the balance of evidence must be carried by marine sediments, which would allow one of these hypotheses to be eliminated. Indeed, the P–B–H hypotheses predict higher than Holocene $\delta^{13}C$ values for planktonic forams grown in the Antarctic during glacial time whereas the B–T hypothesis predicts lower values. Unpublished ^{13}C results by N. Shackleton and R. Fairbanks on planktonic foraminifera from Antarctic deep-sea sediments strongly suggest that the reduction in surface water nutrient content required by the P–B–H hypotheses for glacial time did not occur. Radiocarbon age differences (determined by accelerator mass spectrometry) between planktonic and benthic forams from glacial horizons in deep-sea sediments eventually may provide another basis for making a distinction[25,26]. It is possible that neither of these scenarios is correct.

Although little is known about either ocean circulation or biological cycling in the past, one important clue has been found.

Table 1 Freshwater budget for the North Atlantic Basin, including the Arctic, Mediterranean and Caribbean

Source	Input rate fresh water (m yr^{-1})	Freshwater flux (sverdrup)
River runoff[50]	+0.21	+0 33
Precipitation[50]	+0.87	+1.36
Evaporation[50]	−1.21	−1.89
Mediterranean exchange*	−0 07	−0.10
Net†	−0 20	−0.30

The combined area of the North Atlantic basin (0°–80° N) excluding the Mediterranean Sea is $4\,9\times10^{13}$ m^2. For freshwater flux, 1 sverdrup represents a flow of a million cubic metres per second.

* The exchange between the more salty waters of the Mediterranean Sea and the less salty waters of the open Atlantic leads to a loss of fresh water from the Atlantic. This process is driven by the high evaporation rate (relative to precipitation and runoff) for the Mediterranean Sea.

† Using a deep water production rate in the northern Atlantic of 20×10^6 m^3 s^{-1} and a salinity excess for this water of 0.50‰, a fresh water flux of 0 3 sverdrups would be obtained, indicating that no large discrepancy exists between oceanographic and meteorological budgets

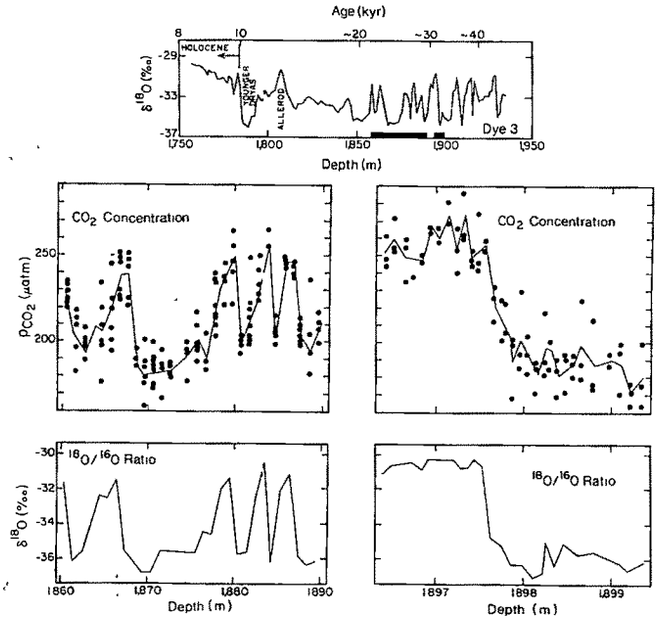


Fig. 5 Detailed P_{CO_2}–$\delta^{18}O$ comparison for two sections of the Dye 3 Greenland core[20,21] The location of these sections are shown in the upper panel by the dark bands. The longer band represents a time interval of ~8 kyr and the shorter a time interval of ~2 kyr The sudden air temperature warmings indicated by the rise in ^{18}O are accompanied by sharp rises in CO_2 content of air trapped in the ice (CO_2 results obtained by Oeschger *et al*)

Evidence from faunal, chemical[27] and isotope studies[23,28–31] of deep-sea sediments suggests that the production of deep water in the northern Atlantic, of great importance to the present-day circulation scheme, was reduced greatly during the peak glacial time. Most convincing is the finding by Boyle that the Cd content of benthic foraminifera from the Atlantic Ocean was higher during glacial time than today[27]. In the present-day ocean, the Cd and PO_4 contents of various water types in the sea are correlated almost perfectly. Thus, the Cd distribution in the glacial ocean is a good substitute for the P distribution. Boyle has demonstrated that the Cd/Ca ratio in benthic foraminifera is proportional to the corresponding ratio in the water in which they grow. In the present-day ocean, the low PO_4 and Cd content of Atlantic relative to Pacific deep water is attributable directly to the formation of deep water in the northern Atlantic. The reduction in this difference (suggested by the glacial foram results) is thus indicative of a reduction in the magnitude of the deep-water production in the northern Atlantic at that time.

Taken together, these arguments for the reduction of deep-water production in the northern Atlantic during glacial time are convincing. Is it possible then that the brief warm events recorded in the ice cores represent periods during which the glacially weakened northern Atlantic deep-water source was rejuvenated?

Distribution of oscillations

Adequate records are available currently to provide a picture of the geographical distribution for only the most recent fluctuation. This last oscillation appears in the records from both the Camp Century and Dye 3 cores from the Greenland ice cap and is found in pollen and $^{18}O/^{16}O$ ratio records from bog and lake sediments in western Europe. Evidence is also found in sediments in the northern Atlantic with an unusually high accumulation rate. Ruddiman and McIntyre showed that ~13 kyr BP the boundary between polar waters and temperate waters moved from its glacial position to near its present position (see Fig. 6), and remained there ~1 kyr before returning to its glacial position for several hundred years[32]. Finally; it returned to its present position where it remained throughout the Holocene.

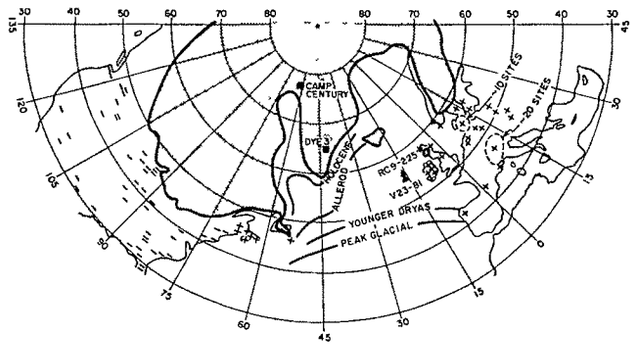


Fig. 6 Map showing the locations of sites at which sediments spanning the 13–9 kyr BP time interval have been studied[33–36]. At sites designated by a+ an oscillation in climate corresponding to the Alleröd–Younger Dryas is found. At sites designated by a− this oscillation has not been reported. The stipling corresponds to the area covered by ice just before the onset of the Allerod[56]. Positions of the polar front in the northern Atlantic during peak glacial, Alleröd, Younger Dryas and Holocene time, as reconstructed by Ruddiman and McIntyre[32], and the locations of the two long ice borings in Greenland and of the two ocean cores referred to in Fig. 4 are shown.

There are >50 European sites whose pollen records contain an oscillation in the 13–10 kyr period[33]. In contrast to the European pollen record is the lack of evidence for this event in the United States[34]. Recent research on cores from lakes and bogs in New Brunswick, Nova Scotia and Newfoundland[35,36], however, indicate that the oscillation was felt in the eastern regions of maritime Canada. This evidence is summarized in Fig. 6. Thus, as proposed by Mercer[37], we may be dealing with a regional change in climate involving primarily the area under the climatic influence of the northern Atlantic Ocean. Because evidence for such an oscillation has been found elsewhere, that is, in the cordillera of South America[38–41] and in New Zealand[42], the situation may well be more complicated. This event is muted or absent in the $^{18}O/^{16}O$ ratio for the Byrd Station Antarctica record (see Fig. 2)[43].

Climate impact of deep-water production

The turning on and off of deep-water production in the northern Atlantic would be expected to produce a regional climate change. The rate of production of deep water is now ~20 Sverdrups (that is, 20,000,000 $m^3 s^{-1}$)[44] The water feeding the source region for the deep water has a temperature of ~10 °C, whereas the new deep water leaving the region has a temperature of ~2 °C. Thus, as a byproduct of deep-water formation ~5 × 10^{21} cal of heat are released to the atmosphere each year, an amount corresponding to ~30% of the solar heat reaching the surface of the Atlantic Ocean in the region north of 35° N.

To test the geographical distribution of the impact of turning on and off this source of heat, an experiment was performed using the general circulation model for the atmosphere developed by the Goddard Institute for Space Studies. A comparison was made between the air temperature for a model run with present-day ocean conditions and for a model run with the surface ocean temperatures in the region north of the glacial polar front (see Fig. 6) cooled to the levels estimated by the CLIMAP (1981) reconstruction for glacial time[45]. The results of this experiment will be published elsewhere. The air-temperature anomaly generated by this change in sea surface temperature spreads across Europe. A cooling is seen in extreme north-eastern North America whereas no corresponding anomaly is seen in the United States or over Antarctica. The results are, therefore, consistent with the pollen and ice-core evidence. Although this experiment does not prove that the Alleröd–Younger Dryas event was caused by changes in the rate of deep-water production in the northern Atlantic, it does suggest that were such changes to have occurred they would have produced climate changes with the observed regional pattern.

Deep-water production changes

The most difficult question is why the production of deep water in the northern Atlantic may have resumed for brief intervals during glacial time. For an answer, we must consider the processes important to the present-day production of deep water in the northern Atlantic and for the absence of equivalent production in the northern Pacific. Oceanographers agree that the contrast between the two northern oceans is rooted in the large salinity difference. The waters of the northern Atlantic are saltier than the mean for the upper ocean, whereas the waters of the Pacific are fresher. The Atlantic's extra salt allows waters of density as great as any found in the deep sea to be generated during the winter months. By contrast, even if cooled to their freezing point, northern Pacific waters achieve a density no greater than that of intermediate depth water.

An approximate fresh water budget for the northern Atlantic and its satellite sea, the Mediterranean, is given in Table 1. Despite the fact that this region receives a sizeable portion of the world's river runoff, it loses an even greater amount of water through an excess in evaporation over precipitation. The resulting net loss of fresh water leads to the salt enrichment observed in the northern Atlantic. The salt budget for the North Atlantic must be balanced by the export of salty deep water to the Pacific and Indian Oceans. On a global scale, the water (and salt) budgets of the surface ocean must balance; thus, the fresh water lost from the Atlantic must re-enter the ocean elsewhere. Polewards of 30° N, atmospheric transport produces a net divergence of water vapour over the North Atlantic and convergence over the North Pacific east of the dateline[46]. Thus, if we are to understand what maintains deep water production in the present-day ocean, we must understand why fresh water is being pumped through the atmosphere from the North Atlantic to the North Pacific.

Warren, addressing this question[47], has shown that this pumping can be explained nicely by the temperature difference between the two oceans. The waters north of 30° latitude in the Atlantic are warmer than their Pacific counterparts. Thus, water is being distilled off the 'warm' Atlantic and condensed on the 'cold' Pacific. If so, then it is the temperature difference on which we must focus. What maintains it?

To some extent the warmer temperatures for the surface of the northern Atlantic must be related to the deep-water formation process. Upper waters in the Atlantic are advected northward into the regions of deep-water production. This advection carries heat to high latitudes. By contrast, in the northern Pacific, deep water upwells to the surface and is carried southward in the upper ocean. In this sense the situation is self-sustaining. Stommel[48] and Rooth[49] have discussed the possibility that thermohaline circulation has more than one stable mode The deep-water circulation pattern maintains surface temperature of the northern Atlantic at a higher value than that for the Pacific. This temperature difference causes the Atlantic to be enriched in salt, whose excess leads to deep-water formation. At steady state, deep-water production must carry away just the amount of excess salt left behind by the atmospheric transport of water from the Atlantic to the Pacific. The standing excess of salt in the northern Atlantic is presumably just high enough to allow the generation of water sufficiently dense to enter the deep Atlantic.

This brings us to the fundamental question of this review—does the ocean–atmosphere system have more than one mode of operation? The idea that the Earth could, under the same solar heating regime, have quite different temperatures depending on its ice albedo or atmospheric greenhouse gas content is not new. Climate modellers have long been aware of the white Earth catastrophe scenario. If somehow the Earth were to be covered with ice, the greatly increased reflectivity of its surface would cause the temperature to fall below the freezing point and thereby stabilize the change. They have also considered the

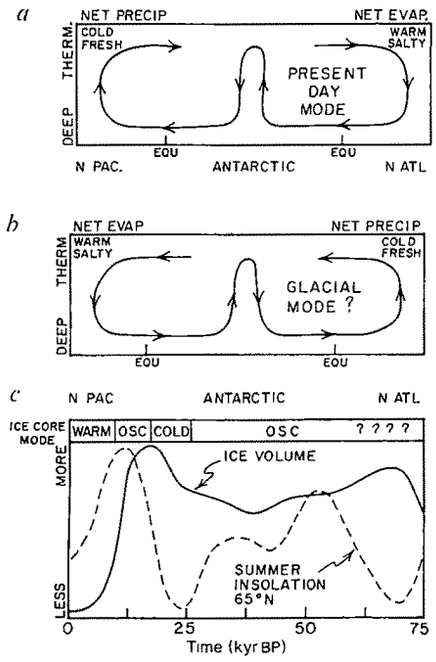


Fig. 7 *a*, *b*, Two possible extreme modes of ocean circulation are proposed here, corresponding to the warm and cold intervals seen in the ice-core record. *c*, The times when the ice-core record shows the system to have been locked in its warm mode, to have been locked in its cold mode or to have been oscillating between these modes are shown with the ice-volume record and the Northern Hemisphere high-latitude summer insolation record. The intervals of warm Greenland climate correspond to intervals of low ice volume and high summer insolation and the intervals of cold Greenland climate correspond to times of high ice volume and low summer insolation. The intervals when Greenland climate oscillated between warm and cold corresponded to times when the ice volume–summer insolation product had intermediate values. This suggests that the atmospheric transport of water vapour between the Atlantic and Pacific changes with ice cover and summer insolation.

greenhouse catastrophe. If somehow the carbon locked up in sediments were to be released to the atmosphere, the enhanced infrared absorption would raise the planetary surface temperature to the point where carbonate minerals and organic residues could no longer form and thereby stabilize the change. Based on the CO_2 results from ice cores, Oeschger suggested that the Earth had two modes of ocean–atmosphere–biosphere–cryosphere operation. Each oscillation observed in the ice cores involves a jump from one mode of operation to the other.

It is tempting to speculate that one of Oeschger's two modes corresponds to the situation when deep-water production in the northern Atlantic is 'strong' and the other to the situation when deep-water production is 'weak'. If this is the case, then the palaeoclimatic record tells us that, for the summer insolation and ice-cover situation that prevailed during the full interglacial time, the system was in the 'strong' mode and for the corresponding situation that prevailed during full glacial times it was in the 'weak' mode. Because in the present-day ocean the deep-water production corresponds to salt enrichment in the Atlantic, it is tempting to postulate that during full glacial time the opposite was true. Then, salt was being enriched in the Pacific and deep-water flow was the reverse of what it is now (see Fig. 7). Based on oxygen isotope studies on North Pacific cores, deep water may have been produced in the Pacific Ocean during glacial time (N. Shackleton, personal communication). On the other hand, it is possible that the rate of salt enrichment in the Atlantic was considerably less during glacial time, causing a reduction in deep-water production rather than a reversal in flow. Boyle's Cd data favours this latter point of view.

The Oeschger climate oscillations occur in intervals with climates lying between those characterizing full glacial and full interglacial time. If, as the summer insolation at high northern latitudes increased and once again the atmospheric vapour transport balance favoured salt buildup in the Atlantic, then the production of deep water would resume (or intensify), releasing excess heat to the atmosphere over the northern Atlantic. This excess heat would lead eventually to increased discharge of ice from the caps surrounding the Atlantic. If 3% of the excess ice present during full glacial time were added to the Atlantic Ocean by melting during a 100 yr interval, the mean freshwater flux would be 0.6 sverdrups (that is, twice the present imbalance between fresh water and loss for the Atlantic). Thus, the melt water generated in this way would significantly reduce deep-water production, returning northern Atlantic climates to their glacial state. This, in turn, would halt ice retreat and allow the northern Atlantic salt buildup to resume. Thus, it is possible that during periods of ice retreat the system oscillates between the North Atlantic 'strong' and 'weak' modes of deep-water production. Although the physics of such an oscillation has yet to be elucidated, two elements are evident. The first involves water storage in and release from the large Northern Hemisphere glacial ice caps adjacent to the Atlantic. The other involves the transport of salt through the deep sea. As water now resides for many hundreds of years in the deep sea, transients in the deep-ocean salinity distribution created by ventilation changes may be an important element in the oscillator.

Climate modelling

Until now, our thinking about past and future climate changes has been dominated by the assumption that the response to any gradual forcing will be smooth. But if, as proposed by Oeschger, the system has more than one quasi-stable mode of operation, then the situation is more complex. Present general circulation models will at best allow us to study only the changes that will take place if the system remains in its current operational mode. Thus, if the changes that characterized glacial time and those that will characterize the coming CO_2 superinterglacial time involve mode switches, investigations of the transient climate response have to allow for this possibility.

Despite the tenuous nature of the information presently available and of the difficulties inherent in thinking in terms of mode changes, we must begin to explore this alternate track. The programme elements required are clear. At least we must have a joint ocean–atmosphere general circulation model that will allow the exploration of several possible connections between planetary radiation, atmospheric water transport, ocean circulation, atmospheric trace gas content and the marine and terrestrial biosphere. If we are to have confidence that these models provide reliable analogues to the real world, we must gather more information about how each of the important subsystems operates in the present. Finally, we must extract all possible information from the palaeoclimatic record. Even given the full use of present resources, it will be several decades before we possess sufficient understanding to predict future climates. Unless we intensify research in these areas, the major impacts of CO_2 will occur before we are prepared fully to deal with them.

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ARTICLES

Powerful extragalactic masers

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Two strong extragalactic OH masers have been detected in the galaxies NGC3690 and Mrk 231 which are in the same class as the megamaser source IC4553 (Arp 220). The amplification model for the background continuum proposed for IC4553 can account for most of the powerful OH maser lines and for part of the H_2O emission. Most masing galaxies have sufficient infrared flux to pump the masing regions, except that the H_2O pumping must occur at a much higher conversion efficiency than the OH pumping.

THE extraordinary strength of the few OH and H_2O extragalactic masers[1–11] suggests that there are emission processes occurring in these sources which differ significantly from those that occur in galactic masers[12,13]. By far the most luminous OH maser detected to date is in the source IC4553 (refs 4, 14), whose maser emission is superposed exactly on the continuum source in the nucleus of the galaxy[14,15]. This strongly suggests the presence of amplification of the flux from the nuclear continuum source by a foreground molecular region. The masing region may have a relatively low gain and may not have been observable in the absence of the background source.

This article presents the recent detection of two powerful OH megamasers in the galaxies NGC3690 and Mrk231. Because of their similarity to the emission in IC4553, most of the powerful extragalactic OH maser emission can be interpreted with the continuum amplification model proposed for IC4553. This concept can be extended to the H_2O maser emission as well.

New detections

A search for OH absorption or emission in 110 galaxies with strong continuum sources in June 1984 used the 91-m telescope of the National Radio Astronomy Observatory (NRAO) in Green Bank, West Virginia, which brings the total number of galaxies searched at Arecibo and Green Bank to 240. A cooled gallium arsenide field-effect transistor receiver with a total system temperature of 27 K was used in combination with a 396-channel autocorrelation receiver, which was split in two banks for the two circular polarizations. The sensitivity of the 91-m telescope is 1.1 K Jy^{-1} at 18 cm. Very gentle third-order baselines were subtracted from the data.

Strong OH emission was detected in two systems, NGC3690 and Mrk231[16]. Several new OH absorption features were detected, among which are the line NGC3079[5] and a tentative detection in the H_2O maser galaxy NGC4258. The absorption results will be published separately (W.A.B. *et al.*, in preparation). The results on the new OH maser sources are summarized in Table 1 and Figs 1 and 2.

NGC3690. The nuclear activity in the interacting galaxy pair NGC3690/IC694 (Mrk171) is one of the prime examples of starburst activity[17]. The pair of Sc galaxies is depicted in plate 299 of Arp's catalogue[18]. NGC3690 exhibits the most luminous optical emission lines of any non-Seyfert Markarian galaxy[19] and is also a very strong infrared source[20–22]. The radio source in NGC3690 has a triple structure and the 10-μm emission and optical line emission are superposed perfectly on this triple[17]. Prominent neutral hydrogen absorption towards the radio nucleus of NGC3690 has been seen at the VLA[23]. This broad and deep absorption line has a double structure with a separation of 160 km s^{-1} and can most readily be interpreted in terms of nearly edge-on absorbing disks (in agreement with the optical image). Optical studies[24] also indicate a velocity spread across the nuclear region of ~125 km s^{-1} increasing to the west. NGC3690 is a strong CO emitter; emission of this gas follows the infrared and radio structure of the galaxy (P. Salomon, personal communication; W.A.B. and R. W. Freund, unpublished results). The CO emission also has two peaks at ~130-km s^{-1} separation.

The OH emission spectrum of NGC3690 is presented in Fig. 1. The 1,667- and 1,665-MHz lines are clearly visible in the spectrum and are merged slightly. The features have been verified recently using the Very Large Array (VLA)-A. The double structure in the 1,667-MHz line agrees quite well with the two H I absorption features. Assuming the high velocity OH component originates at the west continuum component and

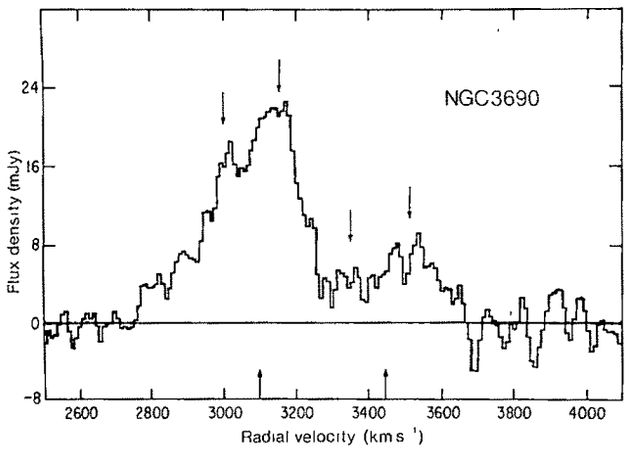


Fig. 1 The OH spectrum of NGC3690 obtained using the 91-m telescope of NRAO. The integration time is 59 min; the velocity scale refers to the 1,667-MHz feature. The 1,665-MHz feature is offset 351 km s^{-1} from the 1,667-MHz line The pair of long arrows indicate the centre velocity of the features. The two pairs of short arrows indicate the double structure found in the H I and CO features

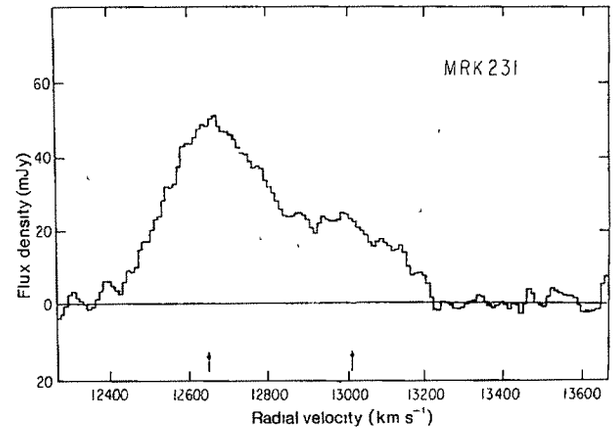


Fig. 2 The OH spectrum of Mrk231 The integration time for the spectrum is 29 min. The arrows indicate the centre velocities for the two features obtained with Gaussian fitting. The H I absorption velocity lies 14 km s^{-1} below this. The velocity scale refers to the 1.677-MHz feature.

the low velocity component at the east, the optical depths for the two components are ~−0.08 and −0.15, using the respective radio continuum fluxes. This suggests a correlation between the infrared flux and the maser gains, because the infrared flux from the west is ~2.5 times that from the east.

Mrk231. This is one of the most prominent Syl galaxies and is considered to be a prime example of Seyfert properties. However, Mrk231 is unique among Syl galaxies for its red colours and its strong interstellar absorption lines[25]. Most of its luminosity lies in the infrared[20,21]. The nucleus of Mrk231, having a strong unresolved radio source coincident with the optical continuum peak, cannot be modelled simply with bursts of star formation[26]. Nevertheless, Mrk231 is one of the few nuclei that shows both Seyfert 1 properties and evidence of star bursts[24].

Mrk231 has optical emission-line velocities that are different from the absorption-line velocities[27]. Broad H I absorption has been found at the lower redshift[23,28], suggesting the presence of an edge-on disk structure. Gaussian fits of the OH emission lines detected in Mrk231 (Fig. 2) indicate a velocity which is 14 km s^{-1} higher than the H I absorption velocity. For an isotropic emission pattern, the emission in the 1,667-MHz line alone is 2,500 $L_\odot$, which is 3.5 times greater than that of IC4553. The luminosities quoted for the masers are isotropic. It is, however, probable that the maser emission is not isotropic and that the beaming is different for the OH and the H_2O masers. Assuming that the maser emission in Mrk231 originates in a cloud that exponentially amplifies the continuum flux[29], the peak optical depth required for explaining the 1,667-MHz lines is −0.3. If all emission in Mrk231 occurs in front of the <0.3″ compact source the brightness temperature of the 1,667-MHz peak is >3.6×10^5 K.

OH megamaser IC4553

The case of the OH maser source in IC4553 may be taken as a basis for the discussion of extragalactic masers. IC4553 (Arp220) has been studied extensively in different wavelength regimes and the data reveal a rather complete picture of the properties of the different masing regions.

The galaxy IC4553 has been detected in emission at all four 18-cm transitions of OH. The two main lines have been presented earlier[4]. The 1,667-MHz feature has a peak strength, which is 0.82 of that of the continuum source, whereas the peaks of the 1,665-, 1,612- and 1,720-MHz lines are respectively 0.24, 0 08 and 0.03 of the 1,667-MHz peak flux (W.A.B., in preparation). VLA-A[14] and MERLIN[15] observations at 18 cm show that the line emission is exactly superposed on a triple continuum structure of 0.6×2.0″.

The high-resolution results indicate that the bulk of the masing material is part of a rotating molecular disk centred on the nuclear continuum source[14,15]. A second distinct emission region exists on the eastern side of the source, which has a radial velocity ~300 km s^{-1} higher than the systemic velocity of the molecular disk[14]. In H I the galaxy IC4553 displays a very strong and broad (740 km s^{-1}) absorption line[30] VLA-A observations[31] at 21 cm reveal that the bulk of the absorption also results from a rotating disk, which belongs to the same large-scale structure as the molecular disk and is centred on the nuclear continuum radio source. The high-velocity OH emission feature has also a counterpart in H I absorption

Table 1 OH megamasers

Name	Alternative names	V_{abs}(H I) (km s^{-1})	V_{OH} (km s^{-1})	Isotropic luminosity L_{OH}(1,667) ($L_\odot$)	Continuum flux (mJy)	Line flux 1,667 MHz (mJy)	Line flux 1,665 MHz (mJy)	Hyperfine ratio R_H	Optical depth τ (1,667)	Brightness temperature T_b (K)	Comments
NGC3690	Arp299	3,115	3,101	85	220	18	4	4.5	−0 08	~5×10^2	East component
	Mrk171	(23)			140 (17)	21	7	3 0	−0 14	~5×10^2	West component
IC4553	Arp220	5,350	5,373	700	278	280	36	5 4	−0 7	3×10^6	Region 1(14)
		5,659 (30, 31)	5,642 (4)			20	8	2 4			Region 2
Mrk231	U08058	12,638 (23, 28)	12,650	2,500	160 (29)	51	22	2 3	−0 28	>3×10^4	

References are given in parentheses

Table 2 Comparison of extragalactic OH and H_2O masers

Name	Alternative name	Distance§ (Mpc)	Nuclear spectral classification	Absorption column density H I N_H/T_s	Absorption column density OH N_{OH}/T_{ex}	Infrared luminosity ($L_\odot$)	Luminosity ratio R_c $L_{(IR)}/L_{(B)}$	Isotropic OH line luminosity 1,667 MHz ($L_\odot$)	Isotropic H_2O line luminosity ($L_\odot$)	Radio continuum	
NGC253		3 1	N	3.4×10^{19}	4.7×10^{15}	1.5×10^{10}	—	2×10^{-1}	—	Extended, compact	Main OH lines
			(24, 36)	(63, 64)	(11)	(20, 21)		(11)		core (56, 63, 64)	
NGC520		42	H	$>2\,0\times10^{19}$	$4\,3\times10^{15}$	$8\,6\times10^{10}$	6	5×10^{-5}	—	Compact, with	1,667-MHz OH
			(24, 36)	(3, 30)	(3)	(49)		(3)		extension (56)	line
NGC3690	Arp299	56	H	$1\,1\times10^{20}$	—	7.4×10^{11}	10	85	—	Triple compact,	Main OH lines
	Mrk171		(24, 36)	(23)		(17, 22)	(22)	(this paper)		extended (17)	
IC4553	Arp220	98	S or H	1.1×10^{20}	—	2×10^{12}	80	700	—	Compact triple	Main OH lines
			(24, 36)	(30,31)		(34)	(34)	(4, 14)	(15)		
Mrk231	U08058	226	S	$2\,8\times10^{19}$	—	4×10^{12}	27	2,500	—	Compact, weak	Main OH lines
			(24, 36)	(23, 28)		(20, 21, 25)	(34)	(this paper)		extension (20)	
M82	NGC3034	3 5	H	2×10^{20}	8×10^{14}	4×10^{10}	8	$3\,6\times10^{-2}$	1.5	Compact source	Main OH lines
			(24, 36)	(1, 2)	(1, 2)	(20, 21)	(34)	(1, 2)	(6)	disk extension (1, 2, 57)	
Circinus		4	S?	—	—	$1\,2\times10^{10}$	—	—	35		
						(52,53)			(10)		
NGC4258		9.5	S, L	—	5×10^{14}	6×10^{8}	$>0\,3$	—	157	Extended S-shaped	
			(24, 36)		(‡)	(54)			(5, 6, 7)	at 20 cm (58, 59)	
NGC4945		8 1	S(?)	$6\,6\times10^{19}$	1×10^{16}	$>2\times10^{10}$	—	3×10^{-5}	240	Extended at	1,612-MHz OH
				(50)	(50, 55)	(52)		(55)	(7, 8, 9)	20 cm	line
NGC1068		20	S	8×10^{17}	$<8\times10^{14}$	3×10^{11}	5	—	320	Compact S-shaped	
				(51)	(39)	(20)			(6, 7)	(56, 60)	
NGC3079		21	L, S	*	$7\,6\times10^{15}$	1×10^{11}	4	†	960	Compact core,	1,667-MHz OH
			(24, 36)		(5)	(53)			(5, 7)	extended structure at 20 and 6 cm (61)	line

References are given in parentheses. The marginal detections of H_2O in NGC6946[6,7] and NGC6240[7] have not been included in the table.
* H I absorption in NGC3079 may be evident from the published spectrum of Shostak[62]
† The strength of the OH maser lines in N3079 is not yet known.
‡ A tentative detection has been made of OH absorption in NGC4258 (W.A.B., A. D. Haschick & J.T. Schmelz, in preparation).
§ $H_0 = 55\ km\ s^{-1}\ Mpc^{-1}$

The optical appearance of the galaxy IC4553 suggests that the galaxy is definitely the result of a recent merger (see plate no. 220 in Arp[18]). Recent optical studies indicate that the dark band, which divides the image in half, is a dust lane[32,33]. The disturbed appearance of the dust lane suggests a disk structure, similar to that seen in M82 (ref. 33). The outer optical structure resembles that of the merging pair in the 'Antennae' (NGC4038/9). The notion of tidal interactions is supported by the high velocity H I gas (in absorption) and OH gas (in emission). No H I emission has been seen in IC4553 (ref. 31).

IC4553 is a very prominent infrared source as has been found recently by IRAS[34] and ground-based instruments[35]. The infrared source in IC4553, with a minimum size of ~1.4" and a spectral temperature of 61 K (refs 34, 35), is centred on the position of the continuum source[32,34] in the centre of the dust lane. IC4553 is unique among the infrared galaxies because it has the highest known ratio of infrared luminosity to blue luminosity ($R_{ib} = 80$). This high ratio could indicate that a powerful burst of star formation is in progress. Optical emission lines suggest Seyfert-like (Sy2) properties, but they would not be inconsistent with an H II region spectrum[24,32,36]. Starburst models as well as Seyfert models (as needed for Mrk231) present difficulties in explaining IC4553 because of the heavy dust obscuration[24,36]. IC4553 exhibits strong H_2 emission[37,38], which could be explained by shock excitation of interstellar clouds. No flux was detected in a 455-min exposure in the short ultraviolet wavelength regime with the IUE telescope (W.A.B. and R. Panek, unpublished results).

There is still controversy as to the nature of the galaxy IC4553. The nucleus may be the advanced stage of a merger of two nuclei or the nuclear activity has been triggered by a tidal interaction, which also resulted in a disturbed outer structure. The radio and infrared sources could be evidence either of Seyfert activity or of a starburst embedded in the very dusty nuclear region.

Working model

On the basis of the observational results on the OH megamaser in IC4553, a model[14,15] has been suggested in which the bulk of the maser emission is caused by amplification of the radio flux from the nuclear continuum source by foreground molecular material. Here I apply this model to the two newly discovered OH masers and to extragalactic H_2O masers. The OH and H_2O molecules in molecular clouds may be inverted partially by the infrared radiation field or other pumping processes. This relatively weak population inversion occurs in relatively extended molecular structures, in contrast to the stronger but more localized population inversions around condensed H II regions which characterize masers in our Galaxy. Because of its low exponential gain, the molecular material is not observable as a maser when observed on its own. However, when a background source is placed behind the cloud, it can amplify the continuum flux to an observable level.

To obtain the correct configuration for continuum amplification the following ingredients are required: (1) the presence of a background continuum source, possibly in the centre of the galaxy; (2) a nearly edge-on molecular disk, thus increasing the probability of seeing a molecular cloud with an inverted population along the line-of-sight; (3) a pump for inverting the molecular material.

Below, I address these requirements in more detail and present the current evidence in support of such a model. Some of these characteristics have already been mentioned in relation to the masers in IC4553 (refs 14, 15, 32) and NGC3079 (ref. 5). The main characteristics of all prominent masing galaxies are given in Table 2.

Continuum sources. Most known masing galaxies are characterized by compact nuclear continuum sources, some of which also have an extended nuclear structure. However, the crucial question is where the maser emission occurs relative to the continuum source. In the case of IC4553, the maser emission is exactly superposed on the continuum structure and the emission and continuum contours are unmistakably the same[14,15]. Spectral line studies of the new OH maser sources at the VLA are not yet available, but the available evidence for NGC3690 strongly suggests that the OH emission originates at the location of the three continuum sources. Mrk231 has a compact nuclear radio source. In the case of M82 the OH maser emission does not

originate at the peak of the continuum map[2], but the continuum flux is still considerable at the maser locations and maser amplification is certainly plausible. For the H_2O masers, a coincidence of maser and continuum emission has been found in three cases. The prominent maser feature in NGC3079 (refs 4, 6) coincides with the nuclear source at 2″ resolution with the VLA-D (A. D. Haschick and W.A.B., unpublished results). Similarly, the prominent feature in NGC4258 falls on a weak source in the extended continuum structure, whereas the high-velocity feature in NGC1068 coincides with one of the eastern continuum knots (M. J Claussen, personal communication). VLBI and VLA-A studies are in progress to determine these relative positions.

Molecular disks. The main observable characteristics (besides the optical image) of seeing a galactic disk edge-on are high absorption column densities. Most masing galaxies show evidence of H I and/or OH absorption and some have the highest known H I absorption column densities. The masing galaxy with a low H I absorption column density, NGC1068, also has a relatively low inclination angle; a severe upper limit has been found for its OH absorption column density (ref. 39; W.A.B. *et al.*, in preparation). The molecular disk in our Galaxy is inclined by an angle of 25° relative to the H I disk[40]; this might be true for other galaxies. A recent compilation of all known OH absorption lines indicates a correlation of the OH absorption optical depth and the estimated inclination angle of the galaxy[3]. This result supports the notion that most of the absorbing OH molecular clouds are confined to a relatively compact disk, approximately aligned with the large-scale disk of the galaxy.

Pumping mechanisms. Here, I assume that the masing molecular gas is contained in molecular clouds that are not necessarily next to compact H II regions. Diffuse molecular gas can have maser action, as has been seen for the galactic molecular clouds along the line-of-sight to the continuum source 3C123 (ref. 41). The 1,720-MHz emission line is found to be stronger on-source than off-source, which can be readily explained with weak amplification at 1,720 MHz of the continuum flux of 3C123 as it passes through the molecular cloud. This is the only known definite example (besides IC4553) of amplification of a background continuum

The pumping agent for the OH in molecular clouds is probably the far infrared radiation from dust[42,43]. For IC4553, all four 18-cm OH transitions have been seen in emission, which is unusual because generally either 1,720 MHz or the other three lines are in emission, but not all four. The pumping of H_2O masers is still subject to study. For H_2O/H II masers it is difficult to explain how such large maser fluxes emerge from clouds of such small sizes. Collisional processes may be the dominant pumping mechanisms for H_2O masers[13].

Most galactic OH and H_2O masers are thought to be saturated[12,13], which means that there is a steady production of photons throughout the masing region. Saturated masers have a high efficiency of converting pump photons into maser photons. Unsaturated masers have an exponential gain but have a lower conversion efficiency. However, an unsaturated maser can amplify background continuum radiation. As the line radiation field in the maser gets stronger, the maser will eventually saturate. The presence of a background continuum may bring this about earlier. An observer will then see the (saturated or unsaturated) masers superposed on the continuum source. Without a background, source the masers may not saturate at all and even be unobservable. This situation may exist in the H_2O sources NGC3079 (ref. 5) and NGC4258, where narrow features could represent (mostly) saturated masing pockets of gas, whereas broader features represent (mostly) unsaturated amplification of the radio continuum. The OH maser lines are generally broad and could mainly result from amplification of the background source. Naturally broad OH lines may also have some high brightness saturated features embedded in them. Using the presently known sizes (upper limits) for the continuum sources, one finds a brightness temperature $T_b < 10^7$ K for the masers, which is considerably below those of saturated galactic masers ($T_b = 10^{10}$–10^{11} K; ref. 12). Much will be learned from VLBI observations as they will reveal the compact and more saturated components.

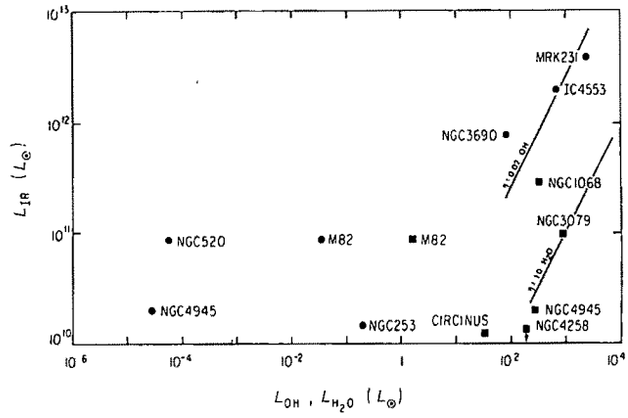


Fig. 3 Relation between the isotropic OH and H_2O luminosities and the integrated infrared luminosity. The circular entries are for OH masers and the square entries are for H_2O masers. The linear relationships in the diagram represent constant conversion efficiency curves for the OH and H_2O masers. For determining these curves the linewidth has been taken to be 300 km s^{-1} for the OH masers and 100 km s^{-1} for the H_2O masers.

A general problem for galactic masers is the absence of an identifiable pump source, in particular of a sufficient infrared flux. However, this is not the case for the extraglactic maser sources, which tend to have prominent infrared sources mostly at the location of the radio continuum sources (that is, NGC3079, NGC3690, IC4553, Mrk231). In Fig. 3 the OH and H_2O maser luminosities have been plotted against the infrared luminosity of the galaxy. The low luminosity masers seem to be independent of L(IR). However, the high luminosity masers show an approximately linear increase in luminosity with increasing L(IR). Such a linear relationship is expected and observed for saturated stellar masers, but seems also plausible for unsaturated masers. Two pump efficiency curves based on the spectra of the prominent maser galaxies (IRAS point-source catalogue) indicate that the OH masers may have an efficiency of a few per cent for converting photons at 30 μm into OH maser line photons. However, if the inferred radiation field at 10 μm pumps the H_2O maser, this should happen at a conversion efficiency of 50–100%. These conversion efficiencies are typical for unsaturated and saturated masers, respectively. Naturally collisional processes can also contritube to the pumping of the water vapour. Evidently, OH and H_2O masers have different pumping mechanisms and efficiencies, or the H_2O masers may be more directional (thus resulting in a overestimate of the luminosity). The low luminosity maser sources may result from similar large-scale processes, but they can also be just like the powerful masers seen in our Galaxy,

OH and H_2O masers

It is apparent from the above discussion that the properties of the OH maser sources and those of H_2O are similar in many ways. Because of these similarities, I suggest that the basic model, amplification of the background source flux, may apply to both types of sources. However, some maser characteristics are different, indicating that different physical conditions prevail in the masing OH and H_2O regions.

In compact H II regions, H_2O masing occurs at higher densities than OH masing (see ref. 12). The H_2O masers are necessarily located closer to the exciting source. If these excitation conditions apply to the situation described here of foreground molecular clouds, then H_2O inversion occurs only in the cores of clouds, whereas OH can be inverted over much large regions. This suggests that: (1) the linewidths of the H_2O maser lines be much smaller than those of the OH maser lines; (2) the H_2O masing regions are likely to be much smaller than

the OH masing regions, and are expected to vary on shorter timescales.

The outstanding H_2O maser features in the spectra of N3079 and N4258 do indeed vary on timescales of months (refs 5, 7; M. J. Claussen, personal communication). The spectra of most H_2O masing galaxies show distinct narrow features, sometimes superposed on some broader feature. Only the weakest OH masers have spectra breaking up into distinct features. However, the prominent narrow H_2O features have linewidths which are significantly larger than those of galactic masers.

Another difference between OH and H_2O maser lines is their velocity relative to the systemic velocity of the galaxy. In IC4553 the H I disk in absorption and the molecular disk in OH emission have the same centre velocity. This is also true for Mrk231 and NGC3690. However, the H_2O masers tend to have velocities away from the centroid velocity of the galaxies. This is strikingly apparent from the comparison of the OH absorption spectrum of NGC3079 and its H_2O spectrum[7]. The H_2O features in NGC3079 are blueshifted by 140 km s^{-1} relative to the OH centre velocity, but the features coincide in velocity with weak OH emission lines, which are superposed on the 1,667-MHz absorption line (B. E. Turner, personal communication). Similarly, the lines of NGC4258 are blueshifted 60 km s^{-1} when compared with the tentative OH absorption detection. NGC4945 has redshifted H_2O lines relative to the OH absorption velocity but these lines also coincide in velocity with a 1,612-MHz OH emission feature. M82 has one blueshifted H_2O feature, which again coincides in velocity with a 1,667-MHz OH emission feature, whereas another feature lies far to the red side of the OH absorption.

The prominent H_2O emission features originate possibly in dense (shocked) pockets of molecular gas with relatively high gains and having high relative velocities. In contrast, the OH emission may originate in more extended regions. Most of the galaxies in Table 2 have been searched for both OH and H_2O, but only in M82, NGC4945 and NGC3079 has emission been seen at both frequencies and at identical velocities.

Properties of masing galaxies

Two of the prime characteristics of the sample of masing galaxies, the presence of a strong nuclear radio continuum source and the infrared flux, are likely to be related[44]. Peculiar and interacting spirals have relatively high continuum-flux densities as well as extended nuclear sources[24,36]. Most of this radio emission can be explained in terms of starburst models[45]. However, it is not clear whether these nuclei have a Seyfert or a starburst nature, since a low ionization or Seyfert spectrum can hide an underlying H II region spectrum completely. The dust can effectively hide from sight the H II regions associated with the starbursts, which in turn may be responsible for the radio continuum sources. Possibly starburst nuclei and Seyfert nuclei are different stages of evolution of objects of one class[46].

The infrared properties of galaxies are still being elucidated, but it seems that double, interacting or merging galaxies are prominent among infrared galaxies[47–49]. Of the sample of masing galaxies, most are strong infrared sources and a few are known to be interacting or merging systems (NGC3690, IC4553, NGC520). It is likely, however, that most of these galaxies have experienced recent bursts of star formation and that the origin of the megamaser phenomenon can be traced to the coexistence of a radio continuum source, an ultraviolet radiation field to be converted into an infrared radiation field by the dust and remnant molecular material.

The detection rates of extragalactic masers have been very low. Four of the OH masers have been detected during surveys of 240 predominantly late-type spiral galaxies with radio continuum (W.A.B. *et al.*, in preparation). Recent searches for H_2O emission in 65 spirals produced four new cases[5–7]. These detection rates of 2–6% have been affected by: (1) a low filling factor for the proper type of molecular clouds (this factor is likely to be larger for the OH gas than for the denser H_2O cloudlets); (2) a relatively short lifetime of masing clouds compared with the lifetime of the continuum and infrared sources; (3) the relative detectability of signals at OH and H_2O; (4) the composition of the sample. The slightly higher probability of detecting H_2O masers could be caused by tighter selection criteria for the sample or by the fact that the gains for some H_2O maser features are higher, leading to better detectable features.

Conclusions

Maser activity in other galaxies is a newly discovered phenomenon and only a small number of cases have been found. Some of the characteristics of the OH and H_2O megamasers can be explained with a model involving unsaturated maser amplification of radio continuum structures (compact or extended) by foreground molecular material. The highest probability for a galaxy to produce observable maser emission is to have a nearly edge-on molecular disk with an embedded radio source and a proper pumping mechanism for the molecules. Most masing galaxies fulfill these requirements.

The nuclear spectra of the prominent masing galaxies have been classified as H II region or Seyfert types. A scenario for masing sources would then involve some sort of nuclear activity (with a burst of star formation or a Seyfert engine), which provides the radio source and an ultraviolet flux. The ultraviolet flux is downgraded to infrared by the surrounding dust. The infrared radiation is used to pump the foreground molecular gas, which in turn gives the maser emission. The starburst activity in several galaxies has probably been triggered by a merger or a galaxy/galaxy interaction in the recent past. All these nuclei could belong to one class, but have different amounts of dust and are at different stages of evolution.

The properties of the OH and H_2O maser lines are sufficiently different to suggest a difference in the excitation of the two molecules. Although they both may amplify the background continuum, the OH masing regions are probably more extended, whereas the prominent H_2O maser features are likely to originate in smaller cloudlets, either falling into the nucleus or flying out. These smaller pockets have a relatively higher gain (more saturated) than the surrounding (unsaturated) material, which results in an H_2O spectrum with narrow lines superposed on broader features.

The observed infrared flux for the masing galaxies seems to be sufficient to pump the molecular gas. However, for the OH masing galaxies, the infrared photon-to-line photon conversion efficiency can be only a few per cent, whereas for the H_2O masers this must be 50–100%. Possibly the bulk of the H_2O maser emission is due to saturated components. An approximately linear relationship between maser and infrared luminosity can be seen for the OH and H_2O masers. Apparently each type runs at its own approximately constant efficiency It is of interest to speculate what this diagram tells us about other galaxies to be detected in OH or H_2O At present H_2O features coincide with weak OH features in three galaxies (M82, NGC3079, and NGC4935). Except for these galaxies, OH and H_2O emission seem to be mutually exclusive.

The similarities and differences between OH and H_2O masers are important for determining the physical conditions of the masing material and provide valuable insight into the nature of these active galactic nuclei. Extragalactic megamasers seem to represent a new form of astronomical 'image processing' Unlike gravitational lensing, the processing is provided by diffuse clouds of interstellar molecules.

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Sources of acidification in Central Europe estimated from elemental budgets in small basins

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Long-term increase in acidification of water and soils in the Elbe river basin is caused mainly by the deposition of dry SO_2 and the application of industrial fertilizers. The direct input of hydrogen ions by acid rain is less significant. The budgets of elements and protons indicate natural and anthropogenic processes that acidify and buffer our environment.

CENTRAL Europe is a region seriously damaged by acid industrial emissions, as demonstrated by increased rates of corrosion[1,2], deterioration in forest productivity[3,4] and release of elements such as aluminium[5] and heavy metals[6] in water. A large-scale application of industrial fertilizers causes acidification of arable land and eutrophication of surface water[7,8]. Central Europe receives one of the highest inputs of sulphur from the atmosphere[9]; the runoff of anions of strong acids in surface water has increased while the runoff of weak acids, represented by alkalinity of stream water, has decreased in the past century[10]. The data in Table 1 present convincing evidence, independent of uncertain historical measurements of *p*H, that acidification has occurred. It may be related to acid rain[11], deposition of dry gaseous SO_2 and NO_x from the atmosphere on plants and soils[12], intensive application of industrial fertilizers[13,14] and depletion of organic matter from topsoil. These processes are of anthropogenic origin and affect the biogeochemical cycles and mass balances of naturally occurring chemical elements.

To collect quantitative data on the environmental changes and their rates, I have evaluated and compared mass budgets of H^+, Na, K, L, Mg, Ca, Fe, Al, Si, N-NH_4^+, N-NO_3^-, C-HCO_3^-, Cl, S and P in small hydrologically and geologically well-defined drainage basins in the watershed of the Elbe river (Fig. 1). Four representative basins considered here have similar bedrock, soil and climate conditions but differ in mean slopes and anthropogenic impacts. Comparison of their elemental budgets will help to elucidate the mechanisms that affect the fluxes of elements and cause acidification of soil and water. The data are used to calculate weathering and erosion rates influenced by acid emissions and agricultural practice. The elemental budgets in basins affected by industry and agriculture serve as background data for a comparison with representative basins in other regions.

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Regional characteristics

Representative basins shown in Fig. 1 are located on highlands composed of gneisses with interlayers of quartzites; the bedrock primarily contains oligoclase, quartz and biotite with <5% muscovite, orthoclase and sillimanite Gneisses are covered by colluvium with well-developed soil profiles to a depth of 120 cm. The soils are typical brown-to-acid brown earths (Food and Agricultural Organization classification: Eutric Cambisol to Distric Cambisol gleyic). Elevation, mean slopes of terrain and major hydrological characteristics of the basins are summarized in Table 2. The mean annual temperature varies from 5 to 7 °C. Two of the basins, X-0 and X-8, are covered with coniferous forest consisting mainly of spruce. The coniferous part of the

Table 1 Long-term change in the runoff of anions from Central Europe, using the Elbe river at Lovosice, Czechoslovakia[10]

Year	1892	1976	Mean annual change in water concentration
	(mmol m^{-2} yr^{-1})		(μmol l^{-1} yr^{-1})
Cl^-	47	177	10
NO_3^-	5.6	40	2.7
SO_4^{2-}	36	170	11
HCO_3^-	360	252	−4.3

forest in basin X-14 died several years before our observations started in 1975, but ~30% of the basin is still covered with deciduous trees and bushes, mainly oaks and beeches. Wheat, rye, barley, oats, maize, potatoes, clover, grass, flax and mustard seeds are rotated crops grown in basin X-7. These fields are intensively fertilized with industrial fertilizers and smaller amounts of organic matter, whereas the forested basins are not fertilized.

Basins X-0, X-7 and X-8 are located in a rural region of the Trnavka river basin (Pacov, Czechoslovakia) without local sources of industrial pollution. The mean concentration of SO_2 in air calculated from daily measurements in 1983 was 8.3 $\mu g\ m^{-3}$, with a monthly variation coefficient of 37%. The concentration of NO_x was 11.2 $\mu g\ m^{-3}$, with a variation coefficient of 31% (ref. 15). The forested basin X-14 is on the eastern slope of the Ertzgebirge Mountains facing the industrial region of the city of Most with open-pit coal mines and power plants in which local coal with a high sulphur content is burned. The 3-yr mean concentration of SO_2 in air was 112 $\mu g\ m^{-3}$ with a variation of 52%, whereas the mean concentration of NO_x was 33 $\mu g\ m^{-3}$ with a summer and winter half-year variation coefficient of 38% (ref. 15).

I have grouped the representative basins in three pairs to illustrate the influence of various factors on their elemental budgets, the first basin of each pair serving as a reference, the second showing the influence of a given factor. The effect of mean slope in forest is demonstrated by the pair X-0 and X-8; both basins are located in the rural region and have slopes of 3.8 and 13%, respectively. The effect of acid industrial emissions in forests is shown by the pair X-8 and X-14 and the effect of modern agriculture by the pair X-0 and X-7. The comparison is based on annual arithmetic means for observation period 1976–82 (7 hydrological years) in basins X-0, X-8 and X-7 and for observation period 1978–82 (5 hydrological years) in basin X-14.

Input and output mechanisms

Atmospheric precipitations were measured at 10 stations by international meteorological methods. Samples for chemical analysis were collected 1.3 m above ground, rainwater through glass funnels in polyethylene bottles and snow directly in polyethylene buckets. The diameters of the collectors were 25 and 30 cm. The containers were exposed only during individual precipitation episodes to determine the composition of wet precipitation. A similar set of collectors was exposed permanently for approximate estimation of total deposition. Samples were stored in a refrigerator immediately after each precipitation epsiode and analysed once a month. They were not filtered or acidified before chemical analaysis[16,17].

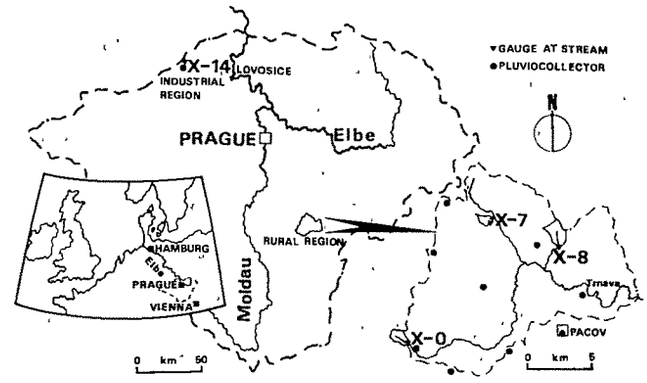


Fig. 1 Location of representative basins in the watershed of the Elbe river. Numbers and symbols correspond to the system established by the Geological Survey of Prague.

Runoff water from the basins was measured continuously at gauge stations in stream channels. The subsurface discharge was found to be negligible by a detailed hydrological and geophysical investigation[18]. Samples for chemical analysis were collected once a month, but more frequently during extreme hydrological episodes such as spring thaw and summer rainstorms. This frequency of sampling was found to be satisfactory because the variation in electric conductivity of the set of collected samples was not statistically different from the variation in the conductivity measured in runoff every day. The samples were filtered through a 0.45-μm filter within 1 day of collection. In the field, water was filtered through a 0.1-μm filter for determination of dissolved Al. This method of sampling gives reproducible results because all sampled streams are very low in concentration of organic matter[30]. Blank samples of deionized water were prepared and analysed by the same methods to check for possible contamination during sampling and analytical procedures. The *p*H of water was measured potentiometrically in the field; the flux of dissolved elements in water was calculated by multiplying the discharge of water between two sampling intervals and the arithmetic mean of the concentrations.

Biological output from the forested basins was determined from detailed records of biomass production in individual plots of the forests and chemical analyses of randomly selected trees. Mean annual production of biomass was multiplied by the mean concentration of elements in fresh wood to estimate the output of biologically fixed elements in basins X-0 and X-8. No data on biological fixation are available for basin X-14 because of

Table 2 Major characteristics of the representative basins in the watershed of the Elbe river

No. of basin	X-0	X-8	X-7	X-14
Locality	Hartvikov	Salacova Lhota	Vocadlo at Velky Jezov	Vysoka Pec at Most
Region	Ceskomoravska vysocina highland			Krusne hory (Ertzgebirge) Mountains
Type of region	Rural	Rural	Rural	Industrial
Type of basin	Forest	Forest	Field	Damaged forest
Drainage area (km^2)	0.98	1.68	0.59	2.7
Forested area (%)	100	100	1.3	30
Arable area (%)	0	0	98.7	0
Clearcut area (%)	0	0	0	70
Elevation above sea level				
Maximum (m)	724	744	635	924
Minimum (m)	672	557	570	335
Mean slope (%)	3.8	13.3	4.9	18.0
Annual precipitations (mm)	781	685	736	812
Annual discharge (mm)	108	128	171	431
*p*H of precipitations	4.27	4.27	4.27	4.19
*p*H of runoff	6.84	7.14	6.15	4.90

Table 3 Precipitation, P_i, and runoff, R_i, in representative basins

No of basin	X-0		X-8		X-7		X-14	
Type of region	Rural		Rural		Rural		Industrial	
Type of basin	Forest		Forest		Field		Damaged forest	
Type of flux	P_i	R_i	P_i	R_i	P_i	R_i	P_i	R_i
H^+	0 42	0.000	0.37	0.000	0.40	0.001	0.46	0.054
Na^+	0.79	6 6	0.69	7.0	0.75	17	2.2	22.5
K^+	0 54	1.5	0.46	1.9	0.51	7.4	1 7	6 8
Mg^{2+}	0 38	2.6	0.33	3.8	0.36	16	1 1	26
Ca^{2+}	3.4	5.9	2.9	9 9	3 2	49	7 4	74
Fe^{2+}	0.16	0 18	0.14	0.19	0 15	0.33	0.15	0.20
Al	0 79*	0.22	0.68*	0 11	0.74*	0.06	1 0*	3.2
Si	0.7	14 4	0.7	15 2	0 7	15.0	0.7	33
Cl^-	2 4	3.2	2.0	4 3	2.2	43	5.1	16
$N\text{-}NO_3^-$	4.3	0 56	3.7	0 58	4 0	19	5.5	12
$N\text{-}NH_4^+$	5 6	0 00	4.9	0 00	5.3	0.00	7 5	0.00
$S\text{-}SO_4^{2-}$	12 2	8.0	10.5	9.0	12	28	19.6	96
$P\text{-}P_2PO_4^-$	0 09	0 025	0.075	0.024	0 08	0.023	0 08	0.042
$C\text{-}HCO_3^-$	0.0	2.9	0.0	6.3	0.0	5.7	0.0	0 0

The values are mean annual fluxes in kg ha^{-1} yr^{-1}; means represent 7 hydrological years in basins X-0, X-8 and X-7 and 5 hydrological years in basin X-14.

* The high atmospheric input of aqueous Al probably results from the hydrolysis of dust in the atmosphere. This hydrolysis buffers the pH of rain before it falls in the basins. This is reflected by the lower values of the precipitation flux of hydrogen ions, P_{H^+}.

dieback of 70% of the tree population. I estimate the biological fixation in basin X-14 to be about one-third of the fixation in basin X-8. The data on biological fixation represent only the long-term output resulting from lumbering. I did not obtain reliable data on the net accumulation of biomass in the basins, therefore, I assume that the biomass remaining in the basins is recycled and that the net annual accumulation is approximately equal to the annual output by lumbering.

Anthropogenic input in field basin X-7 includes fertilization and sowing. The quantities of the applied fertilizers and seeds were recorded and samples collected. The applied quantity multiplied by mean concentrations of elements in fertilizers and seeds was summed over individual hydrological years to represent annual anthropogenic input. Biological output from basin X-7 was determined by recording the harvested amounts of crops and analysing samples from individual fields. Grains and straw, tubers and haulm were analysed separately. Water, rocks and soils were analysed in our laboratory by atomic absorption spectroscopy for Na, K, Mg, Ca and Fe and atomic absorption flameless spectroscopy for Al and photometry for Si. NH_4^+ and Cl^- ions were determined by ion-sensitive electrodes and NO_3^- and SO_4^{2-} by standard methods of wet analysis[19]. Organic samples were analysed by a specialized agricultural laboratory (District Agricultural Testing and Control Station, Brno). Precision of the analyses of water was tested against international standards[20] with good results. The precision of the chemical analyses of organic samples and fertilizers was not reported. The accuracy of the analyses, however, is probably greater than my determination of the mass of organic matter entering and leaving the basins. The accuracy is 7% for precipitation and runoff water analyses and 30% for the anthropogenic input and biological output measurements.

Budgets of elements

The fluxes of elements resulting from wet precipitation and runoff from the basins are summarized in Table 3, anthropogenic input and biological output are given in Table 4.

Inputs, outputs and accumulation affected by acidification but which cannot be easily measured in field include: (1) input of elements by total (that is, chemical plus mechanical) weathering of bedrock; (2) output by chemical erosion, (3) output by mechanical erosion; (4) accumulation of sulphur in regolith; and (5) production and consumption of hydrogen ions (proton balance). These fluxes are estimated with a help of various mass-balance models.

Rate of total weathering of bedrock, rates of chemical and mechanical erosion and related inputs and outputs of elements are calculated using a steady-state model of weathering[10,21] (assuming that Na and Si in regolith are in a steady state). The annual acccumulation or depletion of these elements in weathered zone is negligible compared with their annual inputs and outputs so that the amounts in regolith of the basins are approximately constant with time. In such a case, the input of an element i resulting from total weathering of bedrock, W_i (kg per hectare (ha) per yr), can be calculated from a steady-state mass-balance equation

$$W_i = -(P_i - R_i + A_i - B_i - M_i) \quad (1)$$

where P_i is input of chemical element i by atmospheric precipitation, R_i is output of element i by runoff from drainage basin, A_i is anthropogenic input of element i, B_i is output of biologically fixed element i and M_i is output of element i by mechanical erosion of weathered material.

Two equations (1) written for i = Na and Si contain four unknown values: W_{Na}, W_{Si}, M_{Na} and M_{Si}. These values are related to a rate of total weathering of bedrock, W_{rock}, and to mechanical erosion of regolith, M_{rglth}, by the following equations

$$W_i = W_{rock}\, x_{i,rock} \quad (2)$$

$$M_i = M_{rglth}\, x_{i,rglth} \quad (3)$$

Table 4 Biological output, B_i, and anthropogenic input, A_i, in representative basins

No. of basin	X-0	X-8	X-7	
Type of region	Rural	Rural	Rural	
Type of basin	Forest	Forest	Field	
Type of flux	B_i	B_i	A_i	B_i
Na^+	0.24	0.28	2.3	0.5
K^+	3.4	3.9	84.9	110
Mg^{2+}	0.95	1 1	3.5	7 4
Ca^{2+}	7.6	9 4	64.4	23 5
Fe^{2+}	0 26	0.30		
Cl^-	0.6	0.6	45.9	2
$S\text{-}SO_4^{2-}$	0.56	0 61	18.3	2
$N\text{-}NH_4^+$			57.6	
$N\text{-}NO_3^-$			51.0	
N	7.6	8.7		106
$P\text{-}H_2PO_4^-$	0.84	0 96	36.2	15

Values are mean annual fluxes, kg ha^{-1} yr^{-1}.

where $x_{i,rock}$ and $x_{i,rglth}$ are dimensionless concentrations ($g\ g^{-1}$) of element i in bedrock and its regolith respectively. Including W_{rock} and M_{rglth}, there are six unknown and six independent equations (1), (2) and (3) written for i = Na and Si. This system of equations is solved to yield the rate of mechanical erosion of regolith

$$M_{rglth} = \{[(P_{Na} - R_{Na} + A_{Na} - B_{Na})/x_{Na,rock}] - [(P_{Si} - R_{Si} + A_{Si} - B_{Si})/x_{Si,rock}]\}/[(x_{Na,rglth}/x_{Na,rock}) - (x_{Si,rglth}/x_{Si,rock})] \quad (4)$$

and mechanical erosion of any element, M_i, according to equation (3). Substituting the calculated values of mechanical erosion of Na and Si, M_{Na} and M_{Si} in equation (1), we obtain the rate of the input of the elements resulting from total weathering of bedrock, W_{Na} and W_{Si}. Finally, either of the last-mentioned values substituted in equation (2) yields the rate of total weathering of bedrock, W_{rock} (Table 5).

The rate of chemical erosion of weathered bedrock, C_{rock}, is calculated from equation (5)

$$C_{rock} = -\sum_i (P_i - R_i + A_i - B_i)\ m_{oxide}/n.m_i \quad (5)$$

where $\sum$ includes all elements composing bedrock except O, m_{oxide} is the relative molecular mass of the respective oxide, n is the stoichiometric coefficient of the cation in the oxide (for example, $n = 2$ for Na_2O) and m_i is atomic mass of the cation i. The flux resulting from chemical erosion of weathered bedrock includes direct dissolution of bedrock (that is, chemical weathering) and desorption of exchangeable ions from soil. Mechanical erosion of bedrock is defined by equation

$$M_{rock} = W_{rock} - C_{rock} \quad (6)$$

Note that the rate of mechanical erosion of bedrock, M_{rock}, is different from the mechanical erosion of regolith, M_{rglth}, calculated from equation (4), because weathered material contains components not only derived from bedrock but also others, such as water molecules and organic matter. The calculated rates of chemical and mechanical erosion of gneisses in the basins examined here are summarized in Table 5. The effect of various factors on weathering and erosion are expressed by ratios of the characteristic rates for the individual basins

Accumulation of S in regolith of the basins, which is an important process of environmental acidification, is expressed by a mass-balance equation

$$P_S - R_S + A_S - B_S + W_S + D_S + G_S = \sum_S \quad (7)$$

where $\sum_S$ is accumulation of S in kg ha^{-1} yr^{-1} (this value contains all the uncertainties and errors inherent in the values of fluxes in equation (7)), fluxes P_S, R_S, A_S, B_S were measured in the field, W_S was calculated from the weathering rate of bedrock, W_{rock}, in Table 5 and mean concentration of S in bedrock from equation (2); D_S is the atmospheric input of S during dry periods calculated as a difference between the values measured by permanently exposed collectors and by collectors exposed only to wet precipitations; G_S is deposition of dry S-SO_2 from the atmosphere A small portion of D_S may result from the deposition of SO_2 in the permanently exposed collectors, but this is negligible compared with the deposition of SO_2 on vegetation and soil (G_S)

The deposition of dry SO_2 was estimated from the mean deposition velocity and the mean concentration of SO_2 in the local atmosphere. Deposition velocity was calculated from published estimates for snow cover and for dry and wet periods during which stomata of trees were opened and closed[22]. The deposition velocity in forested basins X-0 and X-8 of the rural region is 5.8 mm s^{-1} The mean concentration of SO_2 is 8.3 μg m^{-3} and the corresponding deposition rate of dry SO_2 is 7 6 kg ha^{-1} yr^{-1} of S. I do not have adequate data on the deposition velocity on fields in basin X-7, but assume that it is the same as the forest basins of the rural region The deposition velocity may be slightly lower in basin X-14 because of the longer duration of snow cover at the higher altitude and the thin biological cover. The velocity is estimated to be 5 mm s^{-1} and the mean concentration of SO_2 112 μg m^{-3}, with the corresponding flux 88.6 kg ha^{-1} yr^{-1} of S (only one digit of the value is significant). The fluxes of S and calculated accumulation according to equation (7) are given in Table 6 I find that the accumulation is approximately the same in all the basins despite the large difference in the concentration of SO_2 in the air of the rural and industrial regions.

Table 5 Rates of total weathering, chemical erosion and mechanical erosion of gneisses

No of basin	X-0	X-8	X-7	X-14
Type of region	Rural	Rural	Rural	Industrial
Type of basin	Forest	Forest	Field	Damaged forest
Total weathering, W_{rock}*	420	732	1,700	1,510
Chemical erosion, C_{rock}†	64	109	160	318
Mechanical ersosion, M_{rock}†	356	623	1,540	1,192
		Determining factors		
Ratio		X-8/X-0	X-7/X-0	X-14/X-8
Wet precipitations		0.88	0 94	1.2
Slope of terrain		3 4	1 3	1.4
Concentration of SO_2 in air		1	1	13
Fertilization		no/no	yes/no	no/no
		Response factors		
Effect of		Slope	Agriculture	Emissions
Ratio		X-8/X-0	X-7/X-0	X-14/X-8
W_{rock}		1 74	4 0	2.1
C_{rock}		1.71	2 5	2 9
M_{rock}		1.88	4 3	1.9

* Calculated according to equations (1), (2), (3) and (4) using fluxes of Na and Si in Tables 3 and 4 and Na and Si mean concentrations ($g\ g^{-1}$) in bedrock and regolith in the individual basins ($x_{Na,rock}$; $x_{Na,rglth}$; $x_{Si,rock}$, $x_{Si,rglth}$) X-0 (0.022; 0 0085, 0 30, 0 30), X-8 (0.016; 0 0070; 0 30; 0 28), X-7 (0 016, 0.0067, 0 31, 0 27), X-14 (0.02; 0 007; 0.3; 0 3)

† Calculated according to equations (5) and (6). Units of rates are kg ha^{-1} yr^{-1} of bedrock Ratios are dimensionless

Table 6 Budget of S in representative basins

No of basin	X-0	X-8	X-7	X-14
Type of region	Rural	Rural	Rural	Industrial
Type of basin	Forest	Forest	Field	Damaged forest
Wet precipitations, P_S	12 0	10 5	12.0	19 6
Runoff, $-R_S$	−8.0	−9 0	−28 0	−96.0
Anthropogenic input, A_S	0	0	13 3	0
Biological output, $-B_S$	−0 56	−0.61	−2 0	−0 3
Weathering of bedrock, W_S^*	0.70	1.2	2.9	3.8
Deposition during dry periods, DS	1 0	3 3	3 0	0.2
Deposition of gaseous SO_2, G_S	7 6	7.6	7.6	88.6
Accumulation in basins, $\Sigma(S)$	12 7	13 0	13 8	15.9

* Calculated from the rate of weathering of bedrock (W_{rock}) and mean concentrations of S in gneiss, $x_{S,rock}$ (0 0017 in X-0, X-8, X-7 and 0 0025 in X-14) acording to equation (2). It is assumed that all S from weathered bedrock dissolves and none is preserved as residual sulphides Units, kg ha^{-1} yr^{-1} sulphur.

Table 7 Hydrogen-ion production and consumption by known geochemical, biological and anthropogenic processes

No of basin Type of region Type of basin	X-0 Rural Forest	X-8 Rural Forest	X-7 Rural Field	X-14 Industrial Damaged forest
Input of H_3O^+ by wet precipitation	42 2	37.3	39.3	45.7
Output of H_3O^+ by runoff	−0.02	−0 01	−0.14	−5 4
Hydrolysis reactions involving alumina; flux calculated from P_{Al}, R_{Al}, pH of precipitation, pH of runoff and complexing constants for Al-hydroxy-complexes[29]	8 8	7 3	7.5	−13.0
Oxidation of pyrite calculated from the rate of release of S from bedrock, W_S (Table 6), according to stoichiometry $FeS_2+3.5\ O_2+H_2O=Fe^{2+}+2\ SO_4^{2-}+2H^+$	2 2	3 9	9 0	11.8
Precipitation of ferric hydroxide calculated from inputs and outputs of Fe^{2+} and the amount of Fe^{2+} released from oxidation of pyrite $Fe^{2+}+0.25\ O_2+2.5\ H_2O=Fe(OH)_3+SH^+$	2.1	3 7	8 7	11 6
Dissolution and dissociation of CO_2 according to stoichiometry $CO_2+H_2O=HCO_3^-+H^+$	23.9	52 5	47.5	0.0
Biological fixation of cations and anions (except nitrogen species) in biomass removed by harvest and lumbering, calculated from data in Table 4	51 4	59 6	232 3	(20)*
Transformation of nitrogen species according to stoichiometry $NH_4^++ROH=RNH_2+H_2O+H^+$; $NO_3^-+ROH+H^+=RNH_2+2O_2$, $NH_4^++2O_2=NO_3^-+H_2O+2H^+$ where R is organic matter	13.3	12 7	192.1	100
Atmospheric deposition of dry SO_2 and oxidation according to stoichiometry $SO_2+H_2O+0\ 5\ O_2=SO_4^{2-}+2H^+$	47.5	47 5	(50)*	554
Release of cations and anions by hydrolysis of bedrock and depletion of exchangeable cations in soil calculated from budgets of Na, K, Mg, Ca, Cl and P in Tables 3, 4	−111	−151	−411	−609
Σ ('excess' production of protons)	80	74	175	116
Deviation from electrical neutrality of aqueous inputs and outputs Σ(cations) − Σ(anions) in precpitations minus Σ(cations) − Σ(anions) in runoff calculated from meq $m^{-2}\ yr^{-1}$	6 0	12	7.9	4.3

Units, mmol $m^{-2}\ yr^{-1}$ of H^+; * uncertain values (see text)

The process of acidification is best represented by the proton balance of all fluxes and reactions which involve an exchange of H^+ such as acid rain, ion exchange, hydrolysis of minerals, biological fixation of ions, oxidation of pyrite and SO_2 in regolith and nitrification[23-25] The inputs, outputs, production and consumption of H^+ in mmol $m^{-2}\ yr^{-1}$ calculated from the measured and estimated fluxes of elements in the representative basins are shown in Table 7. The most important source of H^+ is not acid rain but the deposition and subsequent oxidation of dry SO_2, transformation of nitrogen species and biological fixation of cations. The most important sink of H^+ is hydrolysis of rock-forming minerals and ion exchange in soil. Taking into account all the known processes that involve the exchange of protons, there is an 'excess' production of H^+ in all the representative basins (Table 7). This 'excess' production is not an artefact of electrical imbalance of chemical analyses (Table 7); it must be consumed in the soil by processes that are not apparent in the considered fluxes, probably the protonation of organic active groups in soils, specific adsorption of H^+ on negative surfaces of clay minerals and sesquioxides and hydrolysis of dead organic matter. These processes, together with the depletion of exchangeable cations, gradually exhaust the buffering capacity of local soils.

Discussion

Acidification of soil and water is a dynamic process which depends on fluxes of acidifying chemicals and on geochemical and biochemical reactions involving an exchange of protons in the whole ecosystem. Harmful effects such as the die-back of trees occur when the excess production of protons lasts long enough to exhaust the buffering capacity of soils. The causes of acidification in Central Europe are natural and anthropogenic, the major natural process being the biological fixation of cations and the major anthropogenic processes the emissions of SO_2 and the application of industrial fertilizers. The direct input of H^+ by acid rain is of importance in the forests of rural region while in the field basin it is secondary to biological acidification and in the industrial region it is less important than the input of H^+ resulting from deposition and oxidation of dry SO_2. Geochemical processes such as oxidation of sulphide minerals and precipitation of ferric hydroxide are not important.

The rural region with a relatively low concentration of SO_2 in air and the industrial region with a very high concentration of SO_2 are 160 km apart. The average concentration gradient of SO_2 in air is very steep, 0.65 μg m^{-3} km^{-1}. It corresponds to the increase in H^+ production by the oxidation of adsorbed SO_2, equal to 3.2 mmol $m^{-2}\ yr^{-1}\ km^{-1}$ from the point-source of emissions. This is in contrast to a very small increase in the input of H^+ resulting from acid rain which is only ~0.06 mmol $m^{-2}\ yr^{-1}\ km^{-1}$. This gradient in the input of SO_2 correlates with the observed die-back of coniferous trees and the acceleration of weathering and erosion accompanied by the increased output of nutrients. The total weathering rate of gneisses increases by a factor of 2.1, chemical erosion by 2.9 and mechanical erosion by 1.9 in forest in the industrial region. Part of the increase is caused by the steeper slope of basin X-14 and by higher precipitation and discharge rates. The runoff of all the elements (except Fe) increases (Table 3) The decrease in pH of runoff from basin X-14 causes the alkalinity of water to fall below the detection limit so that water in the basin loses its carbonate buffering capacity. At the same time, alumina starts to behave as a buffer and consumes protons at the rate of 13 mmol $m^{-2}\ yr^{-1}$ (Table 7). This leads to a large increase in the runoff of dissolved Al from 0.11 kg $ha^{-1}\ yr^{-1}$ in basin X-8 to 3.2 kg $ha^{-1}\ yr^{-1}$ in basin X-14 (Table 3). Acidification in the forest of the industrial region is accompanied by a large increase in runoff of nitrates from 0.58 kg $ha^{-1}\ yr^{-1}$ of N in basin X-8 to 12 kg $ha^{-1}\ yr^{-1}$ in basin X-14. The increased flux of nitrates does not originate from polluted air because the mean annual concentration of NO_x in air increases three times (from 11 to 33 μg m^{-3}) while the output of nitrates increases >20 times. Therefore, the increased output reflects the decreasing denitrification capacity of damaged forest in basin X-14. This agrees well with the increased runoff of nitrates from a similar forest basin in New Hampshire which was clear cut experimentally[26].

The consumption of H^+ by ion exchange in soil and hydrolysis of bedrock is not fast enough to neutralize the acidifying inputs

in any of the forest basins described here. The excess production of 80 and 74 mmol m^{-2} yr^{-1} in the forests of basins X-0 and X-8 does not seem to have a damaging effect on trees; it does not lower runoff *p*H and does not increase the output of dissolved Al and nitrates. But the buffering capacity of local soils may soon be exhausted. It is possible that the excess hydrogen ions protonate organic active groups and that they are specifically adsorbed on clay minerals and sesquioxides, causing a reversal in surface charge of some soil particles[27]. If the specific adsorption of excess H^+ is irreversible, then the exhaustion of the buffering capacity of soils and the changes in the surface charge of soil particles may have a sudden negative effect on the productivity of the forest.

The studies reported here support the suggestion[28] that modern agriculture contributes to acidification. The major acidifying mechanism is the application of fertilizers containing the weak base NH_3 and strong acids H_2SO_4 and HNO_3. Agricultural practices in basin X-7 speed up the total weathering of gneiss by factor of four, but most of the weathered bedrock is removed by mechanical rather than chemical erosion. The increased runoff of dissolved substances is caused by fertilization; ~89% Cl, 83% S and 17% N introduced in fertilizers are leached out. On the other hand, cations are retained by plants contributing to acidification of soil and water. The *p*H of runoff from field basin X-7 decreases by ~1 *p*H unit to *p*H 6.15, compared with the runoff from its reference basin X-0. The decrease in *p*H is not enough to mobilize Al, whose flux remains low. The excess production of protons in basin X-7 is 175 mmol m^{-2} yr^{-1}, 95 mmol m^{-2} yr^{-1} more than in the reference basin X-0. Although the uncertainty in this value is large because of the less precise values of biological fixation, the general acidifying trend in the field basin is clear.

The observed differences in the fluxes between the basins representing the agricultural and industrial influence on cycling of elements are not affected greatly by different slopes of terrain. Although the ratio of slopes X-8/X-0 is 3.4, the ratio of chemical erosion is only 1.7. The ratio of slopes X-14/X-7 is 3.7 and the ratio of chemical erosion 2.0, but basin X-14 receives more atmospheric precipitations and the runoff is faster. The hydrological factor increases input and output rates of elements including H^+ and is therefore an important factor of acidification. The fast rate of flushing through basin X-14 may be the cause of the relatively small accumulation of S in spite of an extremely high input of dry SO_2. The similar accumulation rate of S in the same type of soils in the rural and industrial regions indicates that the properties of soils may be important in S accumulation.

The measured and calculated fluxes of chemical elements in the representative basins indicate that observed long-term trends in the flux of dissolved elements from the Elbe river in central Europe are caused mainly by industrial emissions and intensive application of inorganic fertilizers. The runoff from central Europe is still effectively buffered because the mean *p*H of the Elbe river is 7.1. Locally, however, the *p*H of runoff drops below the capacity of CO_2/HCO_3 buffer; here, Al is mobilized and its runoff increased. In the areas with strong input of dry SO_2 and intensive application of inorganic fertilizers, the soils are acidified even if the acidification of runoff is small. Acidification can gradually exhaust the buffering capacity of soils and change surface charge on soil particles. At an 'inflection' point, by analogy with acid–base titration, it may lead to a sudden drop in biological productivity and die-back of coniferous trees. This inflection point is not yet defined; its existence is suggested by the fact that the acidification of soils in Central Europe has proceeded for decades whereas the die-back of forests is a relatively fast phenomenon which takes only a few years.

The data described here are too preliminary to separate quantitatively the distribution of H^+ ions that are generated in excess by natural and anthropogenic acidification. Some of the ions are consumed by ion exchange causing a depletion of the exchangeable cations in soil and loss of nutrients such as Ca^{2+} and Mg^{2+}. Some H^+ ions are used up by hydrolysis of rock-forming minerals; this process increases the rate of chemical weathering and may be ecologically beneficial because it releases nutrient cations from bedrock. Some of the produced H^+ ions are consumed by organic acid groups and may be adsorbed specifically on negative surfaces of clay minerals and sesquioxides. This may reverse the charge of some active surface sites and negatively affect properties of soils. Further research is needed to clarify the distribution of excess H^+ and the mechanisms of their consumption within water–soil–rock systems.

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Three-dimensional structure of calmodulin

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The three-dimensional structure of calmodulin has been determined crystallographically at 3.0 Å resolution. The molecule consists of two globular lobes connected by a long exposed α-helix. Each lobe binds two calcium ions through helix–loop–helix domains similar to those of other calcium-binding proteins. The long helix between the lobes may be involved in interactions of calmodulin with drugs and various proteins.

CALMODULIN is one member of a family of low relative molecular mass acidic calcium-binding proteins[1]. Many of these proteins, such as parvalbumin, troponin-C, intestinal calcium-binding protein and S-100, are found only in vertebrates and, even in these animals, are restricted to a small number of tissues or cells. In addition, the function, when known, is specific and limited to one metabolic process or pathway. Calmodulin is the unique member of this protein class, as it is present in all eukaryotic cells and serves many functions[2,3]. This Ca receptor is a single 148-amino-acid polypeptide chain containing four Ca-binding sites and is highly conserved throughout evolution[3–6].

Although calmodulin exhibits significant α-helicity even in the absence of Ca, binding of this ion results in an increase in the α-helical content[7–10]. It is this Ca-induced conformational change that allows calmodulin to interact with enzymes, peptides and pharmacological agents such as the phenothiazines[2,3]. A major question is how the protein can interact specifically with so many different molecules. It is possible that the regulation of different enzymes by calmodulin is partially a function of the number of Ca ions bound. However, controversy exists regarding the order in which the four sites are filled and the discrete structural changes that accompany Ca binding.

Here we describe the structure of calmodulin at 3.0 Å resolution (Fig. 1). Calmodulin represents the fourth member of the Ca-binding protein family to be characterized crystallographically. (The other three include parvalbumin[11], intestinal Ca-binding protein[12] and troponin-C[13,14].) However, it is the first ubiquitous intracellular Ca receptor to be subjected to such scrutiny and represents the first Ca-binding protein where the gene[6,15] and protein structure can be be compared directly.

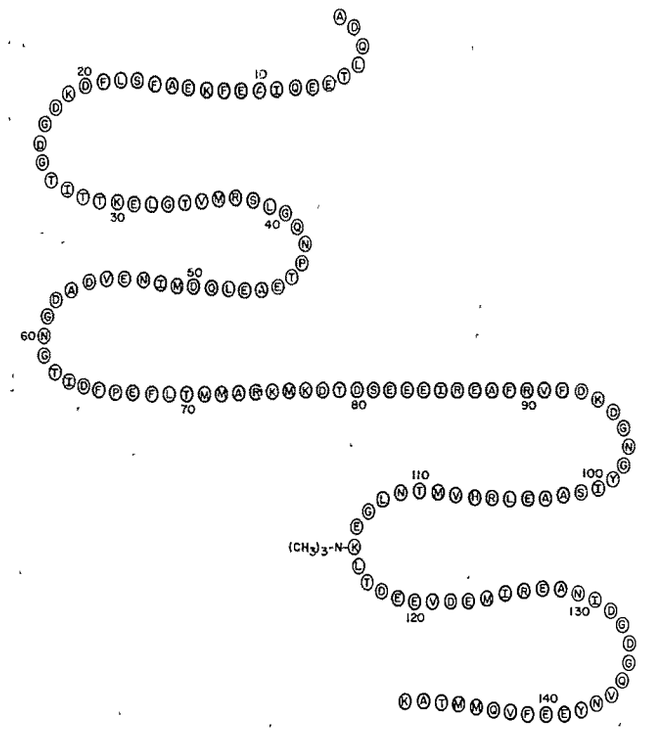


Fig. 1 Amino-acid sequence of calmodulin The sequence is arranged to show schematically the topology of the molecule. Bold circles represent residues in α-helices.

Structure determination

Calmodulin from rat testes was isolated and purified as described previously[7]. The crystallization technique was the same as described by Cook and Sack[16]; all crystals were obtained from solutions of 2-methyl-2,4-pentanediol at *p*H 5.6. The crystals belong to triclinic space group P1 with unit cell dimensions $a = 29.84$ Å, $b = 53.72$ Å, $c = 24.94$ Å, $\alpha = 93.3°$, $\beta = 97.45°$ and $\gamma = 88.77°$; there is one molecule per unit cell.

Two heavy-atom derivatives, lead acetate and potassium platinum tetrachloride, were used in the solution of the structure by the multiple isomorphous replacement method. They were obtained by soaking crystals in 75% (v/v) 2-methyl-2,4-pentanediol that contained 0.05 M cacodylate (*p*H 5.6) and the heavy-atom reagent. Many attempts to replace the Ca ions by lanthanide ions were unsuccessful.

Data for native and derivative crystals were collected with a Picker FACS-1 diffractometer at room temperature using an omega step-scan and nickel-filtered CuKα-radiation. Friedel mates were measured during data collection at negative 2θ values in groups of 10 reflections, alternating with their counterparts. Standard reflections were measured periodically and were used to correct for decomposition. Absorption corrections were applied according to the method of North *et al.*[17].

The heavy-atom positions were determined by a combination of three-dimensional isomorphous difference Patterson and difference Fourier maps. Phases for the difference Fourier maps were calculated by the method of Blow and Crick[18]. Cross difference Fourier maps were used to bring the Pb and Pt coordinates to a common origin and, in conjunction with the use of anomalous data, to establish the correct enantiomeric arrangement of heavy atoms. The heavy-atom parameters were refined by the origin-removed Patterson method of Terwilliger and Eisenberg[19]. This procedure is based on the Patterson-function correlation method of Rossmann[20], except that the origins of the Patterson function are now removed from this correlation and centric and acentric reflections are treated separately. The final model for the Pb derivative consists of seven sites; two Pb ions substitute for Ca. The Pt derivative contains three sites. Various statistics for the heavy-atom derivatives are summarized in Table 1. The protein phases and the figures-of-merit were calculated by the method of Blow and Crick[18].

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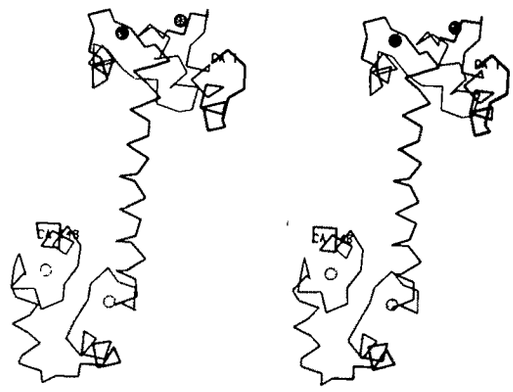


Fig. 2 Stereo drawing of the α-C backbone of calmodulin The four Ca ions are represented by dotted circles.

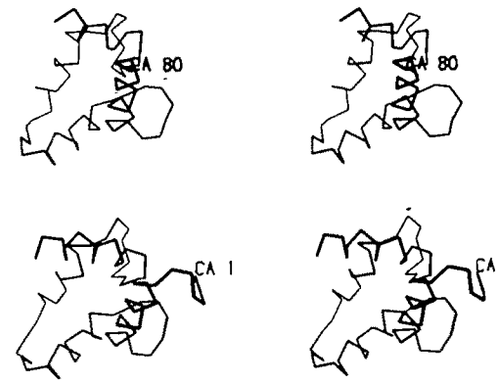


Fig. 3 Stereo drawing of the two pairs of Ca-binding domains. The molecule has been split between residues 79 and 80 and the two halves shown in approximately the same orientation

Contributions from the anomalous differences were included in the phase calculations. The overall figure-of-merit is 0.71 for the complete data set to 3 Å resolution.

An electron density map at 3 Å resolution was computed from the 'best phases' with figure-of-merit weighting factors[18]. This map clearly showed the molecular boundary and most of the secondary structural features, but the polypeptide chain could not be followed completely without ambiguities. Sequence alignment was further complicated by the high degree of internal sequence homology A modified electron density map was obtained by using a tentative chain tracing to assign a molecular boundary, flattening the solvent regions outside the molecular boundary and removing negative electron density values in the molecular boundary. This modified map was back-transformed to produce calculated phases, which were then combined with the multiple isomorphous replacement phases using the method of Bricogne[21]. A map calculated with the resultant phases resolved most of the ambiguities in chain tracing, primarily resulting from better definition of side-chain density; α-C atom positions for most of the residues were assigned from this map. A partial model was traced without residues 1–7, 75–80 and 129–148 These regions either had weak density or contained breaks in the density. The partial model was built and regularized using the computer graphics package FRODO[22]. Phases were calculated from this model and combined with the original multiple isomorphous replacement phases using the method of Bricogne[21]. The resultant map showed the protein density more clearly, allowing the model to be built completely. The four Ca positions were confirmed from a native anomalous difference Fourier map, calculated using the combined phases.

Structure description

A tracing of the α-C backbone of calmodulin is shown in Fig. 2. The molecule looks somewhat like a dumbbell with the two lobes connected by an eight-turn α-helix and no contacts between the two lobes. The length of the molecule is ~65 Å, with each lobe having approximate dimensions of 25 × 20 × 20 Å. The two Ca-binding domains in each lobe are related to each other by an approximate 2-fold axis. The two individual lobes can be brought into approximate register by a rotation of ~90° coupled with a translation. The two halves of the molecule are remarkably similar; the corresponding α-carbon positions can be superimposed with an average difference of ~1 Å (Fig. 3). The molecule contains seven helices, I–VII, consisting of residues 7–19, 29–39, 46–55, 65–92, 102–112, 119–128 and 138–148. The helix content is 63%, which is about the same as observed in troponin-C[13,14]. Calcium-binding domains 1 and 4 have a typical helix–loop–helix conformation and are similar to the two Ca-binding domains in parvalbumin[11] and to the III–IV domain in intestinal Ca-binding protein[12]. Domains 2 and 3 also display helix–loop–helix conformations, but are different from that found in parvalbumin and intestinal Ca-binding protein in that these two domains share helix IV. This long helix provides both the carboxy-terminal helix for domain 2 and the amino-terminal helix for domain 3. Helix IV is two to three times longer than any of the other helices in the molecule. Because most of helix IV is exposed to solvent and forms only a few contacts with the rest of the molecule, it is possible that this part of the molecule retains some helical structure in the absence of Ca.

The Ca-binding segments consist of residues 20–31, 56–67, 93–104 and 129–140. These four segments are similar to the two that are described in parvalbumin[11] and to the calcium-binding region III–IV in intestinal Ca-binding protein[12]. All of these contain 12 residues; the first 9 form the loop and the last 3 the beginning of the second helix in the helix–loop–helix domain. In all of these Ca-binding domains, the Ca ion is displaced from the centre of the loop towards the amino-terminal helix of the helix–loop–helix domain. The second helix of each domain orients the last residues of the Ca-binding segment so that they can participate in Ca coordination.

The molecule is stabilized by multiple interactions between the helices. In addition to these interactions, there is hydrogen bonding between adjacent Ca-binding loops in each half of the molecule. Residues 25–29 run antiparallel to residues 61–65 and these two stretches are joined by H bonds between Gly 25 and Asp 64 and between Ile 27 and Thr 62. In the second half of the molecule, the stretches 99–101 and 135–137 are also antiparallel and are joined by H bonds between Ile 100 and Val 136. Similar arrangements of the calcium-binding loops are found in parvalbumin and intestinal Ca-binding protein. Ca-binding domains in calmodulin are joined by linker segments composed of six residues; this compares with the linker segments of 8 residues in parvalbumin[11] and 10 in intestinal Ca-binding protein[12].

In each half of the molecule the two Ca ions are ~11.3 Å

Table 1 Statistics for the heavy-atom derivatives

	$Pb(C_2H_3O_2)_2$	K_2PtCl_4
Resolution (Å)	3 0	3.2
Merging R (%)	4 7	2.5
Heavy-atom concentration (mM)	0 7	0.2
Fractional change (%)	30	12
No. of heavy-atom sites	7	3
$F_H(\text{calc})/E$	2.7	1 5

$$\text{Merging } R = \frac{\sum ||F(h)| - |F(\bar{h})||}{\sum |F(h)| + |F(\bar{h})|}$$

$$\text{Fractional change} = \frac{\sum ||F(\text{PH})| - |F(\text{P})||}{\sum |F(\text{P})|}$$

where $F(P)$ and $F(PH)$ are observed structure factors for native protein and heavy-atom derivatives, respectively. $F_H(\text{calc})$ is the r m.s value for calculated structure factor contributions of heavy atoms. E is the r.m s. value for the lack-of-closure errors

apart and are coordinated to main-chain oxygen atoms and to oxygen atoms from the side chains of acidic residues. It is not clear at this stage of the analysis if there are any solvent molecules in the Ca coordination spheres. There is disagreement about the location of high- and low-affinity Ca-binding sites, but most recent studies using Ca binding and NMR seem to agree that sites 3 and 4 are the high-affinity sites[23-26]. In the Pb derivative, two of the Pb ions replace the Ca ions in domains 1 and 2. Therefore, our data are consistent with other evidence that sites 3 and 4 are the high-affinity sites.

Discussion

Calmodulin exhibits a high degree of sequence homology with parvalbumin and troponin-C[27,28], but homology with intestinal Ca-binding protein is restricted to the Ca-binding regions. Based on the structure of parvalbumin, Kretsinger and Barry[29] proposed a helix-loop-helix (or EF hand) conformation for the Ca-binding regions in troponin-C. An EF hand is a linear sequence of ~30 amino acids where two nearly perpendicular α-helices flank a 12-residue Ca-binding loop. Parvalbumin contains two such Ca-binding domains. Based on the structural features of these two domains, Tufty and Kretsinger[30] postulated that a stretch of 16 amino acids is essential for the formation of a Ca-binding EF hand. They applied this test sequence to a wide variety of proteins and generalized that all intracellular Ca-modulated proteins contain EF hands. The crystal structure determination of intestinal Ca-binding protein[12] and our results for calmodulin provide structural confirmation of this hypothesis. However, there are significant differences among the three structures, primarily in interhelical angles. A comparison of these angles is given in Table 2. The average interhelical angle in calmodulin is smaller than in parvalbumin and in intestinal Ca-binding protein. The angles in calmodulin are closest to 90°, whereas those in intestinal Ca-binding protein deviate most from 90°.

Table 2 Inter-helical angles in the Ca-binding domains

Calmodulin		Parvalbumin		Intestinal calcium-binding protein	
Helix pair	Angle (deg)	Helix pair	Angle (deg)	Helix pair	Angle (deg)
I-II	92	C-D	103	I-II	121
III-IV	96	E-F	102	III-IV	121
IV-V	97				
VI-VII	107				

The interhelical angles for parvalbumin and intestinal Ca-binding protein are taken from ref. 12.

Rat testis calmodulin contains two tyrosine residues at positions 99 and 138 that reside in the third and fourth Ca-binding domains, respectively[27]. Our studies reveal that these two residues occupy different environments. Tyr 99 is directed into the third Ca-binding loop and is fairly accessible to solvent. Tyr 138 points away from loop four into a hydrophobic pocket where it contacts Phe 89 in helix IV and Phe 141 in helix VII. Thus, although Tyr 99 forms contacts only with residues in domain 3, residues from both domains 3 and 4 interact with Tyr 138. The observed structure supports the earlier evidence that Tyr 138 occupies a hydrophobic environment[8,31-33]. The different environments of the two tyrosine residues could explain why an azido derivative of Tyr 99 can be crosslinked to many more calmodulin-binding proteins than the comparable derivative modified at Tyr 138 (ref. 34). Although Tyr 99 is in one of the Ca-binding loops, it cannot be essential for Ca binding as it is replaced in the spinach protein by phenylalanine and in the protozoan *Tetrahymena* protein by leucine[5].

Vertebrate calmodulins contain a trimethyllysine residue at position 115. Seamon[31] has suggested that this amino acid is exposed to solvent in the absence of Ca. The crystal structure reveals that trimethyllysine is in the region between helices V and VI and is directed towards the solvent with no obvious interactions with other residues in the molecule. Van Eldik *et al.*[35] have shown that trimethylation is not required for Ca-dependent interaction with phenothiazines. Putkey *et al.*[36] have reported that this post-translation modification also is not required for Ca binding, activation of several calmodulin-dependent enzymes or for Ca-induced conformational changes. These facts, together with the absence of a trimethyl group on Lys 115 in several invertebrate calmodulins[3,5], make it unlikely that this rare modification is mandatory for function.

One unusual aspect of the calmodulin structure is the long central helix (helix IV) connecting Ca-binding domains 2 and 3. This long helix seems to serve as the backbone of the protein with the two halves of the molecule attached on either end of the helix. Because this helix is shared by Ca-binding domains 2 and 3, it may have a role in the cooperative binding of Ca. Troponin-C has a similar long central helix that connects the two halves of the molecule; in the crystal structure of troponin-C, there are two Ca ions bound to the carboxy-terminal domain, whereas the cation-binding sites of the amino-terminal domain are Ca free. The amino-terminal portion of troponin-C seems to wrap around the first part of the central helix, it is possible that in a similar fashion the carboxy-terminal portion wraps around the second part of this helix when Ca is removed. Thus, the central helix in troponin-C and calmodulin may be buried in the absence of Ca and exposed when the ion is bound, which would be consistent with proteolysis studies of calmodulin. The greatest sensitivity of calmodulin to trypsin occurs among the residues in this central helix. In the presence of Ca, limited proteolysis results in one cleavage in the centre of this helix that produces two fragments 1-77 and 78-148 (refs 25, 37-39). However, in the absence of Ca the fragments produced are 1-106, 1-90 and 107-148 (refs 25, 37-39). This suggests that Ca binding exposes the residues in helix IV to proteolytic attack.

Several lines of evidence suggest that the residues in helix IV have an important role in the interaction of calmodulin with receptor proteins and drugs. The chemical modification studies of Walsh and Stevens[40] showed that carboxymethylation of methionine residues 71, 72 and 76 prevents the productive interaction of calmodulin with cyclic phosphodiesterase. Also, Newton *et al.*[41] have covalently attached a phenothiazine to the Ca^{2+}/calmodulin complex. The primary site of attachment is Lys 75, which resides in helix IV (D. L. Newton and C. B. Klee, personal communication). Crystallographic studies of calmodulin complexes with drugs and receptor proteins will be of particular interest in elucidating the role of helix IV in these interactions.

One approach that can now be used to study structure/function relationships for calmodulin is to alter appropriate regions of the molecule to see how these modifications affect enzyme binding and activation. This is feasible as the chicken calmodulin gene has been cloned[6,15] and the cDNA has been introduced into bacterial expression vectors to yield large amounts of easily isolatable protein[36]. The chicken calmodulin gene is composed of eight exons and seven introns, five of the introns are in the amino-acid coding region[15]. One intron separates the DNA that encodes amino acids 82 and 83, thus interrupting helix IV and serving to separate the two homologous halves of the protein. Of the other helices, introns split only two: helix I between amino acids 10-11, a region not involved in Ca binding, and helix VII, between amino acids 139-140. This latter intron interrupts Ca-binding site 4. The remaining two introns interrupt Ca-binding loops 2 and 3, respectively. These data reveal that three of the four Ca-binding domains are interrupted by introns and therefore do not totally support the proposal that introns primarily divide the functional domains of proteins[42,43]. The current theory suggests that introns separate regions of proteins that are on the surface of the molecule[44]. The locations of all the introns in the calmodulin gene support this latter theory.

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LETTERS TO NATURE

Low frequency variability and interstellar focusing

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The flux variations of extragalactic radio sources at low radio frequencies have proved very difficult to explain as changes in the luminosity of a simple synchrotron source. To circumvent this problem, several more complicated source models, such as those involving relativistic expansion of the source or coherent radiation mechanisms, have been devised. However, it has been suggested recently that the observed flux changes may be the result of a focusing effect of irregularities in the local interstellar medium. Here we present evidence which suggests that, in accordance with this explanation, low frequency variability is a more common feature of sources at low galactic latitudes. In the sample of radio sources monitored over a period of five years at Bologna, the mean galactic latitude of the variable sources is smaller than that of the non-variables to an extent that would occur with a chance of only 2% were the two groups drawn from the same parent distribution.

It is now firmly established that the low frequency ($\leq$1 GHz) flux density of some extragalactic radio sources varies on timescales of several months: the first conclusive evidence was provided by Hunstead[1] and there have since been several major monitoring programmes to search for variability—notably, those of Fanti *et al.*[2,3], using the Bologna telescope at 408 MHz, and Spangler and Cotton[4]. There are now more than 50 known low-frequency variable sources; most have flat radio spectra, normally associated with compact sources, and many are variable at higher frequencies.

If the flux variations are caused by changes in the source luminosity, the timescale on which they occur may be used to obtain an upper limit for the size of the source. Because the disturbance responsible for the change propagates with a speed less than that of light (c), the source diameter must be less than c (variation timescale)/$(1+z)$ where z is the redshift. However, variations in amplitude of several janskys on timescales of months in sources of moderate or high redshift require very large radiation brightness temperatures, well in excess of those that can be obtained from an incoherent synchrotron source[5]. Recently, however, Rickett *et al.*[6] have suggested that the variations are, at least in part, not intrinsic to the source, but the result of a refractive focusing effect of the general interstellar medium (A similar suggestion was made earlier by Shapirovskaya[7], in which the focusing irregularities were concentrated in galactic loop structures)

Small-scale irregularities in the density of interstellar electrons scatter waves into an angular spectrum of radius θ_{sc} and, for long wavelengths λ (typically $\geq$10 cm), interference among its components causes 100% modulations in the intensity of a point source, on scales of $\lambda/(2\pi\theta_{sc})$ in the plane normal to the direction of the source. It has been noted[8] that these modulations account for the scintillation of pulsars observed on timescales of order 10 min, at frequencies below 1 GHz. Assuming a power law spectrum of irregularities with power per unit wavenumber proportional to (wavenumber)$^{-(2+\alpha)}$, and a Kolmogorov spectrum ($\alpha = 5/3$), recent studies of pulsar scintillation[9] allow, that for a path length L through the galaxy, $\theta_{sc} = 6(\lambda\ \mathrm{m}^{-1})^{11/5}(L\ \mathrm{kpc}^{-1})^{3/5}$ marc s, which applies for a plane-wave source The distribution of scattering irregularities is often approximated by a layer of thickness $\pm$500 pc centred on the galactic plane, so that at galactic latitude b, $(L\ \mathrm{kpc}^{-1}) = 0.5\mathrm{cosec}(b)$ and $\theta_{sc} \simeq 4(\lambda\ \mathrm{m}^{-1})^{11/5}(\mathrm{cosec}\ b)^{3/5}$ marc s. These diffractive scintillations have never been observed for extragalactic radio sources[10,11], which is usually taken to mean that the angular diameters of such sources, θ_0, are sufficiently large that interference patterns from adjacent parts of the source are smeared over a distance $L\theta_0$, much larger than the scale of the patterns themselves, $\lambda/(2\pi\theta_{sc})$. The intensity modulations are thus completely levelled However, the large-scale irregularities present can also modulate the intensity by refractive focusing, which produces caustics in the wavefront from the source that are noticed as intensity fluctuations as the observer passes through them. The largest scale in the medium that

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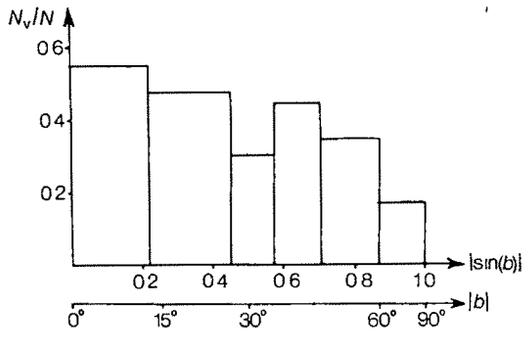


Fig. 1 The height of each bin represents the fraction (N_v/N) of sources from the Bologna sample that show fluctuations of amplitude >5% of the mean. In order of increasing $|b|$, the values for (N_v/N) are. 11/20, 9/19, 5/17, 9/20, 7/20 and 3/18.

can influence the radiation at any one point, is determined by the effective scattering disk of radius $L\theta_{sc}$ at distance L. For a power-law wavenumber spectrum, the increasing power in larger scales results in substantial modulation in the intensity of a point source on scales of $L\theta_{sc}$. The exact level of modulation depends on the power law exponent α and is particularly strong for exponents above four Nevertheless, measurements of laser scintillation in the atmosphere show that even Kolmogorov spectra give rise to 50–100% modulation[12]. Similar results are found for pulsars in the interstellar medium[6]. Compact extragalactic sources of diameter θ_0 should fluctuate as do pulsars if they are small enough ($L\theta_0 < L\theta_{sc}$). Even though this limit is much less stringent than that for diffractive scintillation, low frequency VLBI observations[13] suggest that, at metre wavelengths, most compact sources have $\theta_0 \geq \theta_{sc}$, so the refractive scintillations are at least partially quenched by smearing over a scale $L\theta_0$. The resulting modulation will be[6] $\Delta S_{rms}/S \approx \theta_{sc}/\theta_0$ on timescale $L\theta_0/2v$ where v is the relative velocity of the Earth and the scintillation pattern (10–100 km s^{-1}).

The foregoing theory makes predictions for the dependence of source variability on galactic latitude, which we have examined using the Bologna sample of Fanti *et al.*[3]. Figure 1 shows the most interesting result; the fraction of sources monitored that fluctuate with amplitude >5% of the mean is shown for each of six galactic latitude bins as a function of $|\sin(b)|$. The definite fall in this fraction with increasing latitude suggests that the variations are related to propagation in the interstellar medium. Because a χ^2 test takes no account of the order of the bins, it is not expected to attach great significance to this trend and, indeed, it does not. The Mann–Whitney test[14], which relies on the difference between the ranks of the variable and non-variable sources with galactic latitude, is far more appropriate. This test confirms that the variable sources have statistically lower galactic latitudes than those with steady fluxes; it yields a 2% chance that the two populations would be drawn in the manner observed from the same distribution in b. However, the possibility that this trend is caused by selection effects must be examined.

The Bologna sample is made up of several subsamples, consisting of sources chosen by known variability, flat spectra or interplanetary scintillations To produce the observed trend in a set of intrinsically variable sources by selection, at least one subsample would have to be strongly biased, either in favour of low latitudes, with an excess of variables, or in favour of high latitudes, with a deficiency of variables. Such an effect might arise if one subsample, biased towards low or high latitudes, was selected at very high or very low frequencies, respectively. One would then expect either an excess of flat spectrum sources (likely to be variable) at low galactic latitudes, or steep spectrum and probably extended sources (unlikely to vary) at high latitudes. However spectral information exists for most of the sources and spectral indices −dlog (flux)/dlog (frequency) can be found. Taking a flat spectrum source to have spectral index <0.5 in the region 0.5–5.0 GHz, there is no evidence to suggest that the flat and steep spectrum sources in the Bologna sample are differently distributed in b.

One may also restrict attention to sources in a narrow band of spectral index, across which there is little change in likelihood of variability. Almost all the sources with spectral index <0.2 are variable, but in the range of spectral indices 0.2–0.5, where there is no significant trend in spectral index with $|b|$, the variable sources again lie at systematically lower galactic latitudes than the non-variables, with a chance of <1% that the two populations would be drawn from the same distribution in b. Furthermore, the significance of the trend depends strongly on the dearth of variable sources above $|b| = 60°$, which is apparent (although not highly significant) in the larger subsamples, individually Therefore, it does not seem likely that the observed trend was produced by a dependence of spectral index on $|b|$, such as may arise through compiling the whole Bologna sample from its components. We cannot, of course, eliminate the possibility that unknown systematic effects are responsible for the presence of the trend within the subsamples.

Although the exact form that the focusing mechanism predicts for Fig. 1 depends on the source model assumed and the (unknown) distribution of angular sizes on milliarcsecond scales, the observed trend is clearly in the right sense. At a given latitude, the fraction of uniform sources that fluctuate with amplitude >5% of the mean is, from the above expression for $\Delta S_{rms}/S$, just that with angular size $\theta_0 < 20\,\theta_{sc}$, which will increase with θ_{sc} and hence with cosec(b). However, the sources will probably be inhomogeneous and, if at some $|b|$ θ_{sc} becomes sufficiently large that the scintillating components of most compact sources fluctuate with amplitude >5% of the total flux, then one would expect no further increase in the fraction of variable sources at lower galactic latitudes. This may account for the absence of a marked increase in the probability of variability at low b, where θ_{sc} increases most dramatically.

Using published data for the variable sources in the Bologna sample[15], we have looked for relations between galactic latitude and the amplitude and timescales of variation. None of the expected trends are apparent in these data, which may be because the published parameters describe individual outbursts rather than r.m s. variations. However, wide ranges in the fraction of flux coming from the compact component, in angular size θ_0 and in velocity of the Earth through the scintillation pattern, could also mask these effects. When metre wavelength VLBI data become available, the flux and diameter of a compact component may be used to explore further the relationships between amplitude, timescale, diameter and galactic latitude.

Although Fig. 1 suggests that the focusing mechanism is responsible for low frequency variations in many sources, there remains the possibility that some variations are intrinsic. In this context we note that, of six sources recently monitored[16], five varied almost simultaneously over a range of frequencies between 300 and 1,400 MHz, as the focusing mechanism predicts. One, however, exhibited brightening, with delays at lower frequencies, of the kind often associated with outbursts in a synchrotron source; this source may well be intrinsically variable.

Any source of observed diameter of the order of 10 marc s at a few hundred MHz that shows angular broadening, diminishing in diameter at higher frequencies, has $\theta_0 \leq \theta_{sc}$, and should undergo refractive scintillations with 50–100% modulation as do pulsars, on timescales comparable with other extragalactic sources. Such sources are important candidates for monitoring. It has been noted[17] that at very low galactic latitudes ($\lesssim 5°$), particularly towards the galactic centre, broadening is rather greater than is expected by extrapolating from that at high latitudes using the cosec(b) relation for L. Small-diameter sources seen along such paths should also fluctuate at centimetre wavelengths and it is plausible that several of the variable low-latitude sources, reported by Ryle *et al.*[18] and by Taylor

and Gregory[19], are extragalactic sources whose flux is modulated by refractive scintillation.

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Fermionic and bosonic interference during spin rotation through 2π radians

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Spinor wavefunctions change their sign under 2π rotation, an effect observable in interference experiments[1,2]. Several experiments have used magnetic precession of neutrons to implement rotations[3–7]. Other investigators have observed phenomena in molecular, nuclear or atomic systems with integral spin that have been identified as interference effects produced by 2π rotation[8–11]. I describe here a distinction between experiments on spin-1/2 neutrons and those on bosons, one that is crucial to interpreting these experimental results. Following recent work on interpreting magnetic precession as rotation[12,13], I show how to resolve Zeilinger's thought experiment[12], which actually measures the spin direction of two partial beams, with Byrne's point[13] that the recombined beam of neutrons has been rotated only π rad when the relative rotation between two partial beams is 2π. The essential distinguishing step lies in recognizing the lack of geometric significance, in general, of the mathematical parameter that equals 2π in the boson case.

When one transforms spin-1 states by true spatial rotation, the smallest covering angle (the first angle that restores original behaviour to a continuously rotated two-state subsystem) is π rad, not 2π rad. The observed sign change is nothing more than the (-1) of complete mirror reflection; such a reflection within a plane is exactly equivalent to a π rotation in three-dimensional space. This touches on the central problem of interpretation 'Is there any dynamic interaction that we may interpret as a pure rotation?'[14].

Although static magnetic fields generally cause additional effects, a uniform magnetic field that turns on after the particle enters does implement a pure spin rotation Here I suggest a new symmetrical neutron interference experiment, which, with static fields, clarifies why the spin of the recombined beam has rotated only π rad; with time-dependent fields, this configuration provides a feasible implementation of pure rotation. Indeed, the question posed above is addressed in the context of broader issues in the interpretation of quantum theory at the end of this letter. I begin by considering the relationship between rotations and dynamic interactions.

In quantum theory, certain unitary transformations represent spatial rotations, whereas others are used to represent the time evolution of a system. A rotation through angle θ in the x direction, for example, is $R_x(\theta) = \exp(-\imath\theta J_x/\hbar)$, where J_x is the operator for the x component of angular momentum, which may be calculated from the derivative, or generator, of the rotation

$$J_x/\hbar = i\, dR_x/d\theta|_{\theta=0} \tag{1}$$

Similarly, the hamiltonian, H, is a generator of the unitary time evolution operator, $U(t)$. If $|\Psi(t)\rangle = U(t)|\Psi(0)\rangle$, then the Schroedinger equation states

$$H/\hbar = i\, dU/dt|_{t=0} \tag{2}$$

assuming that H is time independent. The complications introduced to equation (2) by time-dependent interactions are exactly analogous to finding the angular momentum as a derivative at $\theta \neq 0$.

The analogy between equations (1) and (2) underlies all of the work on boson systems. In the simplest case, one finds a two-state subsystem where the hamiltonian induces transformations from one state to the other and vice versa, with equal transition probability, A, given by

$$H/\hbar = \begin{pmatrix} 0 & A \\ A & 0 \end{pmatrix} \tag{3}$$

which has the same form as the Pauli matrix for spin $\frac{1}{2}$,

$$\sigma_x/2 = J_x/\hbar = \begin{pmatrix} 0 & \frac{1}{2} \\ \frac{1}{2} & 0 \end{pmatrix} \tag{4}$$

Indeed, just as the general rotation

$$R_x^{1/2}(\theta) = \begin{pmatrix} \cos(\theta/2) & -i\sin(\theta/2) \\ -i\sin(\theta/2) & \cos(\theta/2) \end{pmatrix} \tag{5}$$

reduces to a phase factor (-1) when $\theta = 2\pi$, so a suitable evolution time for the hamiltonian in equation (3) also produces such a sign change. It is essentially this sign change that is measured by all boson experiments. The analogy between time evolution and rotation is the essence of the work on integral spin systems.

On the other hand, the idea of the neutron precession experiment relies on identifying the hamiltonian with the angular momentum. Indeed, to produce a true rotation, the hamiltonian must be proportional to the actual angular momentum of the system and not just to an analogue of a spin-$\frac{1}{2}$ operator[2,14]. This proportionality to angular momentum has double significance for interpreting the experiments: (1) the correspondence principle teaches that when energy is proportional to angular momentum, the interaction exerts a torque on the system, thereby causing it to rotate; (2) the parameter in equation (5) corresponds (as in the *gedanken* Stern–Gerlach experiments of ref. 12, or in its more feasible experiments using polarized neutron birefringence) to a measurable angle. By contrast, an actual 2π rotation of the analogous boson two-state subsystem produces no change of sign; the measurable rotation angle that results in sign change is π rad instead. This can be seen by considering the spin-1 example.

For a system of spin 1, with the usual basis set $\{|+1\rangle, |0\rangle, |-1\rangle\}$, labelled by $J_z/\hbar$, the generator of a z-axis rotation is diagonal. But transforming to a basis of $|x\rangle = 1/\sqrt{2}(|+1\rangle + |-1\rangle)$, $|y\rangle = 1/\sqrt{2}(|+1\rangle - |-1\rangle)$ and $|z\rangle = |0\rangle$, we find

$$J_z/\hbar = \begin{pmatrix} 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \tag{6}$$

which clearly has a two-dimensional subspace (spanned by $\{|x\rangle, |y\rangle\}$) where it takes the off-diagonal form required by equation (3). Unlike the spin-$\frac{1}{2}$ generator (equation (4)),

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however, the non-zero elements are 1, not $\frac{1}{2}$. This difference makes the physical rotation, restricted to the $\{|x\rangle, |y\rangle\}$ subspace,

$$R_z^{1,\text{restricted}}(\theta) = \begin{pmatrix} \cos\theta & -i\sin\theta \\ -i\sin\theta & \cos\theta \end{pmatrix} \quad (7)$$

in clear distinction to the half-angle dependence in equation (5). Here it is obvious that the physical π rotation reduces to a sign change. Indeed, just as the states $|+1\rangle$ and $|-1\rangle$ correspond to spin fully up and down along the z axis, so the states $|x\rangle$ and $|y\rangle$ represent spin along the x and y axes, respectively. These act like well-behaved vectors in all respects, including complete return (no sign change) under 2π rotation and inversion when rotated by π rad about an axis perpendicular to their plane. Note that under $\pi/2$ rotation $|x\rangle$ becomes $-i|y\rangle$ and $|y\rangle$ becomes $-i|x\rangle$.

This last fact is probably the source of confusion in over-emphasizing the spinor analogy, for it is a π rotation that converts a spinor into its orthogonal complement. If the experiment (or, more exactly, its interpretation) identifies this conversion of $|x\rangle$ into $|y\rangle$ and vice versa with a spinorial π rotation, the analogy of doubling the physical angle is established. As a result, the first return to the initial states is analogous to a spinorial 2π rotation. Equation (7) shows this 'covering' return is actually a π rotation in physical space.

For non-scalar particles travelling at the speed of light, only two angular momentum states exist, which, in the case of photons, are the two distinct linear polarizations (and/or combinations thereof). If the direction of travel is the z axis, then the polarization of a single photon is a complex unit two vector in the x-y plane, essentially the direction and phase of the electric field vector of the corresponding electromagnetic wave. The physical rotation through angle ϕ about $\hat{z}$ (the caret denotes a real unit vector) is

$$R_z(\phi) = \begin{pmatrix} \cos\phi & -i\sin\phi \\ -i\sin\phi & \cos\phi \end{pmatrix}$$

in a basis where

$$\hat{x} = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \; i\hat{y} = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

Once again we see the full-angle dependence.

The space of all distinct polarization vectors, $\binom{a}{b}$, with $|a|^2 + |b|^2 = 1$, can be parametrized by points on a unit sphere in a fictitious three-dimensional space, where rotations are spinorial by definition. One can introduce angles ξ,η, exactly analogous to the usual θ,ϕ angles on a sphere, but the north and south poles of the ξ,η sphere are the spinor basis states, $\hat{x}$ and $i\hat{y}$. Orthogonal directions in space are thus represented by polar opposite points in spinor (ξ,η) space and the spinorial angle between states is twice the angle in physical space. Again, the 2π rotation in a fictitious spinor space is actually a π rotation in real space. This fictitious ('Faraday') space, with its rotation analogies, is of great use in understanding and picturing the relations among states of polarization, but its transformations are not physical space rotations[15].

The distinction between fermions and bosons can be extended to higher spins. For all fermions, true 2π rotations produce a sign change, whereas for bosons they do not. The smallest rotation angle that produces a sign change in a two-state subspace of spin J is π/J. For fermion spin greater than $\frac{1}{2}$ this is an odd submultiple of 2π, which means that a 2π rotation may be composed of these smaller rotations, by multiplying them together. Then the (-1) factor appears raised to an odd-integer power; the full 2π rotation, therefore, also changes sign. For bosons π/J is an integral submultiple of π and a 2π rotation therefore produces the factor $(-1)^{2J}$, that is, no sign change.

In general, taking m (the angular momentum component along the rotation axis) as a label, we have $R(\theta)|m\rangle = \exp(-im\theta)|m\rangle$. The value of m ranges from $-J$ to $+J$ in steps of one unit, where J is the total spin (half-integral for fermions, integral for bosons). Because this includes the values $m = \pm 1$ for non-scalar bosons and $m = \pm\frac{1}{2}$ for fermions, the smallest angle producing a sign change of the entire space of states is always π for bosons and 2π for fermions.

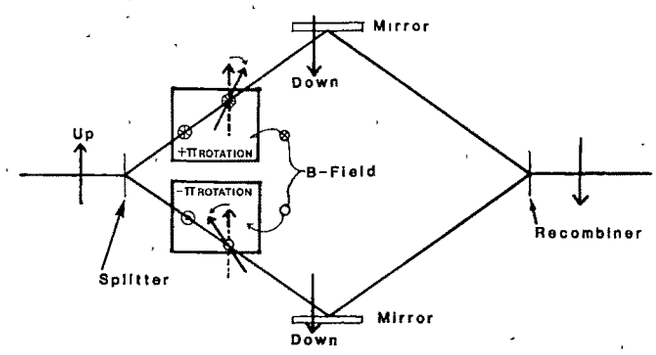


Fig. 1 A symmetric magnetic precession experiment (schematic).

In the highest and lowest m $(= \pm J)$, choosing the basis $|+\rangle = 1/\sqrt{2}(|J\rangle + |-J\rangle)$ and $|-\rangle = 1/\sqrt{2}(|J\rangle - |-J\rangle)$ makes the rotation matrix

$$R_z^{J,\text{restricted}}(\theta) = \begin{pmatrix} \cos(J\theta) & -i\sin(J\theta) \\ -i\sin(J\theta) & \cos(J\theta) \end{pmatrix} \quad (8)$$

from which it follows that sign change first occurs for $\theta = \pi/J$, as stated above. This is particularly important for the case of a particle travelling at the speed of light, because its two polarization states are precisely $|+J\rangle$ and $|-J\rangle$, hence $|+\rangle$ and $|-\rangle$ are a natural basis for the rotations. The cases of $J = \frac{1}{2}$ and 1, corresponding to neutrino and photon respectively, fit formulas given above. The one other massless entity in modern physics, that is the 'graviton', has spin 2. Pictures (p. 230 of ref. 16) of acceleration for linearly polarized gravitational waves clearly show the rotation effect described above: the value of J for bosons denotes a J-fold symmetry of the transverse oscillations in the case of light-like radiation.

What of the interpretation of the neutron spin precession as rotation? Zeilinger's clever thought experiment[12] actually measures the spin direction of two partial beams, providing a physical demonstration of the rotation, whereas Byrne[13] points out that the recombined beam has been rotated by only π rad when the relative rotation between partial beams is 2π.

That both authors are correct is directly demonstrated by a 2π-rotation experiment performed symmetrically, with a positive π rotation along one path and a negative (or opposite-sense) π rotation along the other path. In some cases, this symmetrical rotation scheme could help eliminate possibilities for determining along which path the neutron travelled, for example, by using a common source for the two fields and arranging that any magnetic energy exchange be the same for both possible paths. As is well known in quantum theory, if one can tell which path was taken, the phase coherence between the paths is destroyed and the interference is not observable. If the 2π rotation experiment were done asymmetrically, the passage of a neutron dipole moment through the magnetic field along one path might conceivably produce detectable back reaction, signalling which path it took. Almost certainly there would be some additional phase shift, beyond the expected sign change resulting from 2π rotation, because of the asymmetry.

If we examine the symmetrical experiment (see Fig. 1), we immediately notice that the π rotations produce the same orientation of neutron spin, whichever path is taken. Thus, if the initial spin is 'up' (as in Fig. 1), then the spin of each partial beam will be 'down' when they are about to recombine. The recombined beam is also spin 'down'. Note that this experiment would not require a polarized initial beam because the same π rotation effect occurs with a spin 'down' particle Of course, to follow the spin by, say, Zeilinger's Stern-Gerlach apparatus would require a polarized beam.

These remarks about orientation apply equally to bosons. For example, consider a spin-1 case and let 'up' denote the state $|x\rangle$

defined above. Equation (7), with $\theta = \pi$, shows that positive π rotation transforms $|x\rangle$ to $-|x\rangle$. But so does $\theta = -\pi$; the recombined beam points in the 'down' direction, opposite 'up'. This accords with Fig. 1, but produces no relative phase between the two beams, so there is no difference in the interference pattern from the case of zero rotation An observable sign change occurs at smaller rotation, namely, $\theta = \pm\pi/2$.

The fact that the combined spin direction after a 2π relative rotation is only π rad away from the initial direction is a special case of superposition of two equal-intensity coherent spinors. In general, the spin of the superposition lies in the plane bisecting the angle between the two spin vectors. This can be seen quickly in spinor algebra. For simplicity, take the x–z plane to contain both the combining spinors and let the x axis be their angle bisector (see Fig. 2). This is just a choice of coordinate axes If we use the convention that

$$|\theta, \phi\rangle = \begin{pmatrix} \cos(\theta/2) & e^{-i\phi/2} \\ \sin(\theta/2) & e^{+i\phi/2} \end{pmatrix} \tag{9}$$

where θ,ϕ are the colatitude and azimuthal angles, respectively, we have

$$|\psi^{\text{recombined}}\rangle \propto \left(\left| \frac{\pi}{2} - \eta, 0 \right\rangle + \left| \frac{\pi}{2} + \eta, 0 \right\rangle \right)$$

$$|\psi^{\text{recombined}}\rangle \propto \cos\frac{\eta}{2} \begin{pmatrix} \frac{1}{\sqrt{2}} \\ -\frac{1}{\sqrt{2}} \end{pmatrix} = \cos\frac{\eta}{2} \left| \frac{\pi}{2}, 0 \right\rangle \tag{10}$$

If we take η as the individual rotation angle, π, in the symmetric 2π rotation experiment, then equation (10) implies a vanishing amplitude for the recombined state that reflects the perfect destructive interference attributable to the (-1) phase factor induced by one full rotation. (In the notation of Byrne's paper[13], $\beta = 2\pi$; and $A = B$, that is the parameters characterizing the intensity of the recombining beams are equal, imply the vanishing of the recombined beam itself, for no particles (ideally) would be transmitted. Byrne's equation (3) reflects this—the transmission coefficient drops to zero for these values. His point about the spin polarization is nevertheless correct; it does suffer a rotation of π rad Experimental confirmation would require taking the limits as $\beta \to 2\pi$ and seeing the polarization approximate rotation by π as closely as one likes.)

It is interesting briefly to consider the effects of a relative phase shift, α, on the recombined beams. In this case, spinor addition gives

$$|\phi^{\text{recombined}}\rangle \propto \left(e^{-i\alpha/2} \left| \frac{\pi}{2} - \eta, 0 \right\rangle + e^{+i\alpha/2} \left| \frac{\pi}{2} + \eta, 0 \right\rangle \right)$$

$$= \sqrt{\cos^2(\alpha/2)\sin^2(\eta/2) + \sin^2(\alpha/2)\cos^2(\eta/2)}$$

$$\times \left| \frac{\pi}{2}, 2\tan^{-1}(\tan(\eta/2)\tan(\alpha/2)) \right\rangle \tag{11}$$

Previous work[2,14] has discussed the fact that a static magnetic field introduces precisely such a relative phase shift, as well as the desired rotation, in an asymmetrical relative rotation experiment. This small extra phase shift arises because the static configuration conserves energy. Neutrons with spin parallel to the field increase their kinetic energy, spending less time in the field than those aligned oppositely. The resulting phase shifts are not precisely equal and opposite. That in such an experiment the rotation is not pure, would be revealed in these spin-tracking experiments by arriving at a recombined polarization that lies in the x–y plane, but not precisely along the x axis.

Note that various interactions can produce the hamiltonian of equation (3), which results in a time evolution operator

$$U(t) = \begin{pmatrix} \cos(At) & -i\sin(At) \\ -i\sin(At) & \cos(At) \end{pmatrix} \tag{12}$$

For the slightly more complicated case where A is a function of time, the product At is replaced by the integral $I(t) = \int_0^t A(\tau)\, d\tau$. Then

$$U(t) = \begin{pmatrix} \cos I(t) & -i\sin I(t) \\ -i\sin I(t) & \cos I(t) \end{pmatrix}$$

But the relationship between the product At, or $I(t)$, and a physical rotation angle (if any) depends on the angular momentum, J, of the system. H must be proportional to J to identify $U(t)$ as a rotation. The advantage of magnetic precession as a means of performing rotation lies in the well-established proportionality of the hamiltonian to the magnetic moment and hence to angular momentum. Even so, as stated above (and developed previously[12,14,17]) certain magnetic field distributions produce additional phase shifts. Thus, one must be careful of the theoretical analysis that undertakes identification of any dynamical process with a rotation. For it is well known that interactions produce phase shifts that can mimic those produced by physical rotations of either the reference system (passive rotations) or of the state-preparation devices (active rotations).

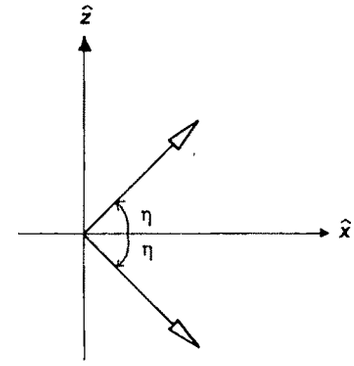


Fig. 2 Addition of spinors.

More directly, to identify a transformation as a rotation requires a physical rotation angle and preferably one that is, in principle, directly measurable. Zeilinger's analysis shows how to make such measurements during magnetic precession. The issue of such a geometric (or macroscopic) correlative of the spin, for polarized neutrons, undergoing precession has been raised in the earlier literature on the interpretation of these experiments[13,17–21]. The suggestion of using a strong static helical field (whose change of direction in space could be easily measured) was an early step in this direction[21]. Such a field entrains the moment of the polarized neutron and guides it around in space. The theory shows that in the asymmetric case such a helical static configuration also produces only approximate rotations[14,20–22]. The approximation involved is so good (often better than 1 part per 10^{12}) that it enters the discussion only at the level of interpretation Nevertheless, every time-independent magnetic field thus far examined requires these approximations in order to be interpreted as a true rotation.

By contrast, the investigation of precession in time-dependent (as opposed to space-dependent) magnetic fields[14] shows exact reproduction of the effect of rotation; that work left only one question—whether such fields could be found that also satisfy Maxwell's equations—now answered affirmatively. An infinite sheet of uniform current density, starting up from zero to a finite value and returning to zero again, produces a plane-wave electromagnetic 'pulse' whose magnetic field fulfills the requirements. The details of this configuration, with discussions of its possible implementation will be published elsewhere (H.J B., in preparation).

The deepest issue of interpretation lies in determining when, if ever, an interaction may be considered to produce a symmetry transformation. The issue is joined in noting that torques must be applied to any spinning object to rotate it. These torques imply (certainly for a quantum mechanical system) entanglement with a macroscopic system, such as the magnetic field in the neutron experiments.

Wheeler[23] terms the sign change of a spinor under 2π rotation its 'entanglement relation', referring to the model of a macroscopic object tied (by elastic strings, ribbons or a strip of material) to its surroundings. Under 2π-rotations the strings become irremediably entangled, but after 4π-rotation they may be topologically restored to the untangled state without rotating the object. This 'entanglement relation' underlies a variety of popularized demonstrations of spinor properties. Dirac was fond of twisting his belt to show it. A triangular lecture-hall model has been described[21], but perhaps the most beautiful demonstration is that of the Phillipine Binasuan dance[24]. The causal connections of interaction may be less surprising than those of correlation (as in recent implementations of the Einstein-Rosen-Podolsky 'paradox'[25]), but even if they turn out to be pictured or controlled more easily, they are no less real.

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Evidence from polar ice cores for the increase in atmospheric CO_2 in the past two centuries

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Precise and continuous measurements of atmospheric CO_2 concentration were first begun in 1958 and show a clear increase from 315 parts per million by volume (p.p.m.v.)[1] then to 345 p.p.m.v. now. A detailed knowledge of the CO_2 increase since preindustrial time is a prerequisite for understanding several aspects of the role of CO_2, such as the contribution of biomass burning to the CO_2 increase and the sensitivity of climate to the CO_2 concentration in the atmosphere. Estimates of the preindustrial CO_2 concentration are in the range 250–290 p.p.m.v. (ref. 2), but the precise level then and the time dependence of the increase to the present levels remain obscure. The most reliable assessment of the ancient atmospheric CO_2 concentration is derived from measurements of air occluded in ice cores. An ice core from Siple Station (West Antartica) that allows determination of the enclosed gas concentration with very good time resolution has recently become available. We report here measurements of this core which now allow us to trace the development of the atmospheric CO_2 from a period overlapping the Mauna Loa record back over the past two centuries.

Air bubbles are a characteristic feature of natural ice. At locations with mean air temperatures well below the freezing point, ice is formed by sintering of dry firn. At the firn–ice transition, the pore volume becomes separated as isolated bubbles having no further interaction with the atmosphere. The transition process is slow, so that the sampling of air into isolated bubbles spans at least several years, the duration depending mainly on the accumulation rate and the mean annual temperature.

Measurements were made on a 200-m ice core drilled in the Antarctic summer 1983/84 at Siple Station (75°55′ S, 83°55′ W) by the Polar Ice Coring Office in Nebraska and our institute. The mean annual air temperature is −24 °C and the annual accumulation rate is 500 kg m^{-2}. Only one clearly identifiable melt layer of irregular thickness (2–10 mm) was observed in the entire core at 7 m below surface (m.b.s.). Counting the seasonal variations of the electrical conductivity of the ice core allowed us to date the core over the past 200 yr with an accuracy of

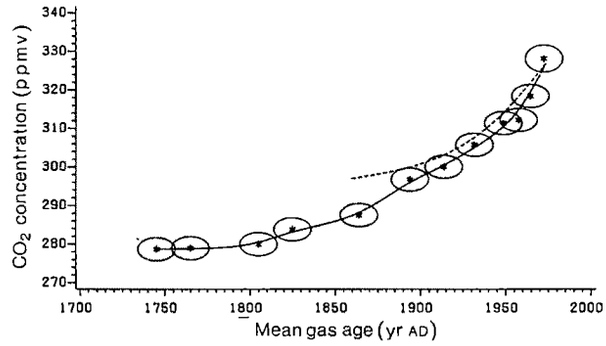


Fig. 1 Measured mean CO_2 concentration plotted against the estimated mean gas age. The horizontal axis of the ellipses indicates the close-off time interval of 22 yr. The uncertainties of the concentration measurements are twice the standard deviation of the mean value, but not lower than the precision of 1% of the measurement. The dotted line represents the model-calculated back extrapolation of the atmospheric CO_2 concentration, assuming only CO_2 input from fossil fuel[2].

Methods. After crushing the ice samples, the gas expands over a cold trap, condensing the water vapour at −80 °C into the absorption cell. Care was taken to minimize the effect of selective transport of CO_2 by water vapour. This effect was discovered while testing the system, when a difference in the measured CO_2 concentration was observed depending on whether the valve between the crusher and the cold trap was open or closed during the crushing process. With the valve closed, the concentrations were systematically higher by ~15 p.p.m.v. Crushing the ice with the valve open causes small ice particles to be transported to warm surfaces, where they immediately evaporate, leading to a considerable amount of water vapour in the system. The part of this water vapour not immediately condensed in the cold trap expands into the absorption cell and then flows slowly back into the cold trap, as indicated by the pressure monitoring in the absorption cell, which leads to a decreased CO_2 concentration in the absorption cell. When the valve is closed, the ice particles created during the crushing process are kept back in the cold crusher and less water vapour reaches the absorption cell after the valve is opened, leading to smaller deviations of the CO_2 concentration. Also, for this slightly modified measuring procedure, the measurements could be controlled by crushing pure gas-free single-crystal ice surrounded by the same amount of standard gas as contained in an average ice sample. With this procedure, small positive deviations in the CO_2 concentration, attributable to water vapour flow from the crusher into the cold trap, can be determined and the measurements corrected accordingly. This correction is below 1% for 10-cm^3 samples and below 4% for 1-cm^3 samples. All Siple samples were measured using the modified procedure and each fourth measurement was a calibration run.

Table 1 Mean CO_2 concentration in air extracted from ice samples

Depth below surface (m)	Samples measured	Ice from (yr AD)	Air enclosed (yr AD)	CO_2 concentration: Extracted air (p.p.m.v.)	CO_2 concentration: Atmosphere (p.p.m.v.)
			Siple Station		
68.2–68.6	8	1891	1962–1983	328±3.5	328
72.4–72.7	11	1883	1954–1976	318±3.0	321
76.2–76.6	11	1876	1947–1969	312±3.0	315
82.0–83.0	28	1867	1938–1960	311±3.0	
92.0–93.0	25	1850	1921–1943	306±3.0	
102.0–103.0	26	1832	1903–1925	300±3.0	
111.0–112.0	26	1812	1883–1905	297±3.0	
128.0–129.0	47	1782	1842–1864	288±3.0	
147.0–147.2	10	1743	1814–1836	284±3.0	
162.0–162.3	9	1723	1794–1819	280±3.0	
177.0–177.3	10	1683	1754–1776	279±3.0	
187.0–187.3	10	1663	1734–1756	279±3.0	
			South Pole		
	IRLS/LES			IRLS	LES
139.0–141.0	12/5	820	1660–1880	278±3.0	280±3.0
160.0–162.0	12/5	610	1450–1670	281±3.0	283±3.0
205.0–207.0	8/4	110	950–1170	279±4.0	280±3.0

LES, Large extraction system.

±2 yr (ref. 3). Based on porosity measurements, the time lag between the mean age of the gas and the age of the ice was determined to be 95 yr and the duration of the close-off process to be 22 yr (ref. 4). These values are, of course, evaluated for one particular core representing the present situation (1983), assuming a homogeneous enclosure process and not taking into account the sealing effect of observed impermeable layers.

The gases from ice samples were extracted by a dry-extraction system, in which bubbles are crushed mechanically to release the trapped gases, and then analysed for CO_2 by infrared laser absorption spectroscopy (IRLS) or by gas chromatography (GC). The combination of a needle crusher and IRLS allows us to analyse samples of ice as small as 1 g (ref. 5) (see Fig. 1 legend). During the past 2 yr, a new large dry-extraction device for ice samples up to 1 kg has been built and extensively tested[6]. The ice samples are ground *in vacuo* and the gases collected on a cold finger (20 K) and later analysed by both GC and IRLS. Applying both procedures to neighbouring ice samples gave the same concentration to within 1%. The measurements using the needle crusher, published previously[5,7,8], were performed using a slightly modified procedure and exhibited generally lower CO_2 concentrations by 15 p.p.m.v. (see Fig. 1 legend). In 1982 an intercalibration study with the Grenoble laboratory[7] was performed using the small crusher with the older measuring procedure. Based on our new results, the agreement of the intercalibration must be viewed as a discrepancy, which we will try to resolve in the near future with a new intercalibration series.

Figure 1 shows the mean CO_2 concentration of the 12 depth intervals, measured on the Siple core, plotted against the mean gas age To extend our data series further back in time, a few samples from the South Pole were also measured (and cross-checked with the large extraction system). These values are listed in Table 1.

Five depth intervals between 82 and 130 m.b.s. of the Siple core were measured with a high spatial resolution. Figure 2*a–e* shows the individual data series together with the $\delta^{18}O$ results. A seasonal structure could not be identified, indicating that the CO_2 concentration in the bubbles of the Siple core is homogeneously distributed and is independent of the seasonality of the surrounding ice.

Based on earlier studies of ice samples from locations in Greenland, we expected to measure enhanced CO_2 concentration in the extracted gas, especially in summer layers[8,9] from locations with a mean annual air temperature above −25 °C. For comparable mean gas ages, the CO_2 concentration measured on samples from the South Pole (−50 °C) and Siple Station (−24 °C) do not differ within the error limit. Therefore, we conclude that the CO_2 level in the bubbles of the Siple core is not influenced by a temperature effect. This is further supported by the lack of seasonal variations in the five data series measured with high spatial resolution.

Between 68 and 69 m.b.s., the porosity measurements showed two layers with bubble volumes above 80 $cm^3\ kg_{ice}^{-1}$, which correspond to complete enclosure. This is clearly above the 40 $cm^3\ kg_{ice}^{-1}$ expected from a smooth homogeneous enclosure process. These impermeable layers have a sealing effect if they are imbedded in firn with reduced permeability[10]. Because of the layers between 68 and 69 m.b.s., the air below is already completely isolated, about 7 m above the depth obtained, assuming a homogeneous enclosure. Consequently, for this core, the difference between ice and mean gas age is only 80–85 yr instead of 95 yr as estimated previously[4].

The enclosure of air occurs in winter layers at shallower depth than in summer layers, which could lead to a seasonal modulation of the CO_2 concentration in the bubbles during a period of changing atmospheric CO_2 concentration in case of a smooth and homogeneous enclosure process. The abovementioned lack of a seasonal variation in the series from 82 m.b.s. therefore further supports the sealing effect of such layers.

If the 328 p.p.m. measured at a depth of 68.5 m.b.s. is matched with the atmospheric South Pole record[11], the mean gas age is 10 yr, corresponding to a difference between mean gas age and ice age of 82 yr, which lies in the above estimated range. This difference is used in calculating the mean gas age for all depths. That the CO_2 concentration measured on the subsequent samples from 72.5 and 76.5 m.b.s. corresponds with the atmospheric South Pole record justifies this age determination and indicates, also, that impermeable layers at a depth of ~68 m.b.s. occur frequently.

There remains the question of the duration of the gas-enclosure time in such an impermeable layer. Assuming, as a first approximation, a constant and continuous enclosure rate ending at the time of core drilling (t_2 = AD1983), the beginning (t_1) can be calculated from the expression $(t_2 - t_1)C_{bubbles} = \int_{t_1}^{t_2} C_{atm}(t)\,dt$, where $C_{atm}(t) = a + b(t-1958) + c(t-1958)^2$ (with t in yr AD). The atmospheric concentration, C_{atm}, is taken from a quadratic fit of the measured CO_2 data in the atmosphere. The time t_1 so determined is 1963, so that the duration of the close-off is 20 yr, in good agreement with the estimate of Schwander and Stauffer[4].

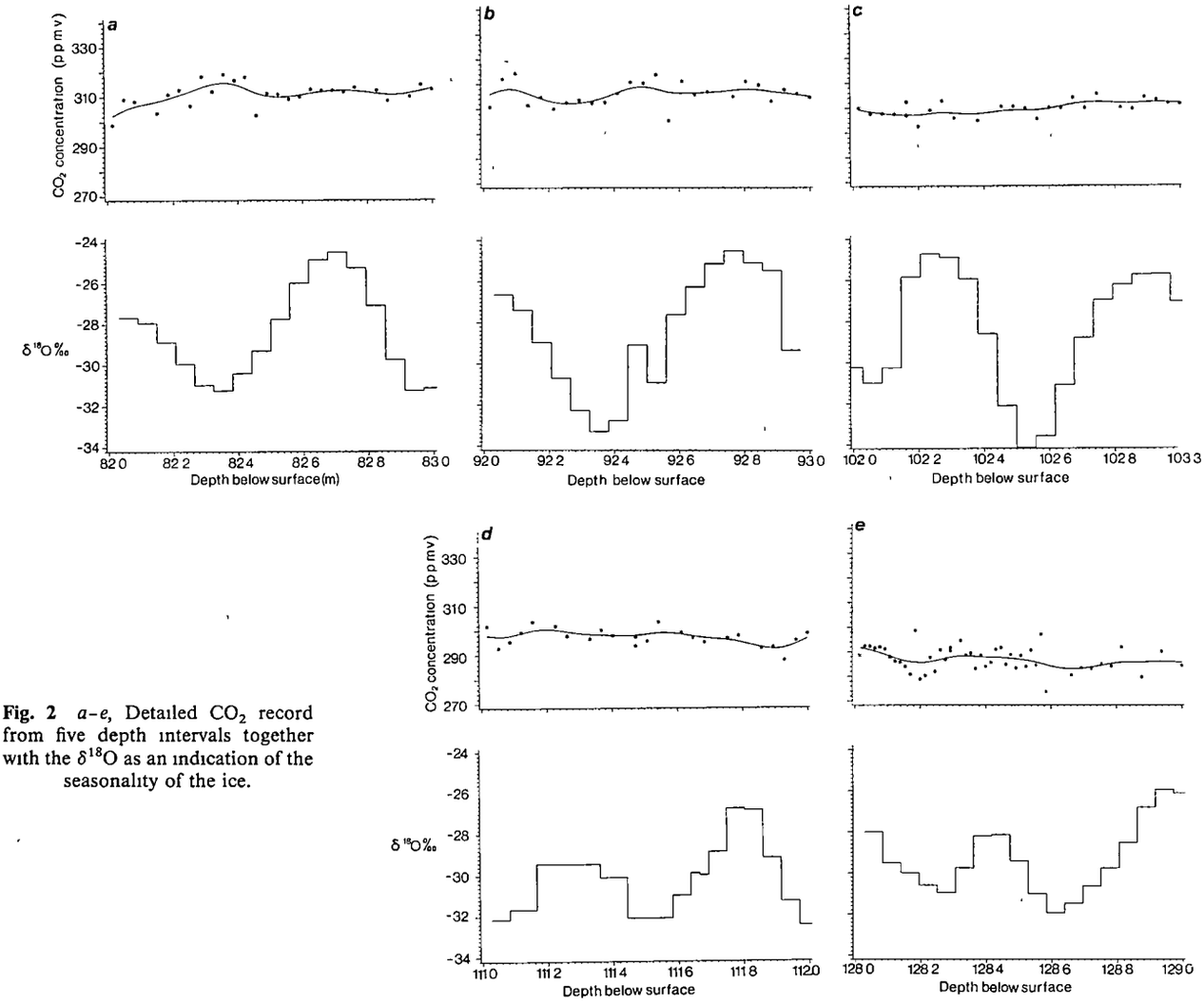


Fig. 2 *a–e*, Detailed CO_2 record from five depth intervals together with the $\delta^{18}O$ as an indication of the seasonality of the ice.

We would have more difficulties explaining values lower by 15 p.p.m.v., as obtained, for example by the open-valve method. This would require a 20–40-yr-older mean gas age in the bubbles, depending on the development of the atmospheric CO_2 concentration. A very much prolonged close-off interval, a possibility which we exclude[4], or an incomplete mixing of air in the firn would be required. Also, the atmospheric CO_2 concentration would have to be increased between 1930 and 1960 by $\sim$1 p.p.m.v. yr^{-1}, a similar rate to that of the past 20 yr.

Based on these results, we conclude that the atmospheric CO_2 concentration around 1750 was 280 ± 5 p.p.m.v. and has increased since, essentially because of human factors, by 22.5% to 345 p.p.m.v. in 1984. Back extrapolation of the Mauna Loa curve, on the basis of the fossil fuel input data obtained by Rotty[12] and assuming zero CO_2 input from biomass burning, suggests a preindustrial level of 297 p.p.m.v. (ref. 2). The difference of 17 p.p.m.v. between this calculated value and that measured indicates a significant contribution from biomass burning, even during the past century when the contribution from fossil fuel consumption was still small. The CO_2 increase in the Siple core will be studied in more detail using $\delta^{13}C$ measurements. Preliminary $\delta^{13}C$ results on an ice core from the South Pole are indeed very promising[13]. The CO_2/air $^{13}C/^{12}C$ record on the Siple ice core and the $^{14}C/^{12}C$ Suess effect from tree rings, together with the fossil fuel input-time history, will then enable us to separate the CO_2 inputs from those attributable to fossil fuels and biomass. It will also allow us to determine the effective airborne fraction for the combined input and to check the validity of the present carbon-cycle models

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Vertical structure of the Brazil Current

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Recent interest in world climate and interaction of the ocean and atmosphere have led to studies of the meridional fluxes of fresh water and heat in the ocean[1,2]. Several such studies[3,4] have noted the asymmetry of heat fluxes between the North and South Atlantic oceans. In particular, the fluxes across both 24° N and 24° S appear to be northward. Crucial to all of the direct calculation techniques is an accurate estimate of the transport of the western boundary currents. In the North Atlantic, useful measurements in the Florida Current[5] over a long period of time are available. For the much less studied South Atlantic no comparable time series has been made. The direct measurements of Brazil Current velocities near 23° S reported here show southward flow of warm water above 400 m depth and northward flow of Antarctic Intermediate Water below that. The upper layer transport was $\sim 6 \times 10^6$ m^3 s^{-1} towards the south offshore of the 200-m isobath, with an indication of comparable flow on the shelf.

The Brazil Current is the South Atlantic analogue of the Gulf Stream. For the present purposes, it is defined as the southward-directed western boundary current, extending downward from the surface, closing the circulation of the subtropical, wind-driven circulation in the South Atlantic. From a consideration of the wind-driven anticyclonic gyres, the two should be quite similar. As Stommel[6] points out, "therefore ... the wind-driven component of the circulation is much the same in the two hemispheres, but ... in the western boundary currents the thermohaline circulation plays quite different roles in the Gulf Stream and Brazil Current, making the former appear much more strongly on the charts of the topography of surfaces, equal pressure, or density". Indeed, the limited historical hydrographical observations indicate that the total transport of the Brazil Current may be no larger than 9.4×10^6 m^3 s^{-1} in the upper 600 m (refs 7, 8). However, as Stommel remarks, the wind-driven component should be as large as $30\text{–}40 \times 10^6$ m^3 s^{-1} to balance the equatorward transport in the gyre. Recent calculations based on balancing the meridional transport due to the wind stress curl[9,10] indicate that up to 30×10^6 m^3 s^{-1} should flow in the Brazil Current near 30° S and $\sim 20 \times 10^6$ m^3 s^{-1} should flow at 25° S.

There are longstanding beliefs that western boundary currents provide an essential means for meridional transport and that the Brazil Current region may be the major conduit for the exchange of at least four major water masses between the hemispheres[11]. In contrast, inverse calculations indicate that the Brazil Current may be important only south of 25° S. In fact, these calculations indicate little southern transport along the boundary north of 30° S (ref. 4). Previous work has relied on water mass boundaries or more arbitrary fixed depths to determine levels of no motion for calculation of geostrophic transport. There have been no systematic investigations of the Brazil Current including direct velocity or transport measurements.

The choice of reference level for geostrophic calculations leads to an ambiguity in even the direction of flow of some of the water masses along the boundary. Traditionally, water mass analysis suggested a layered current system with warm tropical water flowing southward over northward Antarctic Intermediate Water which is in turn above the southward-flowing North Atlantic Deep Water[12]. More recent interpretation of the data has led some investigators to argue that the wind-driven gyre penetrates deep enough to carry Intermediate Water to the south after circulating around the basin[4,13]. Resolution of the issues of transport of the Brazil Current and its vertical structure, that is, direction of flow of the various water masses, motivated the present study. The results presented below indicate that the 'Brazil Current' should be reserved for the flow shallower than ~400 m at this latitude.

A cruise aboard the *Prof. Besnard* of the Institute of Oceanography at the University of Sao Paulo during April 1982 allowed a detailed expendable bathythermograph (XBT) survey and hydrographic study between 19° S and 24° S to be performed. Essentially, the Brazil Current was found to be closely confined to the region near the continental slope. It flowed continuously past the seamount chain at 20°30′ S through the most inshore passage and onto the continental shelf. Geostrophic transports were $< 10 \times 10^6$ m^3 s^{-1}, using either 500 m or 1,000 m as a reference level[14]; they were 3.8×10^6 m^3 s^{-1} and 6.8×10^6 m^3 s^{-1} respectively.

The first direct velocity measurements of the Brazil Current were made in April 1983 aboard RV *Oceanus*. The XBT survey from 19° S to 24° S was repeated in greater detail and extended further offshore than in April 1982. The Pegasus velocity profiler[15] was deployed at two passages in the seamount chain at 20°30′ S and along five sites across the Brazil Current near Cabo Frio between 23° S and 24° S to obtain vertical profiles of horizontal velocity with an estimated accuracy of ± 2 cm s^{-1}. Figure 1 shows the survey area and location of XBT lines and Pegasus sites. Also shown is the depth of the 15 °C isotherm, the gradient of which is indicative of the strength of the baroclinic component of the flow.

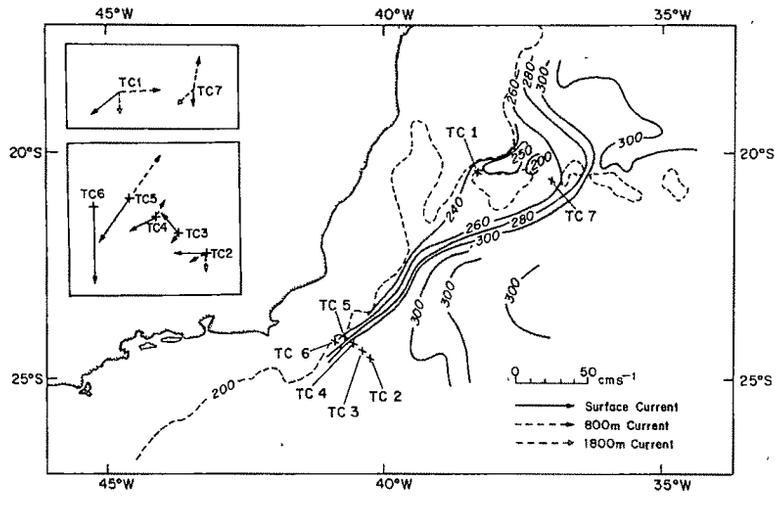


Fig. 1 Survey area for April 1983 cruise showing depth of the 15 °C isotherm and locations of Pegasus stations. Also shown are mean velocity vectors at depths characteristic of surface, intermediate and deep waters

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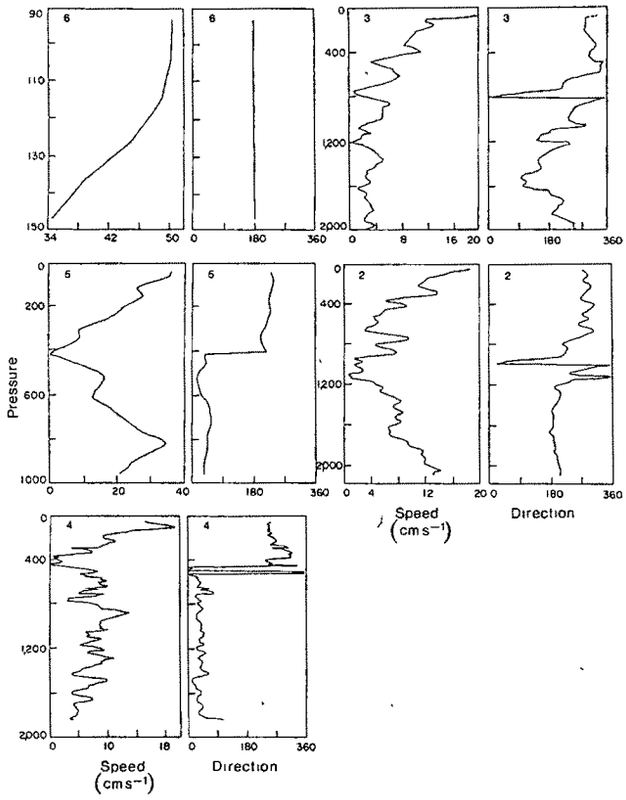


Fig. 2 Vector-averaged profiles of speed and direction at each Pegasus site. Directions are measured as degrees true towards which the current flows (Note the difference in pressure and speed scales among the frames Depths are in decibars.)

Offshore from the 2,000-m isobath and near the Abrolhos Banks, the temperature gradient field is poorly organized and there is no clear indication of baroclinic flow. Based on the temperature gradients alone, the Brazil Current seems to be flowing through the banks with weaker intensity than along the 200-m isobath near Cabo Frio, where it is confined to flow in a narrow stream along the shelf-break. This pattern is essentially the same as found in April 1982 except that there is a suggestion of flow to the east of the banks at 20°30′ S. Earlier work[7,8,14] has shown that a close relationship between temperature (T) and salinity (S) in this region permits inference of geostrophic flow from the temperature field to within an accuracy of ±10%.

Each of the Pegasus sites TC2–TC6 was visited three times during a period of 10 days. Only two measurements were made at TC1 and one at TC7. Although the profiles over the course of the cruise showed small variability, they represent only one snapshot of a field which is likely to have large variability at longer timescales. We may hope that, like the Gulf Stream, the vertical structure is relatively stable. Figure 2 displays the vector-averaged profiles of speed and direction for the section near Cabo Frio[15]. The east and north components of velocity were interpolated to common depths at approximately the original resolution using cubic splines. Vector averages of the three drops at each site were then computed. Figure 3 exhibits all three profiles from site TC5 to illustrate the relatively small variability among the drops.

Site TC2 was selected, based on the isotherm pattern from the XBT survey, to be offshore of the Brazil Current. The surface flow is <20 cm s^{-1} and is directed to the west (or north-west) down to 1,000 m. Between 1,000 and 1,200 m the velocity vector is small (<3 cm s^{-1}) and rotates through 360° with depth. Such flow may be characteristic of the subtropical gyre circulation to the east of the boundary current. However, an XBT line along 36° W failed to indicate significant zonal flow and the surface flow does not indicate an eastern source (Fig. 1). From 1,400 m to the bottom, the flow is directed almost due south. Deep southward flow in the absence of a similar surface current is

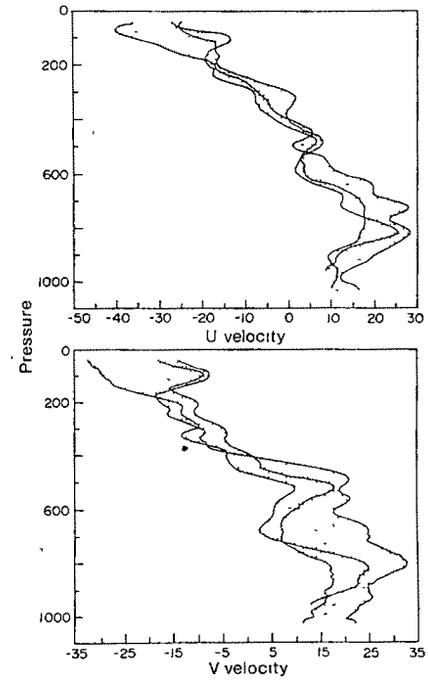


Fig. 3 East and north components of three drops at site TC5. Data were collected over 1 week

not taken to be indicative of the Brazil Current, but is likely to be part of the deep thermohaline mode. At TC3, the next station inshore, all speeds are <20 cm s^{-1}. Surface layer flow is directed to the north-west down to a depth of 500 m. Below 600 m, speeds are quite weak and directions are consequently not well determined. TC4 is the first station definitely in the Brazil Current. In the layer from the surface to 400 m, the current is directed towards the south-west at speeds up to 20 cm s^{-1}. Below 500 m, the water moves northeastward at speeds near 10 cm s^{-1}. TC5 again shows the dramatic change in direction near 400 m depth. The upper layer (traditional Brazil Current water) moves to the south-west at speeds up to 35 cm s^{-1}. Below 400 m, the flow is directed towards the north-east, also at speeds of up to 35 cm s^{-1}. (The salinity minimum, indicative of Intermediate Water, is found near 800 m at this location.) TC6 is from the station made on the shelf in 200 m water depth. This station represents the 'pure' Brazil Current. All flow is directed towards the south-west with speeds decreasing from 50 cm s^{-1} at the surface to 35 cm s^{-1} near the bottom. Ship drift records and navigational charts (from the US Navy Hydrographic Office) indicate that this flow may extend over the entire shelf at comparable speeds.

Data from sites TC1 and TC7 near the seamounts are more difficult to interpret because of the possible influence of the bottom topography. However, both sites show southward surface flow, northward flow of Intermediate Water and southward flow of Deep Water. Note that these data are based on a single profile, not the average of three like those at TC2–TC6. TC1 was revisited at the end of the cruise and speeds were smaller and somewhat rotated from those in Fig. 1. (Surface flow was more westerly and 800 m flow more northerly.) The general sense of the flow of the water masses was, however, the same. All of these profiles support Wüst's mass analysis indicating flow away from known sources, in contrast to the more recent[11,12] view for the Intermediate Water. Note, however, that these measurements do not preclude the possibility that Intermediate Water is transported towards the west, south of 24° S and then turns equatorward. The significance of the direct measurements for dynamic computations is striking. For example, a choice of 900 m as a reference level at TC5 would yield southward surface speeds of 70 cm s^{-1} (twice the observed) and the wrong direction for the Intermediate Water. Thus charts of dynamic topography relative to 1,000 m may be inappropriate for the study of the Brazil Current at 24° S and attention should be paid to the distinct water mass characteristics.

Using these observations to estimate the transport of the Brazil Current down to 400 m yields $\sim 6 \times 10^6$ m^3 s^{-1} towards the south offshore of the 200-m isobath. If TC6 is representative of the flow on the shelf, another 5×10^6 m^3 s^{-1} may flow inshore of the 200-m isobath. Indeed, using the historical T–S relation for the area[14], geostrophic transport computed from temperatures measured by Pegasus is $\sim 5.5 \times 10^6$ m^3 s^{-1} relative to 400 m. While the total transport (11×10^6 m^3 s^{-1}) may not then be significantly different from earlier estimates, its vertical distribution is quite dissimilar in being confined to the upper 400 m. Estimates of the poleward heat flux will also differ due to the higher mean temperature of the water moving towards the pole—an effect which is enhanced by the strong equatorward flow of cooler Antarctic Intermediate Water

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The Somali Current in autumn 1984, before the onset of the north-east monsoon

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The Somali Current undergoes vigorous variations during the course of the south-west monsoon. Although the initial phases of this response were fairly intensively studied during the FGGE Indian Ocean Experiment (INDEX) in 1979[1,2], relatively little is known about what happens in the late summer monsoon and the transition to the winter monsoon. In October 1984, we carried out measurements in the Somali Current regime with a shipboard acoustic Doppler current profiler which showed that a continuous Somali Current did not exist at this time: instead, the cross-equatorial flow with a transport of 12×10^6 m^3 s^{-1} in the upper 100 m identified by relatively low surface salinities, was found to turn offshore south of 2° N. Between 6 and 11° N, a large closed anticyclonic gyre, marked by relatively high surface salinities, was found with a transport of 32×10^6 m^3 s^{-1} in the upper 250 m, which is significantly greater than previously estimated from geostrophic calculations.

In the initial response to the onset of the south-west monsoon, from early May to early June, the Somali Current develops as a shallow cross-equatorial current, turning offshore at about 3° N with a cold upwelling wedge at the turnoff latitude, after the onset of the strong wind stress curl off Somalia in mid-June, a second gyre is generated between 5 and 10° N with a second cold wedge at the coast on its northern end Satellite sea-surface temperature and ship measurements indicate that at some time between late July and September the southern cold wedge starts to propagate northward and the two-gyre pattern seems to collapse[2,3] Ship measurements in different years showed that the water behind the northward-travelling southern cold wedge was low-saline water originating south of the Equator, replacing the higher-saline water found between 5 and 10° N in May to July[2,4,5]. The result of this development is that in the late summer monsoon, the Somali Current is a continuous boundary current from south of the Equator to the Horn of Africa. It is not known in any detail what happens in the transition period between the decay of the south-west monsoon in late August and the onset of the winter monsoon in early November

Nothing is known about the cross-equatorial flow at this time, partly because geostrophy cannot be applied close to the Equator to calculate transports from hydrographical data For the northern Somali Current region, expendable bathythermograph (XBT) measurements (for example, refs 6, 7) suggest that a northern gyre continues in that period, but its water origin, current structure, transports and any relation of this gyre to the current further south are not really known.

During part of this transition period, from 17 to 22 October 1984, we performed measurements in the Somali Current system on board the French RV *Marion Dufresne* with a shipboard acoustic Doppler current profiler, XBTs and continuous surface-temperature and salinity recordings (Fig 1).

The cross-equatorial flow in the Somali Current in October 1984, as the surface-current vectors (Fig. 1*a*) and the surface salinities of 35 0–35 2‰ (Fig. 1*b*) indicate, does not continue northward but turns offshore south of about 2° N Its origin is the South Equatorial Current, the biggest branch of which forms a northward-flowing boundary current east of Madagascar, which then continues westward around the northern tip of Madagascar across to Africa, where it is called the East African Coast Current Through admixtures of the low-saline coastal waters from East African rivers, the surface salinity is slightly reduced relative to that of the inflowing South Equatorial Current

The northward current component through the equatorial section (Fig 2*b*) has a subsurface maximum of more than 150 cm s^{-1} near the coast at about 40 m depth and still velocities of more than 50 cm s^{-1} at 200 m depth The northward transport through that section in the upper 100 m is 12.3×10^6 m^3 s^{-1}, which is almost exactly matched by the transport turning offshore in the top 100 m between 2° N and the Equator, for which we found 12.6×10^6 m^3 s^{-1} from the acoustic velocity-profile measurements The cross-equatorial transport in the upper 100 m is comparable to the transports determined by Leetmaa *et al.*[1] during five repetitions of a transport section normal to the coast at 1° S in the period May–June 1979, for which they obtained a mean transport of 14.0×10^6 m^3 s^{-1}. Even the northward flow in the depth range 100–300 m for which we obtained 8.4×10^6 m^3 s^{-1} (the quality of the acoustic current-profile data deteriorates below ~ 250 m) is comparable in magnitude to that of the 1979 early-summer measurements which showed a mean of 6.0×10^6 m^3 s^{-1} (ref. 1). In summary, the cross-equatorial transport of the Somali Current in late autumn is very similar to that during the onset of the south-west monsoon. The local winds off Kenya/Somalia are quite different during May/June and October (Fig. 1*c*), whereas the trade winds over the subtropical south Indian Ocean are quite similar in these two phases, which suggests that the cross-equatorial flow in the early phase of the south-west monsoon is driven primarily by remote forcing through the inflow from the South Equatorial Current, not by the local monsoon winds off East Africa.

North of 6° N, the ship-drift vectors (Fig 1*a*) indicate a large anticyclonic gyre, which had surface salinities of 35.6–35.8 parts per thousand (p.p.t) (Fig. 1*b*), that is, significantly higher than in the cross-equatorial current. Similar salinity differences between cross-equatorial flow and the northern regime along that longitude were found in earlier autumn cruises with the *Marion Dufresne*[8]. The current in the northern branch of this gyre was surface intensified (Fig. 2*a*) despite the lack of strong local winds during that time (Fig. 1*c*). Transport of this gyre in the upper 250 m was 33.5×10^6 m^3 s^{-1} westward between 6° and

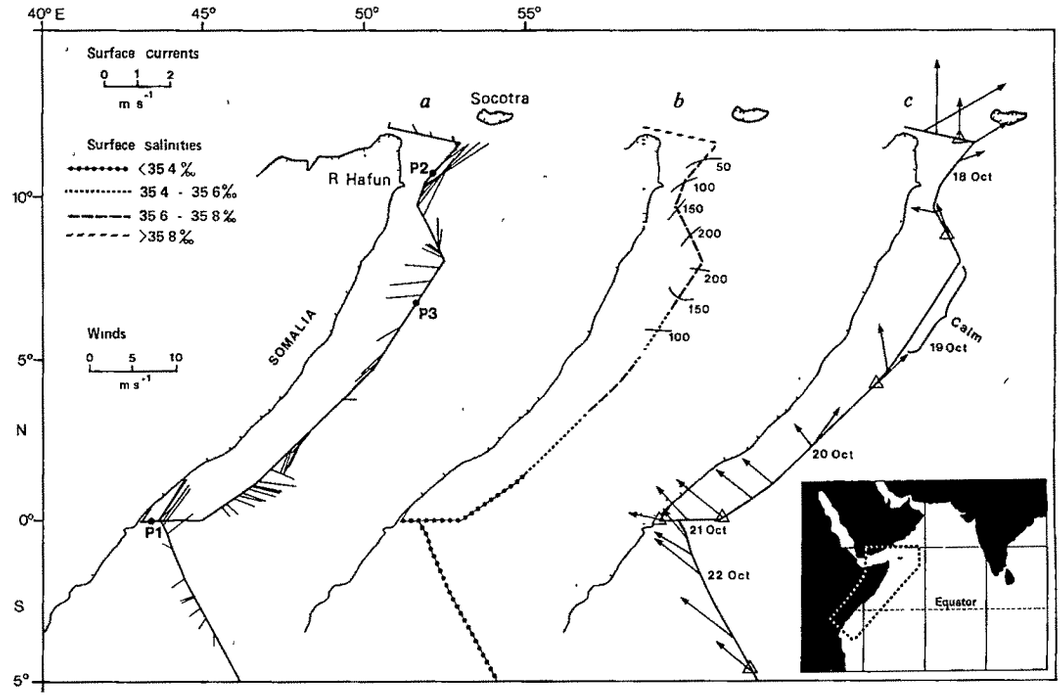


Fig. 1 Measurements along the track of RV *Marion Dufresne* during 18–22 October 1984 The Doppler profiler is an Ametek-Straze Model DCP 4400 operating at 115 kHz. Good data were obtained down to about 250 m depth or more at quiet to moderate sea state. Data storage at sea was in averages of 10 profiles at a time interval of 32 s for the averages. Subsequently, for the purpose of transport calculation, data were averaged to 15-min profiles, that is, over 280 individual profiles. In earlier comparisons in the Florida Current with other direct current measurements, we had determined that for sample sizes of this magnitude the accuracy of the acoustically measured horizontal currents, relative to the ship, was a few centimetres per second. The ship's motion was determined using satellite positioning. *a*, Drift vectors (P1–P3, locations of profiles shown in Fig. 2*a*) determined from the ship's satellite navigation system and the two-component electromagnetic speed log. However, satellite passes occur irregularly and gaps of several hours between fixes actually occurred in that region. Although this is unsatisfactory for calibration of individual profiles, it may be sufficient for the total transport through some section, say the equatorial section of the ship track, because for that calculation only the positions at both ends of the section need to be known. This means that the longer the section, the less the importance of position inaccuracies. *b*, Surface salinities and depth of 20 °C isotherm north of 5° N. *c*, Wind vectors, also indicating midnight ship positions (△, 00Z positions). Inset (lower right) shows area of observations off north-east Africa.

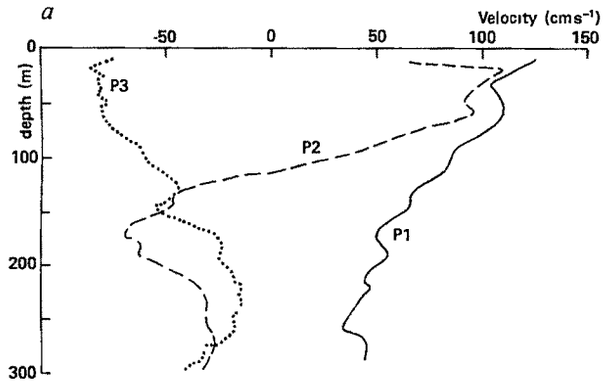
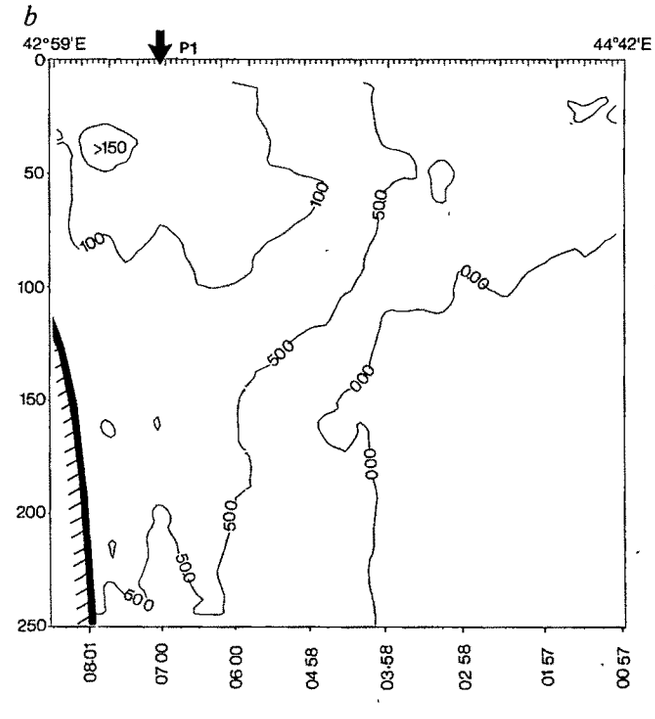


Fig. 2 *a*, Profiles of north component of current at point P1 (0°02′N, 43° 19′E) in cross-equatorial flow and of east components at points P2 (10° 40′ N, 52° 04′ E) and P3 (6°43′N, 51°30′E) in the northern gyre (Fig. 1*a*). *b*, Section of the northward current component along the Equator (for location, see Fig 1*a*) for 21 October 1984 (start 00:57·05; stop 08:45·40)



8.5° N as determined from the acoustic Doppler current profiles, and 31.6×10^6 m^3 s^{-1} eastward between 8.5° and 11.5° N. This is a closed mass budget within a few per cent. This is surprising because it has to be kept in mind that these transport numbers can be significantly affected by a small misalignment of the horizontal axis of the acoustic transducer. We corrected for the misalignment by comparing the vectors from satellite and radar navigation with those from acoustic bottom tracking near the coast, but an error of several 10^6 m^3 s^{-1} is still possible when integrating over long track segments as done here. It appears though, from the good balance both in the northern gyre and the cross-equatorial flow, that the compensation was effective. Earlier geostrophic transport calculations[7] obtained 22×10^6 m^3 s^{-1} in the 0–400-m layer for an XBT section in October, from which densities and geostrophic currents were derived using a mean temperature/salinity relation. When applying the same method[7] to our XBT data, we obtain 17×10^6 m^3 s^{-1}, both significantly lower than our direct measurements for just the upper 250 m. One reason for the difference is that the current at the assumed reference level of 400 m is not zero; another reason is that there is significant deviation from plain geostrophy through the curvature term.

Current profiles in the eastward- and westward-flowing branches of the northern gyre (Fig. 2*a*) show that the northern gyre is not symmetrical, which is also obvious from the ship drifts (Fig. 1*a*) and the depths of the 20 °C isotherms (Fig. 1*b*).

This asymmetry, which is typical and also is visible in earlier XBT sections through the northern gyre[6,7], can be explained by the strong nonlinearity of that circulation system, which causes a shift of high currents into the northwestern corner (for example, ref. 9.)

As mentioned earlier, the Somali Current seems usually to go through a phase (in August–September after the collapse of the two-gyre system where the Somali Current is a continuous boundary current) of carrying low-saline water from south of the Equator to ~10° N[2,4,5]. If this situation also occurred in 1984, we conclude from our observations that the current of low-saline water must retract again in late September–mid-October. Where, then, is all the low-saline water which was found all the way up to Ras Hafun in ~200-m-thick layer between the coast and ~300 km offshore in late August in these earlier studies? The transport of this water was estimated at 13×10^6 m^3 s^{-1} by Schott[10], about equivalent to the cross-equatorial transport found in the early south-west monsoon phase by Leetmaa *et al.*[1]. Another possibility is that in 1984 the two-gyre pattern did not collapse during August/September but remained intact from June to October. It is not known whether this can actually happen, but the possibility will be investigated using satellite surface-temperature data.

It is interesting that only very small currents were observed between Socotra and Cap Guardafui (Fig. 1*a*). This is at variance with recent numerical model results of Luther and O'Brien[11] and G. Philander (personal communication), who, although using quite different models, both show the northern gyre to propagate further northward and to circulate around Socotra in mid-October, that is, with its full transport through the passage between Socotra and the mainland.

A technical conclusion from this first application of the Ametek Doppler profiler in the Indian Ocean is that the system has provided very useful data of the near-surface circulation, which could not be easily obtained by any other means.

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Determination of the age of the Australian continent by single-grain zircon analysis of Mt Narryer metaquartzite

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A fundamental constraint on the evolution of the Earth is the time of the first occurrence of continental crust. The recent publication[1] of ages of 4,200–4,100 Myr for zircons from Australia is therefore of considerable importance. This dating was achieved by single-grain analysis on the SHRIMP ion microprobe at the Australian National University. To substantiate the existence of these old zircons by an alternative method, we studied the same zircon population by conventional solid-source mass spectrometry and isotope dilution analysis of single grains and individual fragments of single grains. Our results confirm neither the 4,200–4,100-Myr ages nor the particular discordancy patterns obtained on the ion probe. The age pattern we obtained can be explained by zircon crystallization 3,800–3,300 Myr ago. Probable primary ages of ~3,750–3,600 Myr coincide with model Sm/Nd ages for the adjacent gneisses[2], the possible source rocks of the Mt Narryer quartzite, and the younger ages are interpreted as those of zircon overgrowth compatible with a Rb/Sr age for the regional metamorphic overprint[2].

In their ion-probe study, Froude *et al.*[1] detected, among the 20 precisely analysed grains, 4 grains with apparent minimum ages older than 4,000 Myr, whereas the others yielded minimum ages between about 3,100 and 3,750 Myr. However, the data for the latter have not yet been published, which precludes a direct comparison with the results presented here. We have analysed 27 single zircon grains and 12 individual fragments of another 5 single grains. Comments on the isotope dilution technique are given in the footnotes to Table 1, which lists the U/Pb analytical data in order of decreasing U/Pb ages. Such high-resolution analyses have been shown to be very useful for deciphering complex zircon populations in igneous and metamorphic rocks (for example, refs 3, 4) and elucidating the age spectrum (provenance ages) of detrital zircons[5,6]. The analysis of fragments of single grains enables us to determine intra-grain ages[7]. Figure 1 shows the location of the sample and indicates previous model Sm/Nd ages[2] from the adjacent gneisses as well as a Rb/Sr age[2], interpreted as dating high-grade metamorphism in the gneisses.

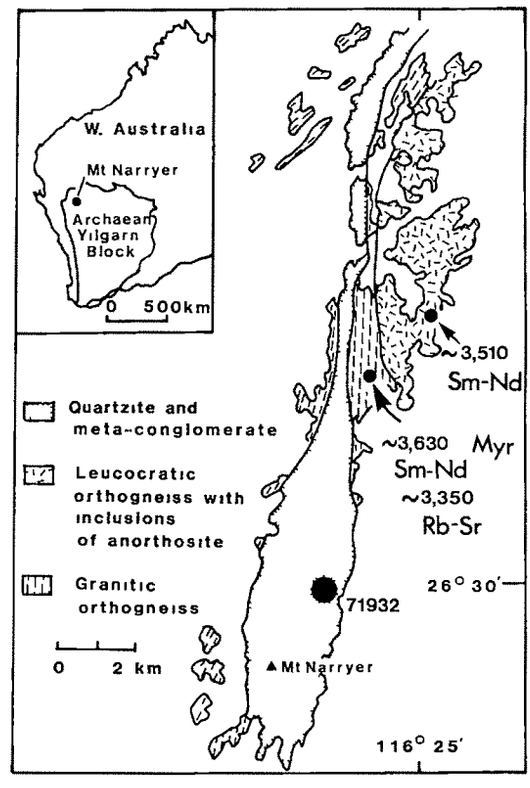


Fig. 1 Geological sketch map (slightly simplified, after ref. 1) with sample locality and previous model Sm/Nd and Rb/Sr ages[2] for gneisses adjacent to the quartzite.

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Table 1 U/Pb analytical results of single zircon grains and single fragments, Mt Narryer metaquartzite, Western Australia

Grain No	Type	U (pmol)*	Pb_{rad}† (pmol)	Pb_c‡ (pmol)	$^{206}Pb/^{204}Pb$ (measured)	Radiogenic Pb (atomic%) ^{206}Pb	^{207}Pb	^{208}Pb	Atomic ratios $^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	$^{207}Pb/^{206}Pb$	Apparent ages (Myr) $^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	$^{207}Pb/^{206}Pb$
1	A	9 108	8 612	0 275	929	70 77	23 05	6 18	0 6740	30 27	0 3257	3,321	3,495	3,597
2	A	12 15	10 92	—§	3,370	71 27	22 44	6 29	0 6450	28 01	0 3150	3,209	3,419	3,546
3	A	6 818	5 848	—	1,400	68 22	21 96	9 82	0 5888	26 14	0 3220	2,984	3,352	3,579
4	B	9 990	8 295	0 203	1,134	73 55	22 05	4 40	0 6156	25 42	0 2995	3,092	3,324	3,468
5	B	11.57	9 686	—	2,542	74 46	21 42	4 12	0 6279	24 88	0 2874	3,141	3,304	3,404
6	B	3 634	3 029	—	912	71 25	20 84	7 91	0 5984	24 13	0 2925	3,023	3,274	3,431
7	C	8 171	7 093	—	1,761	69 13	19 93	10 94	0 6044	24 07	0 2888	3,048	3,271	3,411
8	C	6 390	5 134	—	2,762	74 10	21 28	4 62	0 5985	23 70	0 2872	3,024	3,256	3,403
9	A	15 02	10 98	0 212	1,466	75 46	21 69	2 85	0 5557	22 02	0 2874	2,849	3,185	3,404
10	B	13 65	10 65	0 555	701	74 85	20 28	4 87	0 5884	21 97	0 2708	2,983	3,182	3,311
11	A	14 37	10 69	0 401	887	73 72	20 59	5 69	0 5522	21 26	0 2792	2,834	3,151	3,358
12	C	2 096	1 585	—	493	75 16	19 68	5 16	0 5716	20 71	0 2628	2,914	3,125	3,265
13	C	13 28	8 703	0 102	1,784	74 95	21 15	3 90	0 4946	19 25	0 2823	2,591	3,054	3,376
14	A	2 151	1 556	0 314	170	74 57	19 05	6 38	0 5439	19 12	0 2590	2,800	3,048	3,217
15	B	3 109	2 177	0 135	395	75 87	19 43	4 70	0 5343	18 86	0,2560	2,760	3,035	3,222
16	A	4 844	3 470	—	993	73 33	17 86	8 81	0 5289	17 76	0.2435	2,737	2,977	3,143
17	A	8 642	5 702	0 102	1,278	76 63	19 27	4 10	0 5092	17 66	0 2515	2,653	2,971	3,195
18	A	8 289	5 651	0 101	1,152	72 83	18 86	8 31	0 4998	17 86	0 2592	2,613	2,982	3,242
19	D	31.15	19 52	0 700	1,061	76 84	19 87	3 29	0 4822	17 19	0 2586	2,537	2,945	3,238
20	A	6 306	3 942	—	1 158	76 44	19 59	3 97	0 4829	17 02	0 2556	2,540	2,936	3,220
21	D	33 24	19 77	1 419	600	77 98	17 38	4 64	0 4671	14 35	0 2228	2,471	2,773	3,001
22	D	15.41	9 183	1.009	358	72 87	16 23	10 90	0 4372	13 49	0 2238	2,338	2,715	3,008
23	A	23 09	13 19	2 167	265	75 06	16 10	8 84	0 4316	12 77	0 2146	2,313	2,663	2,941
24	A	21 67	12 83	2 794	192	69 99	15 26	14 75	0 4176	12 56	0 2181	2,250	2,647	2,967
25	D	56 38	29 88	3 770	352	75 41	16 47	8 12	0 4023	12 11	0 2183	2,180	2,613	2,968
26	D	24 36	11 72	1 052	463	77.32	16 34	6 34	0 3747	10 92	0 2114	2,051	2,516	2,917
27	C	51 19	22 77	0 989	992	82 00	15 72	2 28	0 3675	9 711	0 1916	2,018	2,408	2,756
Single fragment analyses														
28	A I	1 248	1 144	0 135	210	71.26	21 61	7 13	0 6582	27 49	0 3029	3,260	3,401	3,485
	II	4 663	4 207	0 174	627	72 23	21 91	5 86	0 6550	27 41	0 3035	3,248	3,398	3,488
	III	3 277	2 923	0 738	157	71 68	21 49	6 83	0 6442	26 59	0 2994	3,206	3,368	3,467
29	D I	4 197	3 150	0 125	581	71 71	20 71	7 58	0 5426	21 60	0 2887	2,794	3,166	3,412
	II	1 622	1 173	0 232	154	73.39	20 53	6 08	0 5358	20 53	0 2779	2,766	3,117	3,351
	III	15 71	10 39	0 347	1,007	75 67	20 45	3 88	0 5034	18 76	0 2703	2,628	3,030	3,308
30	C I	7 474	5 487	1 255	182	72.76	20 46	6.78	0 5368	20 84	0 2816	2,770	3,131	3,372
	II	9 814	6 170	0.174	915	72 75	20 71	6 53	0 4606	18 08	0 2847	2,442	2,994	3,389
31	A I	8 701	5 744	0 251	676	74 07	19 49	6 44	0 4924	17 87	0 2632	2,581	2,983	3,267
	II	7 566	4 755	0 280	512	73 04	18 79	8 17	0 4624	16 42	0 2575	2,450	2,902	3,232
32	B I	13 28	7 342	1 684	188	73 68	14 39	11 93	0 4105	11 04	0 1951	2,217	2,527	2,786
	II	55 27	26 55	3 118	395	79 57	14 80	5 64	0 3848	9 869	0 1860	2,099	2,423	2,707

Types A, B, C, D correspond to the shape characteristics given in Fig 2*b* The constants used are $1\,55125\times10^{-10}\,yr^{-1}$ for $\lambda\,^{238}U$ (ref 15), $9\,8485\times10^{-10}\,yr^{-1}$ for $\lambda\,^{235}U$ (ref 15) and 137 88 for $^{238}U/^{235}U$ (ref 16) Zircons were dissolved in Teflon bombs[17] in 50% HF during ~100 h at 220 °C Element separation and purification were performed as described earlier[18] The isotope ratios and amounts were determined by isotope dilution analyses in a Thomson THN 206 mass spectrometer using a $^{205}Pb/^{235}U$ mixed tracer Both elements were loaded with H_3PO_4 and silica gel on Re single filaments The isotope composition of blank Pb was determined to be 17 84±0 10 (2σ error) for $^{206}Pb/^{204}Pb$, 15 53±0 08 for $^{207}Pb/^{204}Pb$ and 37 41±0 21 for $^{208}Pb/^{204}Pb$ The total Pb blank was $30\pm15\times10^{-12}$ g ($5\text{–}10\times10^{-12}$ g from the chemical procedure and $10\text{–}35\times10^{-12}$ g extracted from the Teflon bombs) The total blank for U was $<10\times10^{-12}$ g with an average of $\sim5\times10^{-12}$ g The maximum errors on the isotope ratios are 0 5% for $^{206}Pb/^{238}U$ and $^{207}Pb/^{206}Pb$ and 0 7% for $^{207}Pb/^{235}U$ These errors take into account (1) the precision of the mass spectrometric measurements, (2) the uncertainties in the isotope composition and amount of blank Pb, (3) the correction for ^{205}Tl interference, (4) the uncertainty of mass-discrimination corrections and (5) the accuracy of tracer calibration

* Absolute amount of U in the sample, corrected for 5×10^{-12} g U blank

† Absolute amount of radiogenic Pb in the sample, corrected for 30×10^{-12} g blank Pb and, where detectable, corrected also for incorporated common Pb (Pb_c) with the following isotope composition 11 83 for $^{208}Pb/^{204}Pb$, 13 87 for $^{207}Pb/^{204}Pb$ and 31 67 for $^{208}Pb/^{204}Pb$ This isotope composition corresponds to a 3,630 Myr BP Pb that evolved from 4,550 Myr BP with a $^{238}U/^{204}Pb$ of 9 3, a $^{232}Th/^{204}Pb$ of 39 1 and a primordial isotope composition identical to the Canyon Diablo meteorite[19], further comments on Pb_c in zircon are given in the text

‡ Absolute amount of Pb_c in the sample

§ No incorporated Pb_c detectable in the sample (total amount of Pb_c in the analyses was $<45\times10^{-12}$ g)

Figure 2*a* is a summary Concordia diagram[8] of single-grain and fragment analyses together with the ion-probe data for the apparently 4,100–4,200-Myr BP grains and Fig. 2*b* shows a more detailed Concordia diagram with a broad grouping of the different zircon types and a distinction between single-grain and individual fragment data Additionally, Fig. 2*b* indicates the upper intercept ages of the regression lines through distinct fragments of single grains (intra-grain ages).

All zircons analysed grain-by-grain were chosen by their habit, colour, degree of transparency, size and particular features such as pronounced core–rim zoning. Those from which the fragments were broken were selected to cover the whole range of different grain types Because Froude *et al.*[1] indicated that the apparently 4,100–4,200-Myr zircons are distinguished by their peculiar angular form and the absence of inclusions, we first investigated perfectly euhedral grains with sharp edges, high transparency (to avoid inclusions) and uncorroded surfaces However, we mention that the grain illustrated for reference in a photomicrograph in Froude *et al.*[1] resembles more a simple piece of broken zircon than a particular angular type Nevertheless, we analysed in a first group of zircons (see Fig. 2*b*) the series of grains that most closely fulfilled the above conditions, followed by a second group (Fig. 2*b*) similar to the first but with much lower degrees of transparency. In a third group we investigated transparent and translucent grains with strongly rounded edges, some of which had significant surface corrosion features. As a last group with distinct grain type, a series of particularly milky translucent zircons was analysed, most of them exhibiting clearly visible zoning between the inner and outer parts of the crystal. This latter feature suggests that some of the zircons grow in two stages

Despite the wide range of grain characteristics, all zircons yield similarly discordant age patterns (Fig 2*a*, *b*) with minimum ages ranging from 2,750 to 3,600 Myr (Table 1), plotting in a data field that intersects the Concordia curve along a sector from ~3,400 to 3,700 Myr. This suggests that the primary ages of the zircons lie in this interval, but because none of the different groups of zircons defines a simple linear array, we cannot distinguish cogenetic zircon populations and, thus, no precise primary ages can be determined.

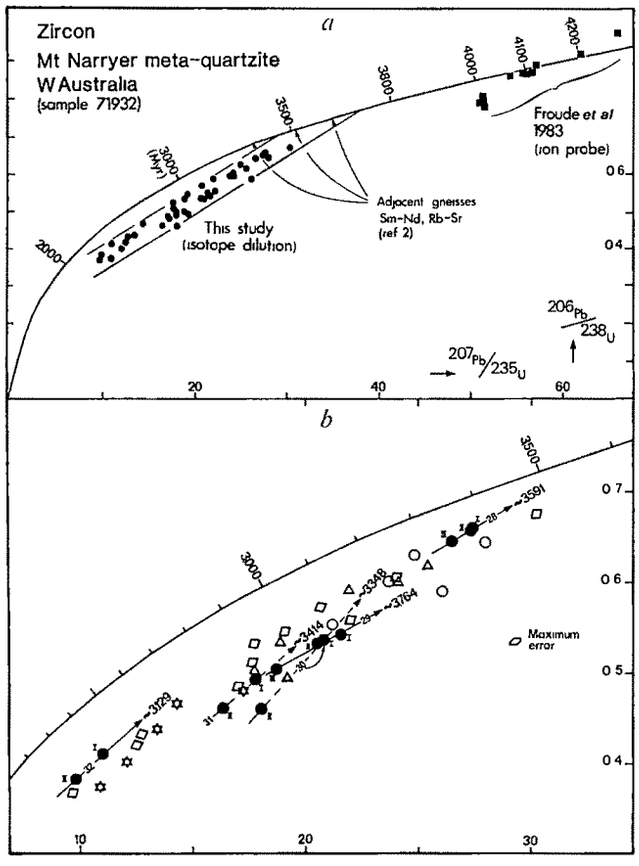


Fig. 2 Concordia diagram of the U/Pb analytical results *a*, Summary diagram of single-grain and fragment analyses compared with ion-probe data[1] for the apparently 4,100–4,200-Myr BP zircons; *b*, detailed diagram showing distinctions between different grain types and single-grain versus fragment analyses. Single zircon grains □, Sharp-edged transparent grains; △, sharp-edged translucent grains; ○, strongly edge-rounded transparent and translucent grains, ☆, milky translucent grains with core–rim zoning; ●, single fragments (from grains 28–32)

A few observations concerning the grain-by-grain analysis may be emphasized: the particularly milky translucent grains yield younger ages than most of the other grain types, plotting in the lowest segment of the discordancy field (Fig. 2*b*). Moreover, these milky grains are, together with some other highly discordant grains, characterized by especially large amounts of incorporated common lead (Pb_c, Table 1), contributing $\leq 15\%$ of total lead in the crystal. This positive correlation between Pb_c content and degree of discordancy supports an earlier conclusion[7] that loss of radiogenic lead may occur by exchange with Pb_c from intergranular phases. The presence of such incorporated Pb_c, also observed to a smaller extent in less discordant grains, requires a correction which necessarily affects the apparent ages of all Pb_c-bearing grains. We performed this correction by assuming the incorporated Pb_c to be on average ~3,630 Myr old, as suggested by the minimum ages of Pb_c-free grains ranging up to ~3,600 Myr, and supported by the model Sm/Nd ages[2] for the adjacent gneisses, the possible source rocks of the quartzite. In the case of sediments, the isotope composition of this incorporated common lead cannot be determined directly because of the lack of Pb-rich/U-deficient cogenetic minerals such as K-feldspar. Therefore, the above assumption is necessary. However, a correction with a significantly younger lead (for example, a 3,350-Myr-old Pb_c) (probable age of metamorphism in the quartzite) would only cause age differences lying within the maximum error of our analytical technique (Table 1, Fig. 2*b*). A correction with a 4,100-Myr-old Pb would influence a few data by 2%, whereas most of the single-grain data would be changed by <1%. This shows that variations in the isotope composition of incorporated Pb_c, relative to the 3,300–4,100-Myr age range, modify the data only to a small extent; even a correction with present-day Pb_c (for example, blank Pb_c, Table 1) would not shift any of the data to ages older than 3,700 Myr.

Our results from the single-grain analyses confirm neither the existence of 4,100–4,200-Myr-old zircons in this sample nor the particular discordancy patterns observed on the ion probe[1]. The discordancy arrays of the ion-probe data all point to the origin of the Concordia curve (zero ages), suggesting recent Pb loss (or U gain) of these zircons (see Fig. 2*a* and ref. 1). Here, we do not show any evidence in favour of such recent perturbation of the U/Pb systems; the lower extrapolation of our discordancy field for all single-grain data intersects the Concordia curve along a sector from ~1,400 to 2,000 Myr, substantiating Pb to be ancient. Moreover, none of our grains yield a result that plots above the Concordia curve as reported for the ion-probe analyses for both apparently 4,100-Myr-old and significantly younger zircons (see Fig. 2*a* and ref. 1).

Because of the discrepancy between the isotope dilution and the ion-probe data, we extended our investigation to analyses of individual fragments of single grains, for two main purposes: (1) to assess whether distinct parts of grains preserved a record of zircon growth before 4,000 Myr BP and (2) to obtain intra-grain ages, which approximate primary zircon ages better than those of arrays of single, and not necessarily cogenetic, zircon grains. Hence, we broke up five zircons representative of the different grain types observed in the quartzite (Fig. 2*b*).

A typical grain of the first group (grain 28), a sharp-edged transparent zircon, was broken into three fragments (I, II, III, Fig. 2*b*, Table 1), where I represents the middle part of this long prismatic grain and II and III the two prismatic ends. The regression line[9] through the fragments (calculated using the individual errors of the fragment analyses) intercepts the Concordia curve at ~3,591 Myr, which is in agreement with the model Sm/Nd age for the possible source rocks of the quartzite. Three fragments of another grain (grain 29), a sharp-edged but translucent zircon (second group in Fig. 2*b*), define an intercept age of ~3,764 Myr. As for grain 28, the central part of the long prismatic zircon (fragment I) is the oldest, suggesting that loss of radiogenic Pb is favoured in the pyramidal ends of the grain either because of crystallographic differences between middle and outer parts or simply because of the higher surface/volume ratio of the pyramidal ends. A third grain (grain 30), a particularly milky translucent and core–rim-zoned zircon (fourth group in Fig. 2*b*), has been analysed in two fragments (I and II) where I consisted mainly of rim material, whereas II was dominated by material from the core of the grain. The two fragments define a line with an intercept age of ~3,348 Myr, which is identical to the probable age of high-grade metamorphism, in the adjacent gneisses[2]. To account for this age coincidence as well as the fact that grain 30 was core–rim zoned, it seems plausible that the intercept age dates zircon overgrowth (that is, new growth) during regional metamorphism, which is recognized clearly in the quartzite[10]. This interpretation seems to be supported by the discordancy trend for single grains of milky zoned zircons (Fig. 2*b*), which points to significantly younger ages than the arrays of most other zircons. A further zircon of the first group (grain 31), a transparent sharp-edged homogeneous grain, broken in two approximately equal fragments, yields an intercept age of ~3,414 Myr, suggesting that some of the smaller grains represent entirely new-grown zircons during high-grade metamorphism. A final zircon, broken in two fragments (grain 32), was a peculiar green milky translucent grain yielding an intercept age of ~3,129 Myr. This age suggests the existence of a second younger metamorphic event in the quartzite. However, the line through the two fragments is too poorly defined to define the intercept age with any confidence and, moreover, no such event is known in the area.

Thus, individual fragments from single grains representing the different crystal types in the quartzite also do not yield any evidence for zircons older than 3,800 Myr. Furthermore, the discordancy trends of the ~3,600–3,800-Myr BP fragments

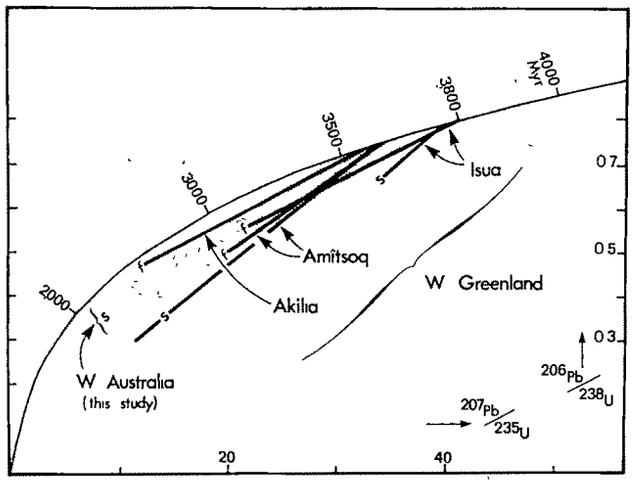


Fig. 3 Summary Concordia diagram including the Australian Mt Narryer quartzite zircons analysed here and, for comparison, zircons from Western Greenland, specifically from the Isua supracrustals[12,13], the Amîtsoq gneisses[12-14] and the Akilia association[13]. All ages have been calculated using the new uranium decay constants[15], f, fractions, s, single grains

(grains 28 and 29) are identical to those for the single zircon grains, again contrary to the discordancy patterns obtained on the ion probe[1]. The question remains as to whether the lack of 4,100–4,200-Myr BP zircons found here is an artefact of grain selection, that is, that we have not chosen the correct crystals. This possibility cannot be completely eliminated. Furthermore, there is an independent argument to be considered. the single grains and individual fragments analysed here yield clearly discordant ages, proving that all zircons were open systems for Pb either during a distinct period or continuously. This contrasts strongly with the apparently concordant ages of the 4,100–4,200-Myr BP zircons identified by the ion-probe technique. Such closed-system behaviour of the 4,100–4,200 Myr BP zircons, which would necessarily have a higher degree of radioactive damage than the younger ones, is difficult to explain. Closed-system behaviour would be particularly surprising given that the quartzite from which the zircons have been extracted suffered high-grade metamorphism probably ~3,400 Myr ago; in general, even low-grade metamorphism, for example, under lower greenschist facies conditions, produces significant Pb loss in detrital zircons (for example, ref. 11).

In conclusion, we emphasize that the complete data set for single grains and fragments can be explained by zircon growth between 3,300 and 3,800 Myr BP. The upper intercept ages of both the overall discordancy field and two of the intra-grain arrays point to primary ages of the order of 3,600–3,800 Myr. Because the quartzite has been affected by high-grade metamorphism, probably ~3,400 Myr ago, these upper intercept ages may be slightly too young relative to the real crystallization ages of zircon, because of an early Pb loss during metamorphism. From this point of view, the possibility that parts of the Australian continent are older than 3,800 Myr cannot be excluded completely. However, the present study does not produce any evidence in support of this idea. On the other hand, the zircon ages reported here show the Australian continent to contain crustal rocks 3,600–3,800 Myr in age, analogous to those in Greenland. This similarity is illustrated in Fig. 3, which is a summary Concordia diagram including all reliable zircon ages from Western Greenland (Isua, Amîtsoq and Akilia; refs 12–14) together with our data from the Mt Narryer quartzite. The overlap between possible primary ages and discordancy arrays of the Australian and Greenland zircons is consistent with the hypothesis that both cratons in the present-day Northern and Southern Hemisphere developed from a continental nucleus that grew ~3,600–3,800 Myr ago.

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Halomethane from halide ion—a highly efficient fungal conversion of environmental significance

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Globally, at least 5 Mt yr^{-1} of chloromethane (CH_3Cl) must originate from natural sources, according to estimates based on environmental concentrations, man-made emissions of 26 kt yr^{-1} being insignificant in comparison[1-3]. Although CH_3Cl is a natural product of several fungal species[4,5], no quantitative investigations of its biological production have been reported. We have measured CH_3Cl production by a common wood-rotting fungus, and report that on glucose-based media, the chloride ion (Cl^-) at concentrations <4 mM was methylated with >90% efficiency. The pattern of CH_3Cl biogenesis was typical of a secondary metabolite and paralleled loss of Cl^- from the medium. With cellulosic substrates, CH_3Cl yields ranged between 80 and 95% at Cl^- concentrations as high as 25 mM, although production extended over a longer period. Bromo- and iodomethane were formed in high yield from the corresponding halide ions. Such high-efficiency methylation of the halide ion indicates that fungi could make a substantial contribution to the atmospheric CH_3Cl burden.

Assessments of CH_3Cl production were made during growth of *Phellinus pomaceus* (Pers.) syn. *Fomes pomaceus* (Forest Products Research Laboratory no. 33A), a white-rot fungus of worldwide distribution reported to synthesize CH_3Cl (ref. 4). When the fungus was grown with glucose as the main carbon source, CH_3Cl production was clearly confined (Fig. 1) to the period after exponential growth and before autolysis, a pattern characteristic of secondary metabolites, the formation of which is usually retarded until the trophophase approaches completion[6,7]. Whereas fungal growth continued for ~10 days, CH_3Cl biosynthesis extended for another 8 days. Chloride uptake from the medium closely paralleled CH_3Cl biogenesis, showing that there was little accumulation of Cl^- in the mycelium before CH_3Cl release, a conclusion confirmed by determination of Cl^- content in the mycelium after ashing at various stages of growth. Chloromethane yield, expressed as a

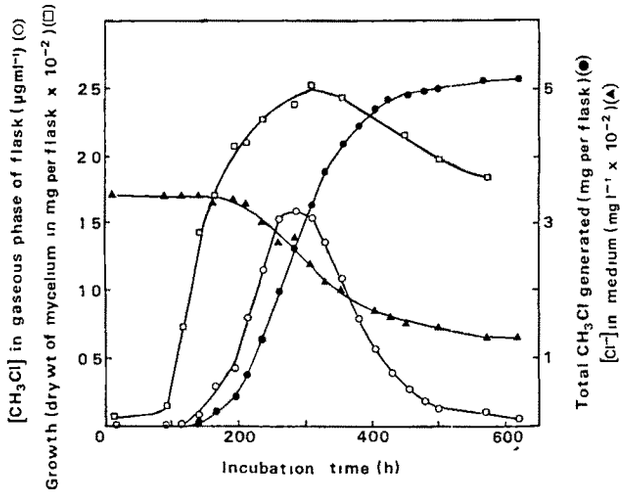


Fig. 1 Growth of *P. pomaceus* on glucose-based medium containing 9.6 mM NaCl in relation to CH_3Cl production and chloride loss from the medium. Results are expressed as follows: □, growth measured as mycelial dry wt; ○, CH_3Cl concentration in gaseous phase in the culture flask, ●, total CH_3Cl generated per culture flask; ▲, chloride ion concentration in the culture medium.

Methods. Cultures of *P. pomaceus* were grown on a solid medium *p*H 6.5 (20 ml) containing ($g\,l^{-1}$): glucose, 30; mycological peptone, 5, agarose '10' (BDH), 10; NaCl, 0.5 in conical flasks (25 × 250 ml) at 25 °C. As a precaution against bacterial contamination, chloramphenicol (25 mg l^{-1}) was also added to the medium, experiments having indicated that the presence of the compound did not affect production of CH_3Cl or chloride levels in the medium during growth. The inoculum, which was spread uniformly over the surface of the medium, was a mycelial suspension (1 ml) prepared by homogenizing mycelium (600 mg wet wt) in sterile distilled water (20 ml) for 30 s with an Ultra Turrax homogenizer. The mycelium used for inoculation was grown on 5% malt extract agar containing not more than 1 mM chloride. Each conical flask was provided with a polytetrafluoroethylene (PTFE)-coated rubber bung through which passed a glass tube (10 cm × 0.8 cm internal diameter, i.d.) packed uniformly with cotton wool and fitted so that the lower end was flush with the lower surface of the stopper. The bung was also equipped with a sampling port comprising a glass tube of 1 mm i.d. projecting 0.5 cm into the interior of the flask and flared at the upper end protruding from the stopper so as to accommodate a small PTFE-coated rubber bung. CH_3Cl production during growth was assessed by a modification of a headspace technique described previously[14]. Initially, to calibrate the system, the rate of loss of gaseous halomethane from flasks equipped with outlet tubes as above was measured by introducing aqueous solutions of halomethane (20 ml) and determining headspace concentrations (by gas chromatography with a flame ionization detector) of aliquots (2 ml) withdrawn through the sampling port at intervals over 8 days. As diffusion of halomethane from the liquid to the gaseous phase within the flask was rapid compared with the rate of loss from the flask, the system could be regarded as single-phase in subsequent calculations. The loss rate obeyed first-order kinetics, the half-lives of chloro-, bromo- and iodomethanes being 13.8, 19.6 and 26.2 h respectively. Therefore, the total amount of halomethane generated by a fungal culture at any time during growth could be calculated from empirical measurements, at regular intervals, of the halomethane concentration in the flask headspace and the half-life of the halomethane using a computer integrating program[14]. Growth was measured as the dry wt of washed mycelium obtained by filtration of the agarose medium after solution in hot water. Chloride ion concentrations were determined on the diluted medium with a Corning Eel 921 chloride meter.

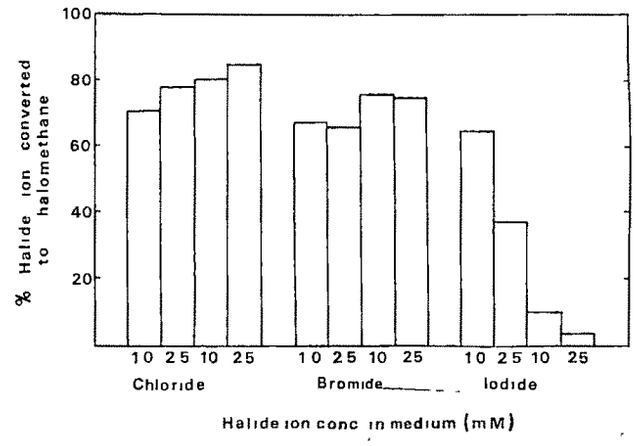


Fig. 2 Effect of halide ion concentration on yield of corresponding halomethane for *P. pomaceus* grown on cellulose as main carbon source.

Methods. Cultures of *P. pomaceus* were grown in conical flasks (in the conditions described in Fig. 1 legend) on filter paper (Whatman No. 540) (6.9 g) augmented with a chloride-free mineral salts medium of *p*H 6.5 (20 ml) containing pure L-amino acids (3.6 $g\,l^{-1}$) in similar proportions to those in mycological peptone and thiamine mononitrate (1.2 mg l^{-1}). The medium was supplemented with 1–25 mM NaCl, NaBr or NaI, as indicated. The results shown are the mean of duplicate treatments.

percentage of the Cl^- originally present in the medium, varied between 90 and 100% for Cl^- concentrations <4 mM, falling to 6% at 170 mM Cl^-. Between 1 and 170 mM Cl^-, total CH_3Cl production was linearly related to the logarithm of Cl^- concentration, although slight deviation occurred at the higher end of the concentration range.

As the fruiting bodies of *P. pomaceus* are typically found on trees of the Rosaceae, especially *Prunus*, CH_3Cl yields were assessed when the fungus was cultured with supplemental NaCl (20 mM) on sawdust derived from the wood of *Prunus domestica*, the common plum. Chloromethane production with both wood and other cellulosic substrates (such as filter paper, microcrystalline cellulose, cotton wool and newspaper) as main carbon sources extended over long periods, from 4 weeks (cotton wool) to 6 months (wood) but yields of CH_3Cl were much higher than with glucose-based medium at similar chloride concentration, ranging from 86% (cotton wool) to 95% (newspaper).

Figure 2 illustrates the effect of different concentrations of halide ion on halomethane yield, with cellulose (filter paper) as the substrate. Both bromide and iodide acted as acceptors for the methylating system. In contrast, with glucose-based media, yields of both chloro- and bromomethane did not decline with increasing halide ion concentration; indeed, a slight trend in the reverse direction was discernible with Cl^-. The suppression of secondary metabolite production by glucose and other readily utilizable carbon sources has been noted in many studies on secondary metabolism[7]. Iodomethane production decreased with increasing iodide concentration but this is probably attributable to the manifestly detrimental effects of this ion on growth. When equimolar concentrations (5 mM) of chloride, bromide and iodide were present, the preferred substrate was iodide, the respective halomethanes being formed in the proportion 1:4:19.

The magnitude of CH_3Cl production in nature by fungi is difficult to estimate. Chloromethane has been identified as a volatile product of six *Fomes* species[4] and of *Agaricus bisporus*[5], the common mushroom. Future studies may reveal the presence of this biosynthetic ability in many more cellulose-degrading species growing on wood, litter and soil worldwide. The high affinity of the methylating system for Cl^- ensures that even when environmental Cl^- concentrations are low, appreciable quantities of CH_3Cl are generated. Thus, despite a concentration of only 34 mg kg^{-1} in *P. domestica* wood, yields of CH_3Cl when *P. pomaceus* was grown on the unsupplemented substrate were between 40 and 50%. However, wood-rotting fungi are not confined to such low-chloride environments, being capable of mycelial growth on many plant substrates under a broad range of environmental conditions. The normal Cl^- content of plant material varies from 200 to 10,000 mg per kg dry wt[8,9] and many terrestrial soils exhibit appreciable salinity. Growth of fungi possessing such a high-affinity methylating system in these

circumstances could lead to production of substantial quantities of CH_3Cl and must therefore be considered alongside other postulated sources of atmospheric CH_3Cl such as combustion of vegetation[10] and reaction of iodomethane with Cl^- in sea water[11]. That such a biological source may be a major contributor to atmospheric CH_3Cl levels is of considerable interest in view of the importance ascribed to CH_3Cl and other man-made halocarbons in controlling the levels of stratospheric ozone[1,12]. Quite apart from the environmental significance, the enzymatic incorporation of halide ion apparently directly into a one-carbon compound deserves attention as it must involve a mechanism quite distinct from the haloperoxidase-mediated halide ion incorporation previously regarded as the major route to organohalogen compounds in nature[13]. The biotechnological potential of this novel system warrants investigation; indeed, a role for the fungal gene involved can be envisaged in the development of halotolerant crop plants, although environmental considerations may limit such an application.

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Use of numbers by a chimpanzee

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Recent studies have examined linguistic abilities in apes[1–6]. However, although human mathematical abilities seem to be derived from the same foundation as those in language, we have little evidence for mathematical abilities in apes (but for exceptions see refs 7–10). In the present study, a 5-yr-old female chimpanzee (*Pan troglodytes*), 'Ai', was trained to use Arabic numerals to name the number of items in a display. Ai mastered numerical naming from one to six and was able to name the number, colour and object of 300 types of samples. Although no particular sequence of describing samples was required, the chimpanzee favoured two sequences (colour/object/number and object/colour/number). The present study demonstrates that the chimpanzee was able to describe the three attributes of the sample items and spontaneously organized the 'word order'.

Before the present study[11,12,14], Ai had been trained to name 14 objects and 11 colours by choosing among the set of symbols shown in Fig. 1. For the first experiment, five objects and five colours were selected from Ai's 'vocabulary' to form groups of sample items to be identified. The experimenter presented the sample items (for example, three red pencils) in the display

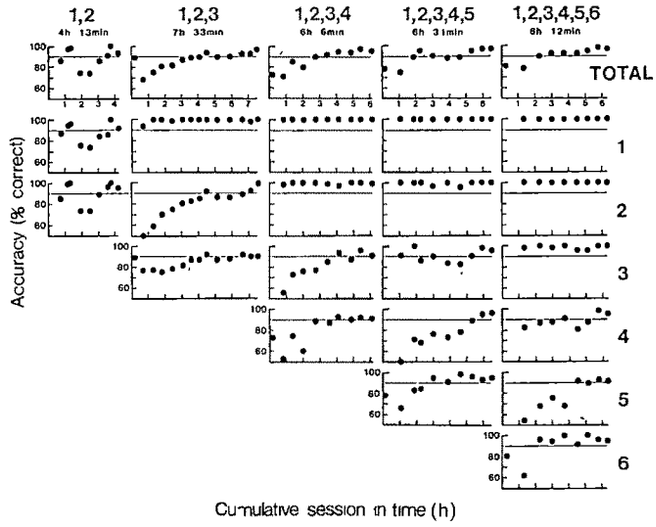


Fig. 2 Acquisition of number names for the training item, red pencils. The top row represents overall accuracy, the other rows accuracy for individual numbers. Each dot shows the performance in each session, with percentage correct plotted against the time elapsed since the beginning of training on each number set. In the first session of each stage, except for training on the number names 1 and 2, the chimpanzee received intensive training limited to the newly introduced number.

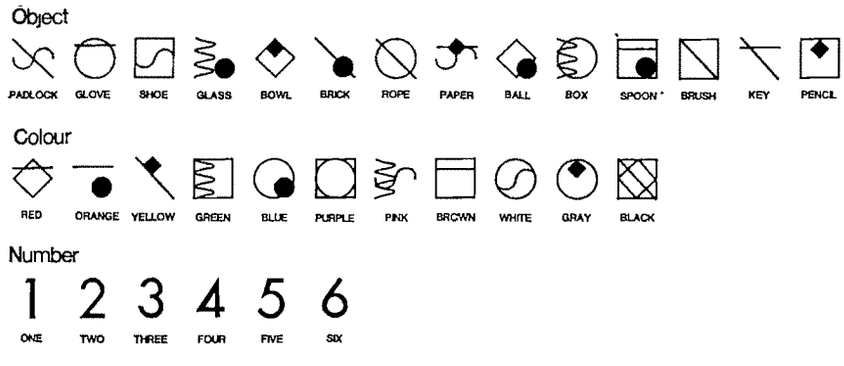


Fig. 1 Symbols used in these studies In the symbolic matching-to-sample task, the chimpanzee was trained to identify the sample item that appeared in the display window (24 × 17 × 24 cm) by pressing an illuminated key (2 × 2.5 cm) showing the assigned symbol These symbols could appear in various positions in a 5 × 6 key matrix on a console located below the display window This console was interfaced with a PDP11/V03 minicomputer. The set of operative-illuminated keys was changed from trial to trial and the positions of the keys were also changed from session to session so that the symbol, rather than the position of the correct key, had to be learned The trial ended when the chimpanzee pressed a blank key to the right of the matrix on the console In numerical naming, two methods of displaying the sample items were used to minimize the possibility that the chimpanzee would associate each numeral with a particular display pattern On some trials the samples were laid out in a row The position and the spatial density of items were changed within the area of the window in each trial so that area covered was not correlated with number of items. On other trials the items were held in one hand in front of the display window The orientation and the angular separation of items was varied from trial to trial Depending on type of object, either or both methods were used. In the first experiment, five objects (pencil, paper, brick, spoon and tooth-brush) of five colours (red, green, blue, yellow and black) were used to form groups of items to be identified

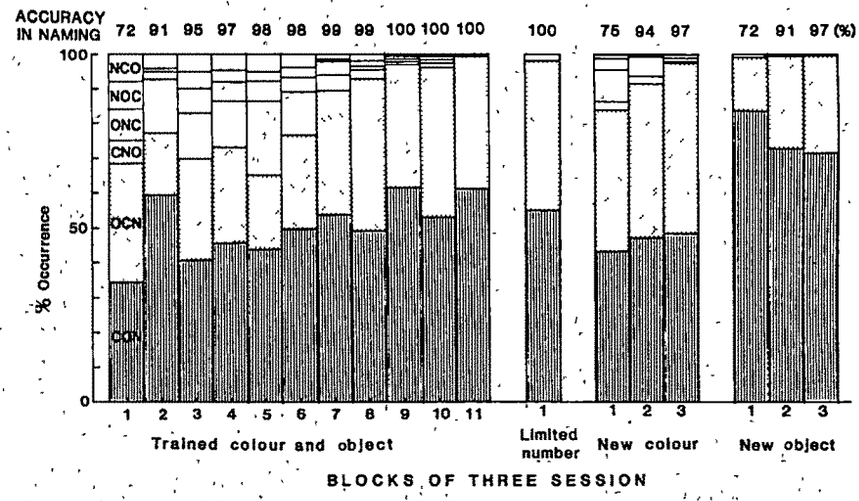


Fig. 3 Percentage of six possible sequences in three-term naming. N, number; C, colour; O, object. For example, NCO refers to trials in which the sequence 'number/colour/object' was used correctly. The open bars show the percentage of trials on which number was the first or second attribute named. The percentage of trials on which all three attributes of the sample items were named correctly is shown at the top of each column. The left panel shows the change of use of the six possible sequences as a function of three-term-naming training. A session ended when 100 rewards had been delivered. Each reward required two consecutive correct responses. Each block consists of three consecutive sessions. In the final block of three sessions of the task, the chimpanzee received 604 problems during 127 min and made only three mistakes. To investigate the determinant of the particular sequences, three kinds of tests were done. In the first test ('limited number'), the number of sample items was not varied but restricted to one of the five alternatives within a session. The objects and colours used in this first test were the same as those used in previous training. In the second test ('new colour'), new colours were introduced. The sample items consisted of the numbers from one to five, the five trained objects and five new colours, pink, brown, purple, white and grey. In the third test ('new object'), new objects were introduced. The sample items consisted of the five trained colours and five new objects, padlock, glove, glass, ball and key. In this test, the number of items to be named was restricted to one and two. Although those new samples had never been used in three-term naming, or even in simple numerical naming, the chimpanzee correctly described the three attributes of the samples. Decreasing the difficulty of numerical naming by using the same number of items in all samples in a given session, or increasing the difficulty of object or colour naming by introducing new colours and objects, had little effect on the favoured sequences. The chimpanzee seemed to have established the order of describing the three attributes of the sample items. However, the introduction of new samples had a temporary impact on the ratio of these two sequences. The introduction of new colours increased one sequence, object/colour/number. The introduction of new objects increased the use of the other sequence, colour/object/number. As those new samples were presented repeatedly, the ratio of the two favoured sequences gradually returned to the original level.

window and then pressed a start switch to light a set of numeric keys on a console. Correct and incorrect key choices were followed by different sounds. A piece of apple or a raisin was delivered after a number of consecutive correct responses. A session ended when 100 such rewards had been delivered or when 60 min had elapsed, whichever came first. Ai received one or two sessions a day. On the average, 303 trials were given in a 43-min session, with trials spaced ~8.5 s apart.

At the beginning of the first stage of training, only two numbered keys were available and only one or two red pencils were shown. The number of red pencils and corresponding number keys was increased successively. Training was continued on each number set until accuracy exceeded 90% in at least two consecutive sessions. Acquisition of number naming with red pencils only as exemplars is shown in Fig. 2. The introduction of a new number was always accompanied by poorer identification of the maximum number learned in the previous stage. For example, in the course of initial training to name six items in addition to 1, 2, 3, 4 and 5, Ai made 274 errors out of 3,004 trials, 63.1% of these confusion between 5 and 6. Moreover,

Table 1 Generalization of numerical naming to a new colour, a new object or both a new colour and object in a chimpanzee

Numbers	Accuracy on probe trials: Samples — New colour, trained object	Trained colour, new object	New colour, new object	Accuracy by chance	Accuracy of trained samples on probe trials
1, 2	0.83 (5/6) Blue Pencil	0.50 (3/6) Red Paper	0.50 (3/6) Blue Paper	0.50	0.97
1, 2, 3	0.60 (18/30)* Yellow Pencil/paper	0.33 (10/30) Red/blue Brick	0.33 (5/15) Yellow Brick	0.33	0.99
1, 2, 3, 4	0.71 (34/48)‡ Black Pencil/paper/brick	0.54 (26/48)† Red/blue/yellow Spoon	0.56 (9/16)* Black Spoon	0.25	0.99
1, 2, 3, 4, 5	0.79 (79/100)‡ Green Pencil/paper/brick/spoon	0.57 (57/100)‡ Red/blue/yellow/black Tooth-brush	0.56 (14/25)* Green Tooth-brush	0.20	0.98

The generalization was tested by inserting probe trials in which the new colour, new object or both, was used to illustrate the number. These probe trials were included among trials during which trained samples were used. On trials with trained samples, the chimpanzee was informed whether her choice was correct or incorrect. On probe trials, which were inserted once every 10 trials on the average, the choice response was followed by neither the 'correct' nor 'error' feedback sound. Proportion correct is shown for the trained items as well as for the new colour and/or object introduced in each stage of training. The numbers in parentheses show the number of trials on which the proportions are based. The last column shows the proportion correct on the trials for the trained sample items, including all the colours and objects previously used. Generalization of the number name to new objects of familiar colour, to new colours of familiar objects, and to new objects in a new colour was at first poor. Less generalization was observed with new objects than new colours in each stage of training. As additional numbers were introduced and new objects and colours were used as exemplars, the correct number names were applied not only to new colours but also to new objects.

* $P<0.05$, † $P<0.01$; ‡ $P<0.001$ significantly above the chance level (χ^2 test).

most errors (94.9%) were confusions between neighbouring numbers.

Following acquisition of a new number name, generalization to a new object and/or a new colour was tested. The specific objects and colours used as well as test performance are summarized in Table 1 and reported in more detail elsewhere[13].

Accuracy during the final two sessions of naming 1, 2, 3, 4 and 5 with all five colours and five objects exceeded 98.5% in 830 trials. To reach this level of performance, a total of 95 sessions were required. In other words, 68 h 21 min (28,799 trials) elapsed from the beginning of training. It took an additional nine sessions (6 h 12 min, 3,004 trials) for Ai to reach criterion in selecting the correct Arabic numeral for groups of up to six red pencils.

How could Ai's newly acquired numerical skills be combined with object/colour naming? To answer this question, the chimpanzee was required to name not only the number of items, but also the colour and objects used to illustrate the number.

Fifteen keys, each of which represented one of five numbers (1, 2, 3, 4, 5), five colours (red, blue, yellow, green, black) and five objects (pencil, paper, brick, spoon, tooth-brush), were operative at one time. The sequence in which the keys were depressed was free, but the chimpanzee was required to select the three keys correctly describing each of the three attributes of $5 \times 5 \times 5 = 125$ types of sample items. For example, when five blue tooth-brushes were shown in the display window as a sample item, it was necessary for Ai to press keys of '5', 'blue' and 'tooth-brush' in any order. When the chimpanzee pressed an incorrect key, the 'error' sound followed and the trial ended. The pressed key was darkened and inoperative, so that the repeated response such as 'red/red' was inhibited.

Although no particular 'word order' was required, the chimpanzee favoured two particular sequences among the six possible alternatives, colour/object/number and object/colour/number (left panel of Fig. 3). In both sequences, numerical naming was always last. In the final sessions, it generally took less than 3 s for Ai to describe the object, colour and number of the sample items. She seldom looked back at the display between each component response. The response latency in the first component was the longest and the third component (numerical naming in almost all cases) was longer than the second one. The previously favoured sequences continued to be used for new samples with new colours and/or new objects (details in the right panel of Fig. 3).

These results suggest that the readiness with which the attributes are named is a partial determinant of the favoured sequences. Accuracy in numerical naming was always lower than that in object or colour naming in the three-term naming task. Moreover, number names were learned more slowly than were the names of objects and colours. It took 1,052 trials (171 min) for Ai to reach criterion in her first object discrimination task ('padlock' and 'glove'), 538 trials (105 min) for the first colour discrimination ('red' and 'green') and 1,821 trials (253 min) for the first number discrimination ('1' and '2'). In the previous stage of two-term naming, Ai had experienced both sequences, object/colour and colour/object. The previous experience may be the factor facilitating the reversible bonding of object and colour names in three-term naming. It seems that the favoured sequences in three-term naming are determined by complex factors, which include readiness with which the attributes are named and her previous experiences.

The range of numbers (1-6), the variety of sample items (at least 125 types), generalization to new items and the complex sequence of responses make it highly unlikely that the naming depends on the mere association of particular sample items and alternative keys. The present study suggests that the chimpanzee was able to match the sample items on the basis of numerosity in addition to colour and types of object.

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Co-localization of corticotropin releasing factor and vasopressin mRNA in neurones after adrenalectomy

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The discrete anatomical distribution of arginine vasopressin and corticotropin releasing factor (CRF) immunoreactivity in the paraventricular nucleus (PVN) of the rat hypothalamus is altered after adrenalectomy[1-3]. Not only is the immunostaining of both peptides enhanced, but vasopressin immunoreactivity, normally confined to the magnocellular subdivision, becomes clear in a large percentage of CRF neurones in the parvocellular subdivision[2]. These changes in immunoreactivity may reflect changes in post-translational events, peptide metabolism or genomic activity that lead indirectly or directly to the enhanced expression of vasopressin. Here we report that levels of transcripts homologous to vasopressin messenger RNA increase in the PVN after adrenalectomy, in parallel with increases in vasopressin immunoreactivity. In fact, after adrenalectomy, vasopressin mRNA can be detected in CRF-immunoreactive neurones. These results indicate that a considerable degree of plasticity is retained by the adult neuronal genome of the rat and that this plasticity may be modulated by the endocrine environment.

CRF and vasopressin act synergistically to release adrenocorticotropic hormone (ACTH) from the anterior pituitary[4]. Recently, immunohistochemical studies have demonstrated that CRF-containing fibres in the median eminence arise from cells in the parvocellular subdivision of the PVN[5], whereas the greatest density of vasopressin-containing neurones is found in the magnocellular subdivision of the PVN[6]. After adrenalectomy, the anatomical distribution of these peptides becomes less discrete; vasopressin and CRF can be found co-localized in >70% of parvocellular neurones[2]. It has been suggested that the removal of adrenal steroids accounts for these changes in neuropeptide expression[7]. Because steroids regulate peptide synthesis by modulating genomic activity[8], it seemed possible that adrenal hormones regulate neuronal synthesis of vasopressin by influencing some process preceding mRNA translation. Therefore, we examined the effects of adrenalectomy on vasopressin mRNA levels in the PVN by applying *in situ* hybridization techniques in conjunction with standard

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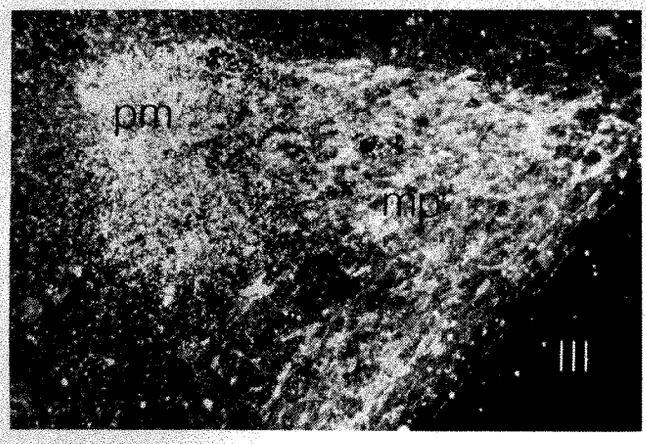


Fig. 1 Simultaneous detection of vasopressin mRNA and CRF-like immunoreactivity in the PVN of a control rat photographed under dark-field illumination. CRF-like immunoreactive neurones are confined to the medial parvocellular division (mp), whereas vasopressin mRNA (silver grains) is largely confined to the posterior magnocellular division (pm). III, Third ventricle. Scale bar, 200 μm.
Methods. Colchicine-treated (75 μg) rats were perfused with phosphate buffered saline (*p*H 7.0) followed by buffered 10% formalin containing 15% sucrose. Transverse 35-μm sections containing the PVN were cut on a freezing microtome and processed for CRF immunoreactivity using the avidin-biotin method[9] and diaminobenzidine as the chromogen. Immunostained sections were then delipidated in ethanol, rehydrated, rinsed in diethylpyrocarbonate (1 μl 5 ml^{-1}), slide-mounted and incubated overnight at room temperature with ^{32}P-labelled probe (5×10^4 c.p.m. per section) diluted in hybridization buffer (2 × SSC, 10 × Denhardt's, 50% formamide, 0.1% SDS and 0.1% denatured salmon sperm DNA). The next day, slides were rinsed in 2 × SSC for 4 h with solution changes every 15 min. Slides were then air dried and dipped in Ilford L4 nuclear research emulsion diluted 1:1 with water. Because this emulsion is fine grained, slides were exposed for 4 weeks in light-proof boxes at 4 °C. Development was performed with Kodak D-19 (diluted 1:1) for 5 min at 20 °C and followed by fixation in 30% sodium thiosulphate.

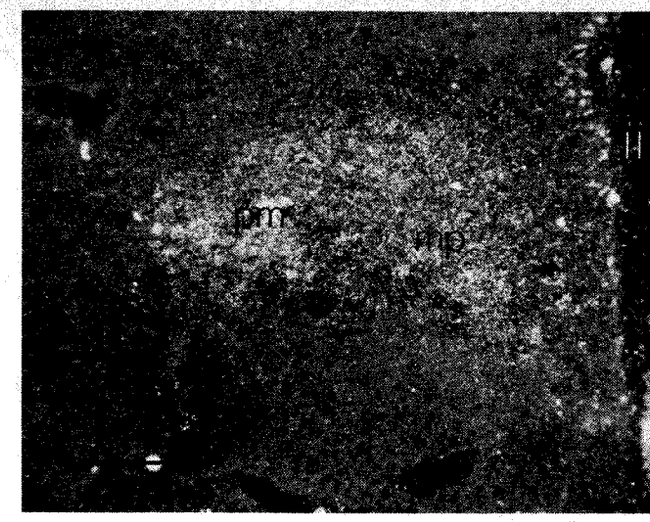


Fig. 2 Localization of vasopressin mRNA in the parvocellular PVN of an adrenalectomized rat photographed under dark-field illumination. The highest density of grains is still seen in the posterior magnocellular division (pm), but there is also a considerable amount of hybridized probe in the medial parvocellular division (mp). III, Third ventricle. Scale bar, 230 μm.
Methods. A 48-base oligonucleotide complementary to the glycoprotein region of vasopressin mRNA was prepared according to previously published methods[10]. Two different 24-mers were synthesized on an Applied Biosystems (Model 380A) DNA synthesizer and purified by reverse-phase HPLC on a μBondapak C18 column (Waters Associates). One of the 24-mers was labelled at its 5′ end with ^{32}P by reaction with [γ-^{32}P]ATP and T4 polynucleotide kinase. The 3′ end of this 24-mer was then ligated to the 5′ end of the second 24-mer, phosphorylated with ATP and T4 polynucleotide kinase, with the aid of a 17-mer holding fragment and T4 DNA ligase. The resulting 48-mer was separated from excess reactants and the complementary holding fragment by polyacrylamide gel electrophoresis in denaturing conditions. The final activity of the probe was ~10^8 c.p.m. μg^{-1}.

immunocytochemical methods. (For simplicity, the terms vasopressin mRNA and CRF- or vasopressin-immunoreactivity used here imply their putative nature.)

Adrenalectomized (5 days) and sham-operated control male Sprague–Dawley rats were colchicine-treated, perfused 48 h later and processed for vasopressin or CRF immunostaining on alternate sections, using the avidin–biotin method[9] with commercially produced antisera (Immunonuclear). *In situ* hybridization techniques with radiolabelled, synthetic oligodeoxyribonucleotide probes were then applied to detect vasopressin mRNA in the immunostained sections. The hybridization procedures used and controls for the specificity of the resultant signal were, with minor modifications, as described previously[10]. Conventional autoradiographic procedures were used to visualize hybridized probe. No differences in the hybridization signal were observed between colchicine- and non-colchicine-treated animals.

In sections from control animals, vasopressin immunoreactivity and the hybridization signal were largely confined to the magnocellular subdivision of the PVN, as expected if the probe specifically hybridized to transcripts homologous to vasopressin mRNA (Fig. 1). This localization of vasopressin mRNA was seen in all control tissue, regardless of whether vasopressin or CRF was immunostained in the section. Since vasopressin and CRF are normally distributed discretely in the PVN, the consistency of the distribution of the hybridization signal indicates that the immunoreaction product did not lead to the production of false-positive chemographic images.

Adrenalectomy induced the appearance of vasopressin immunoreactivity in dorsal and medial regions of the parvocellular PVN. Hybridized probe could also be detected in these same parvocellular regions (Fig. 2). Indeed, vasopressin mRNA could be seen in the cytoplasm of parvocellular CRF-immunoreactive neurones (Fig. 3). Adrenalectomy did not lead to the appearance of vasopressin mRNA in CRF neurones in all regions of the central nervous system (CNS) (data not shown).

These results demonstrate the ability of the endocrine environment to regulate transcript levels in a discrete region of the adult CNS. Certainly, the changes in genomic expression described here need not have been a direct consequence of decreased circulating adrenal hormones. The loss of feedback inhibition of both pituitary corticotrophs[11] and CRF-secreting neurones[5] upon adrenalectomy leaves open the possibility that elevated concentrations of ACTH or CRF contribute to the enhanced production of vasopressin mRNA. CRF has been shown to contribute significantly to the elevation of proopiomelanocortin mRNA levels seen in the pars intermedia of adrenalectomized rats[12]. Furthermore, changes in neuronal activity that might occur in response to endocrine manipulations[13,14] could themselves lead to changes in neuropeptide expression. For example, chemical depletion of adrenergic terminals in the hypothalamus leads to enhanced CRF immunoreactivity in the PVN[15]; depolarization of sympathetic ganglia is known to result in changes in their neuropeptide content[16]. Whether such indirect actions consequent to adrenalectomy also affect the observed redistribution of vasopressin mRNA remains to be determined. In contrast to the increase in vasopressin immunoreactivity reported here in the PVN after adrenalectomy, a dramatic decrease in vasopressin immunoreactivity in the adult rat habenula has been found after gonadectomy[17]. Whether this effect of gonadectomy reflects changes in genomic activity

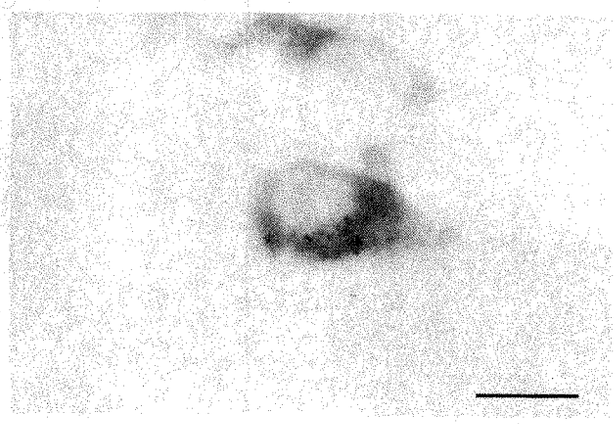


Fig. 3 CRF-immunoreactive neurone containing vasopressin mRNA. Note the confinement of the silver grains to the cytoplasm. Scale bar, 20 μm.

remains to be demonstrated. In any case, the observation that regional levels of the same neuropeptide may be differentially regulated by different humoral factors is particularly striking.

Our results show that changes in the endocrine composition caused by adrenalectomy promote the expression of vasopressin mRNA in parvocellular regions of the rat PVN. Of particular note is the induction of vasopressin mRNA in CRF-immunoreactive neurones under these conditions. These results demonstrate that differential regulation of gene expression occurs in the adult CNS in response to physiological stimuli.

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Stimulation of connective tissue cell growth by substance P and substance K

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Connective tissue cells proliferate actively when cultured in the presence of serum. Platelet-derived growth factor (PDGF), a basic protein of relative molecular mass ~30,000, has been identified as the major serum mitogen for these cells[1]; its main physiological/pathophysiological role may be to initiate wound healing in connection with tissue injury. However, growth of cultured cells is also influenced by several other factors, including epidermal growth factor[2], fibroblast growth factor[3], insulin and somatomedins[4]. Furthermore, Rozengurt and Sinnett-Smith recently showed that bombesin, a neuroendocrine peptide isolated from frog skin, stimulates DNA synthesis and cell division in cultures of a specific subtype of 3T3 cells[5]. Substance P and substance K (also known as neurokinin A or neuromedin L) are mammalian peptides belonging to the tachykinin family[6]. Substance P has been studied extensively; it is distributed widely throughout the central and peripheral nervous system[7], including primary sensory neurones[8-10], and can be released in the periphery from axon collaterals of stimulated pain fibres[11,12] and contribute to the inflammatory response[13]. Substance K is a member of the tachykinin family isolated from mammalian spinal cord[14,15]; Nawa *et al.*[16] determined the primary structure of two types of substance P precursors, one of which contained a sequence homologous to substance K, as well as the sequence of substance P. We report here that substance P and substance K stimulate DNA synthesis in cultured arterial smooth muscle cells and human skin fibroblasts, and that this stimulation is inhibited by the substance P-antagonist spantide[17].

Smooth muscle cells were isolated enzymatically from the aortic media of adult Sprague-Dawley rats[18] and fibroblasts were isolated by outgrowth from human skin explants[19]. Secondary cultures of muscle cells and cultures of fibroblasts in the 10th to 12th passage were growth-arrested by transfer to serum-free medium for 24 h. The rate of entrance of the cells into S phase of the cell cycle was determined by counting the number of autoradiographically labelled nuclei after a 24-h incubation with ^{3}H-thymidine. Exposure to medium containing 10% newborn calf serum initiated DNA synthesis in 71.8 ± 3.8% (±s.d.) of the muscle cells and in 47 ± 5.3% of the fibroblasts, compared with 18.6 ± 3.0% and 17.1 ± 2.7%, respectively, in unstimulated cultures.

Addition of substance P or substance K to serum-free medium stimulated DNA synthesis in cultured muscle cells in a dose-dependent manner, whereas addition of bombesin had no effect (Figs 1*a*, 2). Substance K was a more potent stimulator than substance P, at 10^{-6} M producing 62.0 ± 1.4% labelled nuclei while 10^{-5} M substance P produced 38.3 ± 4.4% labelled nuclei. PDGF at 25 ng ml^{-1} produced maximal stimulation of DNA synthesis in muscle cells grown in serum-free medium (42.6 ± 4.7% labelled nuclei; see ref. 18), whereas 2 ng ml^{-1} PDGF produced only a small increase (Fig. 1*a*). DNA synthesis in muscle cells stimulated with 2 ng ml^{-1} PDGF could be further stimulated by the addition of substance P or K. However, the combined effect of substance P or K and suboptimal concentrations of PDGF was less than the sum of their individual effects. Furthermore, at concentrations $>10^{-8}$ M, substance K was more active alone than in combination with suboptimal concentrations of PDGF (Fig. 1*a*).

Substance P and substance K also stimulated initiation of DNA synthesis in fibroblast cultures; no effect was observed after treatment with bombesin (Fig. 1*b*). Again, substance K was more potent than substance P. The rate of DNA synthesis in fibroblasts stimulated with suboptimal concentrations of PDGF was further enhanced after treatment with substance P or substance K, but the enhancement was lower than the individual effects of either substance (Fig. 1*b*).

To determine the specificity of the stimulating action of substance P and substance K, we performed control experiments with cultures preincubated with spantide ([D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P; Bachem), a substance P antagonist[17]. We found that the addition of spantide (3×10^{-6} M–3×10^{-5} M) to serum-free medium caused a limited stimulation of DNA synthesis, 3×10^{-5} M producing 26.4 ± 2.0% labelled nuclei, and a significant reduction of DNA synthesis induced by 10^{-7} M of substance P or substance K in both muscle cells and human fibroblasts. Spantide did not reduce the rate of DNA synthesis in cultures stimulated with 25 ng ml^{-1} PDGF (Table 1).

There is accumulating evidence that many factors are involved in the regulation of cell growth. The present results demonstrate

Table 1 Effect of the substance P analogue spantide on DNA synthesis induced by substance P (SP), substance K (SK) and PDGF

	Smooth muscle cells		Human fibroblasts	
	% Labelled nuclei	s.d.	% Labelled nuclei	s.d.
MCDB 104	18.6	3.0	17.1	2.7
MCDB 104/3×10^{-6} M spantide	17.2	2.7	15.7	3.2
MCDB 104/10^{-5} M spantide	21.1	2.1	23.8	0.4
MCDB 104/3×10^{-5} M spantide	26.4	2.0	26.1	2.3
10^{-7} M SP	40.9	0.6	32.0	1.1
10^{-7} M SP/3×10^{-6} M spantide	32.0*	2.2	28.7	2.0
10^{-7} M SP/10^{-5} M spantide	33.9	5.3	28.5*	0.4
10^{-7} M SP/3×10^{-5} M spantide	25.8	1.2	24.6	0.2
10^{-7} M SK	50.1	1.3	38.2	1.4
10^{-7} M SK/3×10^{-6} M spantide	41.5*	0.8	28.7*	3.4
10^{-7} M SK/10^{-5} M spantide	33.3	4.2	27.2	2.9
10^{-7} M SK/3×10^{-5} M spantide	27.6	1.6	23.1	1.2
PDGF 25 ng ml^{-1}	40.8	3.3	45.9	4.3
PDGF 25 ng ml^{-1}/3×10^{-6} M spantide	44.5	4.1	45.0	5.0
PDGF 25 ng ml^{-1}/10^{-5} M spantide	41.1	1.1	43.2	1.3
PDGF 25 ng ml^{-1}/3×10^{-5} M spantide	41.9	0.9	43.2	2.5

Medium MCDB 104 (Gibco) was used as control. The fraction of labelled nuclei was determined as described in Fig. 1 legend. Each value represents the mean of triplicate cultures.

* The first value in each group, significantly different ($P < 0.05$) from the control.

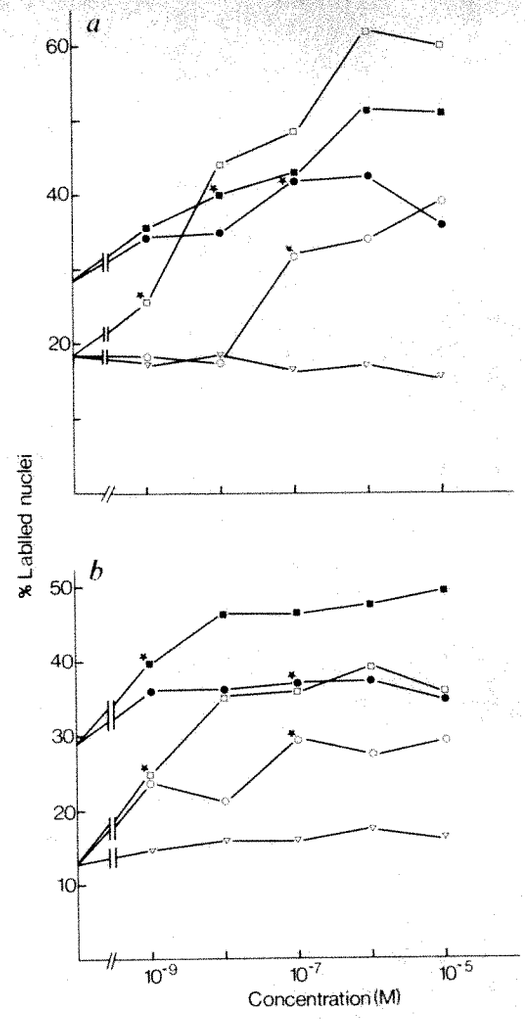


Fig. 1 Effects of substance P and substance K on initiation of DNA synthesis in smooth muscle cells (*a*) and skin fibroblasts (*b*). Subconfluent, secondary cultures of muscle cells were growth-arrested by transfer to medium MCDB 104/0.1% bovine serum albumin (serum-free medium) for 24 h. They were then incubated with 2.5 μCi ml^{-1} of ^{3}H-thymidine and various concentrations of substance P (Bachem) alone (○) or with (●) 2 ng ml^{-1} of PDGF[25] added, various concentrations of substance K (Peninsula) alone (□) or with (■) 2 ng ml^{-1} of PDGF or with various concentrations of bombesin (Peninsula) alone (▽) added for another 24 h. The cells were fixed in 3% buffered glutaraldehyde and processed for autoradiography as described elsewhere[18]. The fraction of labelled nuclei was determined by counting 500 randomly selected cells in each specimen. Values are given as means of triplicate cultures (s.d. < 20%). * Indicates the first value in each group, which is significantly different ($P < 0.05$) from the control.

that substance P and substance K, two structurally related neurotransmitters found in both the central and peripheral nervous system, may act as mitogens for connective tissue cells. The addition of 10^{-7} M of substance P alone or together with suboptimal concentrations of PDGF stimulated initiation of DNA synthesis, whereas substance K stimulated entrance to S phase at concentations as low as 10^{-9} M. Note that substance K was 100 times more potent in stimulating DNA synthesis than substance P, indicating an action via a substance P-E receptor subtype[20,21]. Substances P and K were less effective in stimulating DNA synthesis if the cells were simultaneously stimulated with suboptimal concentrations of PDGF. This lack of potentiating effect may result from the existence of similarities in the mechanisms of action of neuropeptides and growth factors. Indeed, our results indicate that PDGF decreases the stimulatory effect of substance K in smooth muscle cells, which suggests a complex interaction between the mitogenic effect of PDGF and substance K. DNA synthesis induced by substance P or substance K was markedly reduced by incubation with spantide, an analogue of substance P with antagonistic activity, suggesting a competitive inhibition of receptor binding. Spantide alone had a small stimulatory effect, consistent with the previously reported agonistic activity of the substance P analogues[7]. It is interesting that bombesin, which has two C-terminal amino acids in common with substance P and substance K, failed to induce DNA synthesis. We therefore conclude that the stimulatory effect is mediated via specific tachykinin receptors.

The mechanisms by which substance P and substance K stimulate DNA synthesis remain to be determined. However, it is interesting that both substance P[22] and PDGF[23] have been found to stimulate hydrolysis of phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol trisphosphate and that inositol trisphosphate has recently been shown to function as a second messenger for mobilization of intracellular calcium[24], which is believed to participate in regulation of cell proliferation.

The present data suggest a possible role for tachykinins in wound healing. It is tempting to speculate that substance P and/or substance K released from peripheral neurones in connection with tissue injury not only contribute to the inflammatory response but also could help other mitogens, such as PDGF, to stimulate proliferation of surrounding connective tissue cells and thus initiate a healing response.

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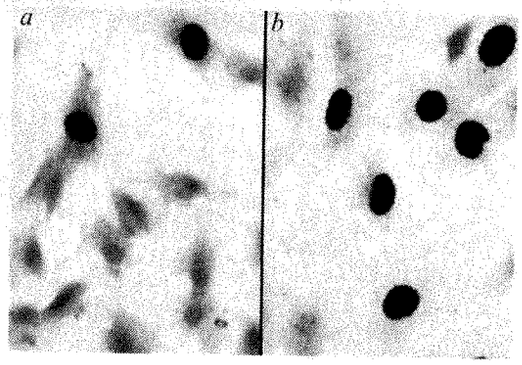


Fig. 2 Micrographs of representative fields of autoradiographs from a culture of smooth muscle cells treated with serum-free medium (*a*) or 10^{-6} M substance K (*b*). ×600.

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Voltage dependence of Na/K pump current in isolated heart cells

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The Na/K pump usually pumps more Na^+ out of the cell than K^+ in, and so generates an outward component of membrane current[1,2] which, in the heart, can be an important modulator of the frequency and shape of the cardiac impulse[3-5]. Because it is electrogenic, Na/K pump activity ought to be sensitive to membrane potential, and it should decline with hyperpolarization[6-9]. However, such voltage dependence of outward pump current has yet to be demonstrated[6-10], one reason being the technical difficulty of accurately measuring pump current over a sufficiently wide voltage range. The whole-cell patch-clamp technique[11] allows effective control of both intracellular and extracellular solutions as well as membrane voltage. Applying this technique to myocardial cells isolated from guinea pig ventricle, we have measured Na/K pump current between −140 mV and +60 mV, after minimizing passive currents flowing through Ca^{2+}, K^+ and Na^+ channels. We report here that strongly activated pump current shows marked voltage dependence; it declines steadily from a maximal level near 0 mV, becoming very small at −140 mV. Pump current–voltage relationships will provide essential information for testing models of the Na/K pump mechanism and for predicting pump-mediated changes in the electrical activity of excitable cells.

We used wide-tipped patch pipettes to modify intracellular ionic composition so as to reduce outward K^+ currents[12], by lowering the pipette K^+ concentration ($[K^+]_{pipette}$) to 10 mM (K^+ replaced by Cs^+) and by adding 20 mM tetraethylammonium (TEA). To diminish inward currents in Ca^{2+} and K^+ channels, external solutions were nominally Ca^{2+}-free and included small amounts of Cd^{2+}, Ba^{2+} and Cs^+. The resulting low membrane conductance facilitated the control of membrane potential over an extensive range and enhanced the signal-to-noise ratio for detection of Na/K pump current.

Rod-shaped, quiescent ventricular cells were obtained by collagenase digestion of guinea pig hearts, followed by enzyme-free incubation in high $[K^+]$, low $[Ca^{2+}]$ medium[13,14]. The cells were superfused at 36 °C with normal Tyrode's solution containing (in mM): 144 NaCl, 5.4 KCl, 0.3 NaH_2PO_4, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.5 dextrose, 5 HEPES (*p*H 7.4). Giga-ohm seals were obtained using wide-tipped (4–5 μm), fire-polished pipettes filled with the same Tyrode's solution (pipette resistance ~1 MΩ), then, before rupturing the membrane patch, the pipette solution was exchanged[15] for one containing (in mM): 100 Cs-aspartate, 10 $Tris_2ATP$, 5 K_2-creatine phosphate, 1 NaH_2PO_4, 20 TEA-Cl, 5.5 $MgCl_2$, 5 EGTA, 5 HEPES (*p*H 7.4). After rupture of the patch, the holding potential was set at −40 mV to inactivate Na^+ channels and, after allowing ~5 min for the cell interior to equilibrate with the pipette solution (see Fig. 1*A*), the cells were superfused with nominally Ca^{2+}-free Tyrode's solution ($CaCl_2$ replaced by 1.8 mM $MgCl_2$, 0.9 mM $BaCl_2$, 0.2 mM $CdCl_2$ and 1 mM CsCl). In these conditions, membrane currents elicited by voltage steps were small and largely time-independent (Figs 1*B*, 2*A*), confirming that channel currents were depressed.

Figure 1*A* shows that a sudden increase in $[Na^+]_{pipette}$ from 1 mM to 34 mM (Na^+ replacing Cs^+) caused a rapid outward shift of holding current which was completely abolished by external application of 10 μM ouabain. The fact that ouabain specifically inhibits the Na/K pump suggests that, at −40 mV, the entire outward current shift caused by raising the pipette (and, hence, intracellular) $[Na^+]$ results from Na/K pump activation. To determine effects at other voltages, we applied 300-ms clamp pulses to potential levels between −140 mV and +60 mV. Figure 1*B* shows samples of the currents obtained in the presence of high pipette $[Na^+]$ immediately before (*a*) and just after (*b*) pump inhibition by ouabain; *c* shows the ouabain-sensitive components obtained by digital subtraction of the corresponding traces in *a* and *b*. These difference traces already indicate that the size of the ouabain-sensitive current varies with membrane potential.

The detailed voltage dependence of the ouabain-sensitive current is illustrated in Fig. 2. The different symbols in Fig. 2*A* show current levels measured during the four data-collection periods numbered in Fig. 1*A*. The currents in the presence of ouabain (●) are practically superimposable on those recorded with $[Na^+]_{pipette}$ of 1 mM (△), arguing that, over the entire voltage range examined, the outward current elicited by raising the intracellular $[Na^+]$ results from activation of the Na/K pump. The data points in Fig. 2*B* show the amplitudes of the difference currents, obtained by digital subtraction of the currents in Fig. 2*A*: triangles show the Na^+-activated current, and the open and solid circles show the ouabain-sensitive current determined, respectively, by washing on, and washing off, ouabain. All three sets of points demonstrate that the ouabain-

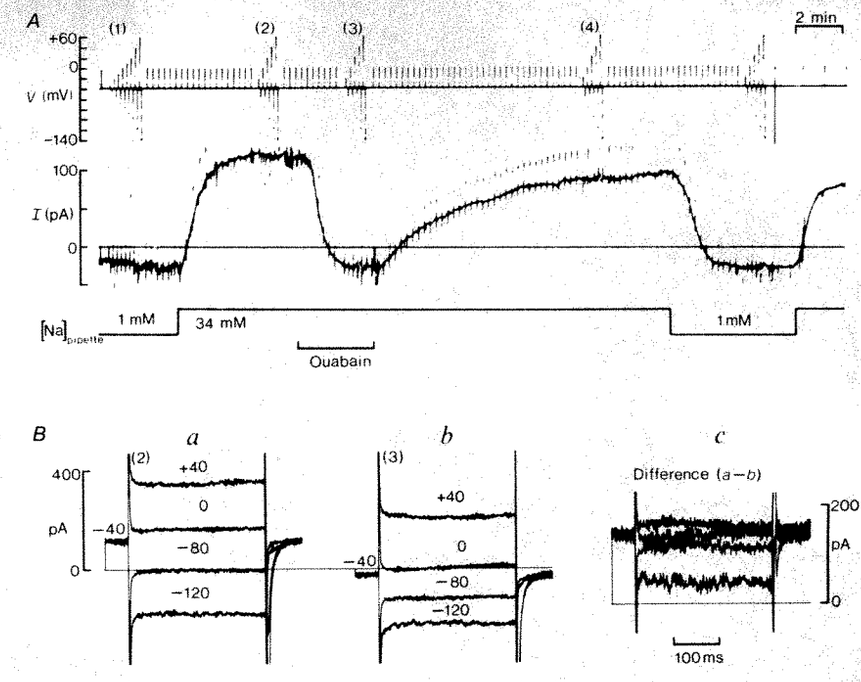


Fig. 1 Current changes due to activation or inhibition of the Na/K pump in a ventricular cell superfused with 5.4 mM K^+, Ca^{2+}-free Tyrode's solution. *A*, Chart recording: upper trace, membrane potential; middle trace, membrane current; the lower line indicates approximate timing of step changes in $[Na^+]_{pipette}$ between 1 and 34 mM, and the bar beneath it marks the exposure to 10 μM ouabain; the numbers above the voltage trace indicate acquisition of four sets of current–voltage data. To change the pipette solution[15], negative pressure (~ −30 cm H_2O) draws fluid from a miniature reservoir through fine silastic tubing and delivers it to within 200 μm of the pipette tip via a narrow, tapered polythene catheter attached to a 25-gauge stainless steel tube. A constriction device closes the silastic tube during transfer between reservoirs. To exchange the <20 μl volume between pipette tip and reservoir takes ~30–60 s. *B*, Superimposed sample current records taken from *A*, just before (2)(*a*) and during (3)(*b*) exposure to ouabain. The pulse potential is indicated for each record; holding potential −40 mV. Records were low-pass-filtered at 2 kHz and digitized at 0.4-ms intervals. *c*, Superimposed difference currents obtained by computer subtraction of each record in *b* from its counterpart in *a*; pulse potentials were, from top to bottom, 0, +40, −80 and −120 mV. Note the expanded current scale in *c*.

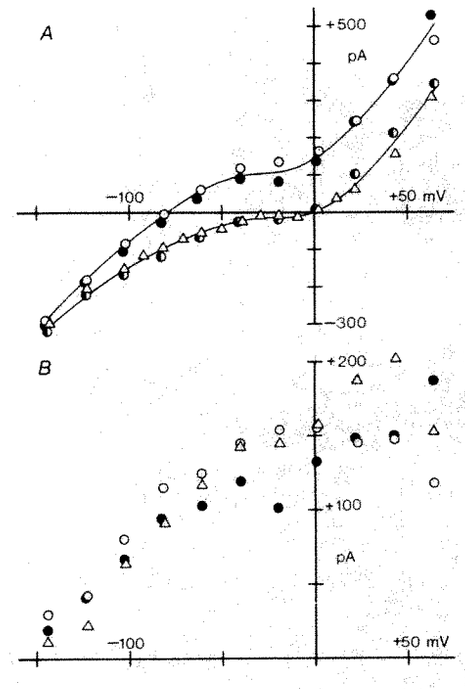


Fig. 2 Voltage dependence of current associated with Na/K pump activity. Data from the experiment in Fig. 1. *A*, Net membrane currents in the presence and absence of Na/K pump activity. Ordinate, steady current levels, obtained by averaging digitized values over the final ~100 ms of each 300-ms pulse. Abscissa, membrane potential during the pulse. The data were recorded during the periods numbered in Fig. 1*A*: △ (1), 1 mM $[Na^+]_{pipette}$; ○ (2), 34 mM $[Na^+]_{pipette}$; ◐ (3), 34 mM $[Na^+]_{pipette}$ during exposure to ouabain; ● (4), 34 mM $[Na^+]_{pipette}$ after recovery from ouabain. Arbitrary curves were fitted to the points by eye. *B*, Steady-state levels of difference currents derived by subtracting currents recorded in the absence of Na/K pump activity from those recorded, at the same voltage, in its presence. Ordinate, amplitudes measured as in *A*; abscissa, membrane potential. The three sets of points are: △ (2)−(1), current activated by raising $[Na^+]_{pipette}$; and ouabain-sensitive current on ouabain application [○, (2)−(3)] and ouabain withdrawal [●, (4)−(3)]. Because these ventricular cells have a flat, rectangular shape, the apparent cell surface was approximated as 2 × mean width × mean length. Comparison with cell capacitance measurements, however, suggests that actual membrane area is 2–3 times greater than this approximation, presumably because of T-system and sarcolemmal caveolae. The true pump current density is thus probably 2–3 times smaller than that estimated here.

sensitive current attains a maximal amplitude near 0 mV and, at negative potentials, declines roughly linearly with voltage towards an extrapolated zero-current potential near ~ −160 mV. Very similar current–voltage characteristics were obtained in a total of nine cells ($[Na^+]_{pipette}$ 34–41 mM) exposed to 5.4 mM K solution to which 10–20 μM ouabain or 50 μM strophanthidin was added to inhibit the Na/K pump; the zero-current potential estimated by linear extrapolation averaged −156 ± 14 mV (±s.d.), and the current amplitude at 0 mV was +160 ± 69 pA per cell (equivalent to 3 ± 1 μA cm^{-2} if related to apparent cell surface, but see Fig. 2 legend).

Before we can conclude that cardiotonic steroid-sensitive current is simply Na/K pump current, possible contributions from two other sources must be considered. One possibility, that cardiotonic steroids alter membrane current other than via their effect on the Na/K pump, is eliminated by the current–voltage relationships in Fig. 3*A*, which show that if the pump is first inhibited by exposure to K^+-free solution, application of 50 μM strophanthidin has no effect. A second possibility is contamination by an indirect effect of pump inhibition. Thus, despite our precautions to minimize K^+-channel currents, the cardiotonic steroid-sensitive current (and also the Na^+-activated current) may still contain a component reflecting changes in K^+ concentration in an unstirred extracellular space, caused by the

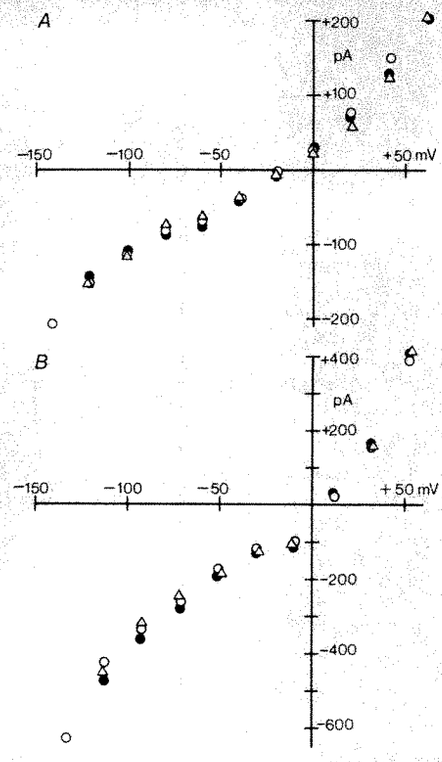


Fig. 3 Control current–voltage relationships demonstrating that, in the absence of Na/K pump activity, membrane current is unaffected either by application of strophanthidin (*A*), or by a 10-fold change in external $[K^+]$(*B*). *A*, 54 min after raising the $[Na^+]_{pipette}$ to 41 mM, the Na/K pump was inhibited by exposure to K^+-free solution, causing a 143-pA reduction in current at 0 mV. Current–voltage relationships were then determined before (○), during (△) and after (●) exposure to 50 μM strophanthidin. The points show steady current amplitudes measured as in Fig. 2. Holding potential −40 mV. *B*, 9 min after raising $[Na^+]_{pipette}$ to 34 mM, 20 μM ouabain inhibited the Na/K pump, causing a 118-pA inward shift of current at 0 mV. Current–voltage relationships were then determined in 5.4 mM K^+ (○), 1 mM K^+ (△) and 10 mM K^+ (●) external solutions, Na^+ replacing K^+ or vice versa. The points show steady currents. Holding potential −30 mV.

changes in pump activity. Figure 3*B* confirms, however, that once the pump is inhibited (in this case by 20 μM ouabain), a 10-fold change in external $[K^+]$ has virtually no effect on membrane current.

We conclude, therefore, that Fig. 2*B* illustrates faithfully the Na/K pump current–voltage relationship at high pump rates; its general shape conforms to thermodynamic expectation[6–8], as do our preliminary results indicating that a rightward shift of the pump current–voltage relationship accompanies the reduction in pump current caused by lowering either $[Na^+]_{pipette}$ or external $[K^+]$. Detailed analysis will require higher resolution current measurements to be made, probably using averaging techniques, under various ionic and metabolic conditions.

That such marked voltage dependence of outward Na/K pump current (Fig. 2*B*) has not been documented previously is probably attributable both to the limited voltage ranges explored (for example, −20 to −90 mV for cardiac preparations[4,10]), and to the difficulty of clearly distinguishing pump current from other currents. For example, pump-mediated changes in extracellular $[K^+]$ should act via inwardly rectifying K^+ channels to distort current–voltage relations of the type shown in Fig. 2*A* by making inward K^+ currents smaller at high, than at low, pump rates. This would result in more parallel current–voltage relations than those in Fig. 2*A*, so that the difference currents (see Fig. 2*B*) would show less voltage dependence. Voltage sensitivity of inward current generated by the backward-running Na/K pump in squid axons has been reported recently[16], albeit in the opposite direction to that expected on simple thermodynamic grounds.

The rapid reversal of ouabain action shown here (Fig. 1*A*; strophanthidin is still severalfold faster) contrasts with its poor reversibility in intact Purkinje fibres from sheep and dog hearts (for example, see ref. 17), but it is not yet clear whether this reflects a mere species difference or results from collagenase treatment of the cells.

Na/K pump current contributes significantly to the electrical activity of cardiac cells[4,5]; a sustained increase in heart rate, for example, leads to Na/K pump-mediated hyperpolarization and abbreviation of the cardiac action potential[3,5]. Full analysis or simulation of such effects in heart, nerve or muscle cells requires knowledge of the voltage dependence of the Na/K pump current. Accurate determination of pump current–voltage relationships under various conditions is thus vital to our understanding of both the mechanism of the Na/K pump and its role in modifying electrical activity.

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NMR technique for assessing contributions of heavy and light chains to an antibody combining site

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Nuclear magnetic resonance (NMR) has been used extensively to study the structure of antibody combining sites[1,2]. In recent studies we have observed the proton resonance spectra of the Fab fragment of a monoclonal anti-spin-label antibody derived from a hybridoma grown on various specifically deuterated amino acids[3,4]. The broadening of the proton resonance signals by the paramagnetic hapten, together with selective deuteration, has allowed the identification of most of the amino acids in the combining-site region of this antibody and has also provided estimates of distances between amino-acid protons and the unpaired electron[5]. Here we show how recombination of specifically deuterated heavy and light chains permits the assignment of single amino-acid proton resonance signals to either the heavy or light chain. In addition, the spectra of such recombinants demonstrate that their combining-site structures must be almost identical to the native structure.

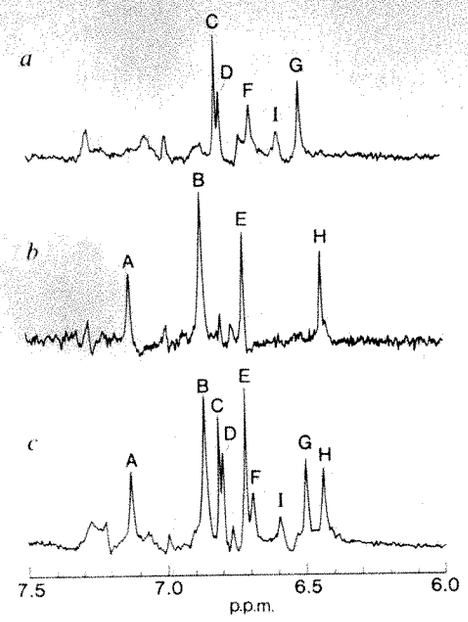


Fig. 1 NMR difference spectra for selectively deuterated Fab fragments of a monoclonal anti-spin-label antibody. *c*, Signals A–I are each due to pairs of protons in the 3,5 ring positions of tyrosine residues in the combining-site region of the ANO2 antibody. Spectra *a* and *b* give the corresponding signals from protons in the light chain and heavy chain, respectively. Note that the spectrum in *c* is, to a good approximation, the sum of the spectra in *a* and *b*. (See text for details.)

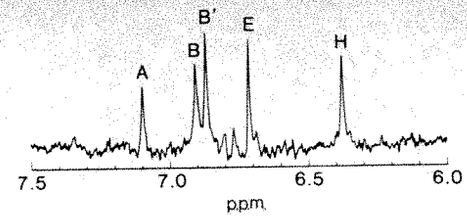
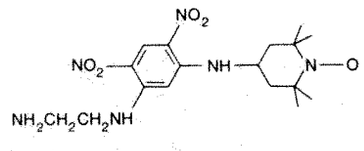


Fig. 2 NMR difference spectrum for a selectively deuterated Fab fragment of a monoclonal anti-spin-label antibody. The proton resonance signals arise from pairs of protons in the 3,5 positions of the tyrosine residues of the Fab heavy chain. These resonance signals are the same as those in Fig. 1*b*, except that in the present case the combining site is occupied by the chemically reduced non-paramagnetic form of the spin-label hapten (the hydroxylamine) whereas the signals in Fig. 1*b* arise from a combining site that is unoccupied. Note that the presence of the hapten leads to splitting of signal B in Fig. 1*b* (due to two tyrosines and four protons) into two signals (B and B') in Fig. 2.

The murine IgG_1 monoclonal anti-spin-label antibody (ANO2) was derived from a hybridoma grown on specifically deuterated amino acids, as described previously[3]. Two preparations of Fab were obtained: one (Fab I) was obtained from the hybridoma grown on perdeuterated tryptophan, perdeuterated phenylalanine and perdeuterated tyrosine; the second (Fab II) was obtained from the hybridoma grown on perdeuterated tryptophan, perdeuterated phenylalanine, and tyrosine deuterated at the 2,6 ring positions. The Fab fragments, prepared as described previously[3], were partially reduced and alkylated. The heavy (H)-chain fragment and the light (L) chain were separated on a DEAE column under denaturing conditions (8 M urea, Tris-glycine buffer, *p*H 8.2), then the heavy-chain fragments and light chains were recombined to produce the recombinants H(I)L(II) and H(II)L(I). The urea solution was made 1 M in propionic acid. The recombinants were dialysed against 1 M propionic acid and then twice against each of the following: water, 5 mM acetate buffer *p*H 5.8, 5 mM acetate buffer *p*H 5.8 containing 0.15 M NaCl, and phosphate-buffered saline *p*H 7.2 (at least 12 h for each). A similar procedure for reconstitution of a whole antibody molecule has been described previously by several groups[6,7].

Figure 1 shows the proton resonance difference spectra ΔH(II)L(II), ΔH(II)L(I) and ΔH(I)L(II), where Δ = proton resonance spectrum of Fab − proton resonance spectrum of the Fab fragment with paramagnetic spin-label hapten bound. The paramagnetic spin-label hapten has the formula



The proton resonance signals in Fig. 1*a* arise from the 3, 5 protons of tyrosines in the light chain that are in the combining-site region (within 17 Å of the unpaired spin[5]). The signals in Fig. 1*b* are due to the 3,5 protons of the tyrosines of the heavy chain in the combining-site region.

That the spectrum in Fig. 1*c* is very nearly the sum of the spectra in Fig. 1*a* and *b* represents compelling evidence that the combining-site structures are virtually the same in all three cases; this is because resonance positions, intensities and linewidths are sensitive to conformation, motional freedom and environment. A similarity in structure between native and reconstituted IgG was observed previously using circular dichroism and optical rotatory dispersion[7,8].

Our spectra show that there are five tyrosines in each chain in the combining-site region. Signal I from the light chain is weaker because it is farther from the unpaired electron. In each tyrosine signal the two proton resonance lines are equivalent due to rapid rotation of the aromatic ring. Similar conclusions are reached from the proton resonance spectra of the Fab fragment containing tyrosine deuterated in the 3,5 positions (data not shown), that is, there are five tyrosines in each chain, and the conformation of the combining site in this reconstituted Fab is very similar to the conformation in the native Fab.

Signal B in Fig. 1*b* arises from two tyrosines, as is evident from the observed intensity. Figure 2 shows that this signal is split in a difference spectrum, Δ = spectrum of Fab containing the reduced spin label (nitroxide to hydroxylamine) − resonance spectrum of Fab to which the paramagnetic spin label is bound. Interestingly, all the tyrosine proton resonance signals in Fig. 2 remain sharp, and some shift in resonance position when the non-paramagnetic hapten is in the combining site. Presumably, some of these tyrosine residues are in contact and/or are hydrogen-bonded to the hapten. Nonetheless, the motional freedom remains large enough to yield sharp resonance signals (4–7 Hz linewidth at half height).

In previous studies using double difference spectra, we demonstrated the presence of three to five threonines in the combining-site region[4]. In the present study we have used double difference spectra involving two recombinant Fab fragments to establish the presence of two threonine residues on the light chain and two to three threonine residues on the heavy chain in the combining-site region. One Fab fragment was not labelled and the other had deuterated threonine in the light chain (data not shown).

Note that the 'combining-site region' is defined as the region within 17 Å of the unpaired electron, and that proton resonance signals in this region are obtained from the difference spectra Δ = proton resonance spectrum of Fab − proton resonance spectrum of Fab with paramagnetic spin-label hapten bound. The geometry of the hapten is such that the protons of all amino acids in contact with the hapten are included in this combining-site region. The 'combining site' is thus fully included within

the 'combing-site region.' Integration of the difference spectra and assignment of the resonances to specific types of amino acids reveal that the difference spectra represent contributions from ~50 amino acids[3,4], while the entire Fab fragment contains 440 amino acids. It is probable that some, or all, of the tyrosine residues have a significant role in affecting the structure of the combining site, irrespective of whether they are in contact with the hapten or not.

The present study demonstrates that NMR, coupled with specific amino-acid deuteration and with heavy- and light-chain recombination, can be used to establish the contributions of the heavy and light-chain amino acids of an IgG monoclonal antibody to the combining-site region, provided the relevant hapten or antigen produces a suitable perturbation of nuclear resonance signals. Therefore, NMR studies offer the possibility of obtaining a working model of the combining-site structure of the ANO2 monoclonal antibody when the complete amino-acid sequence beomes available. The difference between the spectra in Fig. 1*b* and Fig. 2 indicates the information potentially available for non-paramagnetic haptens/antigens. Some of the resonances in the spectrum in Fig. 2 undergo changes in chemical shift relative to their positions in the spectrum in Fig. 1*b*. These resonances show up in a difference spectrum; Δ = spectrum with reduced spin label − spectrum without hapten, implying that useful information on combining-site structure may sometimes be obtained even with non-paramagnetic haptens.

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Comparison of α-tropomyosin sequences from smooth and striated muscle

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Tropomyosins are a closely related family of proteins with a dimeric α-coiled-coil structure[1]. Skeletal isoforms are composed of two types of subunits, α and β[2-4] which, in turn, are assorted into two main molecular species αα and αβ[5-7]. Both isoforms are present in different molar ratios in individual skeletal muscle types[8,9]. In small mammals, however, only α-chain is expressed in cardiac muscle[8]. Tropomyosin, in association with the troponin complex (troponin-I, -T and -C) plays a central role in the Ca^{2+}-dependent regulation of vertebrate striated muscle contraction[10-12]. On the other hand, despite structural similarities with the striated isoforms, the function of this protein in smooth muscle and non-muscle cells remains unknown, because in these cells contraction is thought to be regulated by myosin-linked processes independently of tropomyosin[13-15]. Here we report the nucleotide sequences of cloned complementary DNAs for rat striated and smooth muscle α-tropomyosin. Comparison of the derived amino-acid sequences reveals the existence of tissue-specific peptides that delimit the putative troponin-I and troponin-T binding domains of tropomyosin. S_1-nuclease mapping studies reveal the existence of three distinct α-tropomyosin messenger RNA isoforms each encoding a different protein; these isoforms are tissue-specific, developmentally regulated and most probably encoded by the same gene.

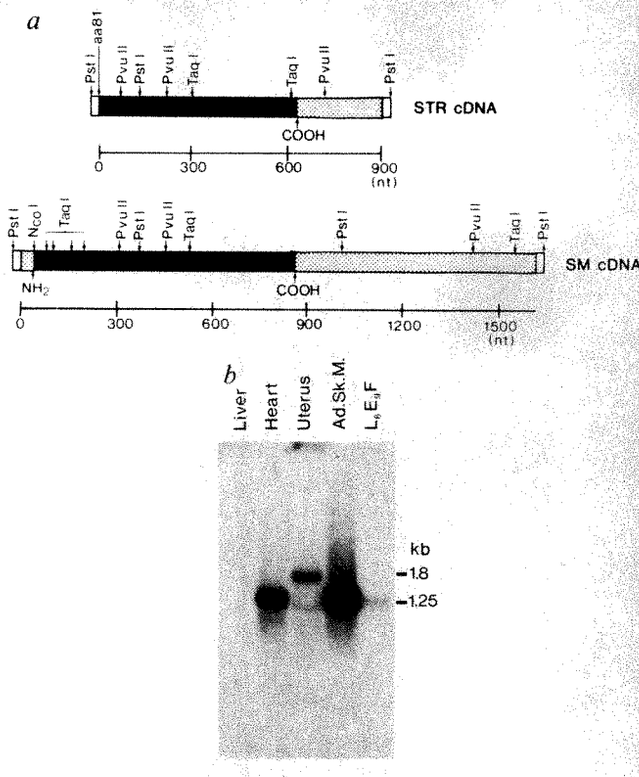


Fig. 1 Isolation and characterization of rat smooth and striated muscle α-tropomyosin cDNA clones. *a*, Partial restriction endonuclease map of striated (STR) and smooth (SM) muscle cDNA clones. Solid, stipled and open bars indicate amino-acid coding, 3′ and 5′ untranslated regions and GC tails, repsectively. The position of the first (residue 81, $-NH_2$) and last ($-COOH$) codons are indicated. The uterus-derived cDNA clone (~1.7 kb) contains mRNA sequences starting at the poly(A) track and extends all the way through the 5′ untranslated region. The striated muscle-derived cDNA clone (~0.9 kb) spans the mRNA from the poly(A) track up to codon 81. *b*, Hybridization of the smooth muscle cDNA clone to a panel of total cellular RNAs (20 μg per lane) isolated from different tissues. The ^{32}P-labelling (nick-translation) hybridization and blotting procedures were performed as described previously[40]. The size of the two hybridizing mRNA species is indicated in kb. Ad. Sk. M. and L_6E_9F represent hind leg adult skeletal muscle and L_6E_9 myotubes[16], respectively. The cloning of striated and smooth muscle cDNA was performed with mRNA–DNA hybrids annealed to pBR322 essentially as described[41,42] using RNA isolated by the hot phenol procedure[43] from adult rat hind leg and gravid uterus. One α-tropomyosin cDNA clone was isolated from each cDNA library using a previously characterized cDNA clone which cover residues 80–120 in the α-tropomyosin sequence[44].

Partial restriction maps of smooth and striated muscle α-tropomyosin cDNA clones are shown in Fig. 1*a*. The uterus-derived cDNA clone, of some 1.7 kilobases (kb), contains mRNA sequences starting at the poly(A) track and extends all the way through the 5′-untranslated region. The striated muscle-derived cDNA clone (~0.9 kb) spans the mRNA from the poly(A) track up to residue 80. To ascertain the tissue-specificity of the two cDNA clones, we performed blot analysis of RNA isolated from different tissues (Fig. 1*b*). Using the smooth muscle cDNA clone as probe, two size-classes of α-tropomyosin mRNA were detected: a 1.25-kb mRNA present in adult ventricular

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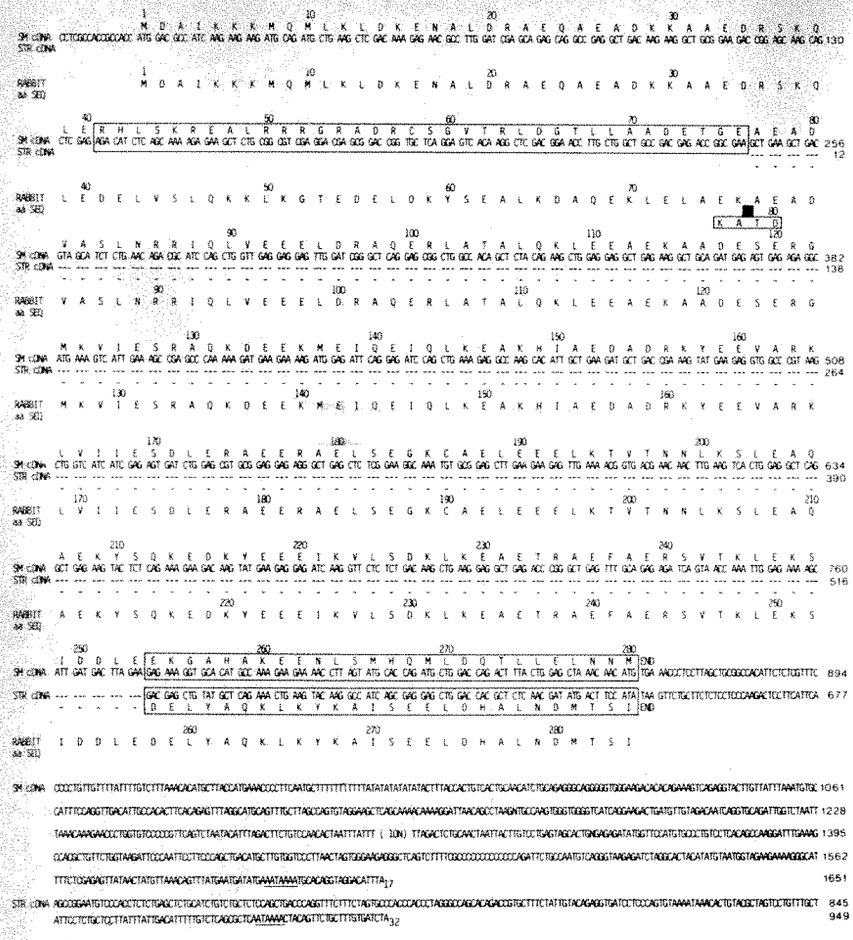


Fig. 2 Nucleotide and derived amino-acid sequence comparison of smooth and striated muscle α-tropomyosin cDNA clones. Nucleotide sequence of the smooth and striated muscle α-tropomyosin-cDNA clones were determined by cloning of gel-purified restriction fragments from both cDNA clones into M13 mp cloning vectors and subsequently sequenced according to Sanger *et al.*[45]. The deduced amino-acid sequences for the smooth and striated muscle α-tropomyosins are shown on top and underneath, respectively, of the corresponding nucleotude sequences. The AATAAA sequence of both cDNAs are underlined. Note that a second AAATAAA sequence is located 75 nucleotides upstream of the used one in the striated muscle cDNA sequence, suggesting the existence of two different poly(A) addition sites. The isotype-switch peptides sequences are boxed (residues 41–76 and 254–280 of the smooth muscle isoform). Most of the striated muscle cDNA sequence (residues 77–253, smooth muscle isoform) is shown as dotted lines indicating identity with the analogous region of the smooth muscle isoform. The rabbit α-tropomyosin residue sequence[3] has been aligned with the rat α-tropomyosin isoforms by boxing the extra four amino acids out of the linear arrangement of the sequence.

myocardium, uterus, skeletal muscle and L_6E_9 myotubes[16] (L_6E_9F) and a 1.8-kb-long mRNA expressed mainly in uterus. Uterine cells contain the highest concentration of the 1.8-kb mRNA and the lowest of the 1.25-kb species, whereas the converse is true for heart and skeletal muscle. Longer exposure of the L_6E_9F lane also shows that, although both mRNAs are present in the myotubes of this clonal cell line, the 1.25 kb species is predominant. Based on the differences in size and tissue distribution, the 1.25- and 1.8 kb mRNA species probably represent the mRNA coding for the striated and smooth muscle α-tropomyosin mRNA isoforms, respectively.

Examination of the nucleotide sequences shown in Fig. 2 reveals several new structural features of tropomyosin proteins. First, the smooth muscle α-tropomyosin is 280-residues long and 4 residues shorter than the rabbit skeletal muscle isoform[3]. Second, the rat striated muscle isoform is identical in amino-acid sequence to its rabbit counterpart, at least throughout the regions which have been compared (residues 81–284 of the rabbit molecule). Third, the comparative analysis between the smooth and striated muscle isoforms uncovers 'isotype-switch peptides' that reside in different positions along the protein molecule. The striated muscle α-tropomyosin is identical in amino-acid sequence to the smooth muscle isoform except for residues 258–284 (striated muscle sequence) that defines a carboxy-terminal 'isotype-switch peptide'. In addition, comparison of the rabbit striated muscle α-tropomyosin with the rat smooth muscle isoform shows them to be identical in sequence except for two regions: from residues 41–80 (striated muscle sequence), which depicts an amino-proximal isotype-switch peptide, and the carboxy-terminal isotype-switch peptide discussed above. By S_1 nuclease mapping, we have demonstrated that the rat striated muscle α-tropomyosin also has the amino-proximal isotype switch peptide in common with rabbit striated muscle (data not shown). Further inspection of the smooth muscle α-tropomyosin amino-acid sequence in the amino-proximal isotype-switch peptide (smooth muscle residues 41–76) reveals that the 3–4–3–4-periodicity of hydrophobic residues characteristic of the tropomyosin proteins[17,18] is interrupted significantly at positions 46 (R), 50 (R), 53 (G), 57 (R) and 60 (G) (Fig. 2). The occurrence of such residues, especially those that are charged, could lead to a local disruption of the α-helix, which has been suggested to affect the stability of the coiled-coil at the protein level[19]. These local differences in structure could alter the interaction of the smooth muscle α-tropomyosin with other components of the contractile apparatus.

Recently, it has been suggested that the binding of troponin-I to tropomyosin enhances cooperatively the binding of troponin-T to tropomyosin[20]; however, the location of the troponin-I binding site on tropomyosin has not yet been determined. The interaction of tropomyosin with troponin-T seems to occur near Cys 190 (site 2)[21–27] and at a site close to or at the carboxy-terminus (site 1) of the tropomyosin molecule[28,29]. As shown in Fig. 2, the only difference in primary sequence between the smooth and striated α-tropomyosins resides on the isotype-switch peptides. Therefore, it is likely that these two peptides specify the troponin-I (residues 41–80) and troponin-T site 1 (residues 258–284) binding domains, respectively. These isotype-specific peptides also can explain the observed differences in actin binding and in the actin activation of myosin between the two α-tropomyosin isoforms[30]. At the nucleotide level, the most striking observation from the comparative analysis between the striated and smooth muscle cDNA clones is their identity in the region coding for residues 81–257 (striated muscle sequence) (Fig. 2). In contrast, sequences located 3′ from codon 257 are unique for each cDNA. The fact that in the common amino-acid coding sequence of the two mRNAs there is not a

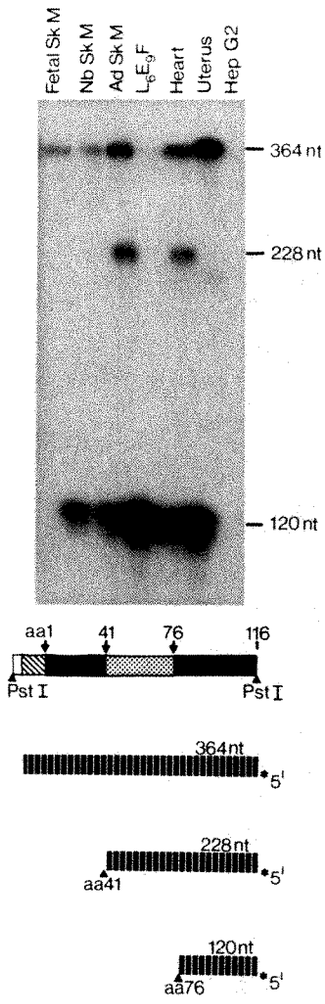


Fig. 3 Developmental and tissue-specific expression of α-tropomyosin mRNAs. The S_1 nuclease mapping studies were done under condition essentially described by Mahdavi *et al.*[46]. The 5′ end-labelled fragment was hybridized as a double-stranded probe to 30 μg total RNA under RNA-loop conditions[47,48]. After hybridization, the mixture was incubated with 600 U m^{-1} of enzyme and the S_1 nuclease-resistant products were analysed by 6% polyacrylamide/8.3 M urea gel electrophoresis[46] and their sizes estimated by comparison with 0X174 size standards. RNA samples isolated from 18-day-old fetal skeletal muscle (fetal Sk.M.), newborn skeletal muscle (Nb.Sk.M.), adult skeletal muscle (Ad.Sk.M.), adult ventricular myocardium (heart), gravid uterus (uterus), L_6E_9 myotubes (L_6E_9F) and hepatocellular carcinoma cell line ($HepG_2$) as a negative control were analysed. The 5′ end-labelled probe was generated by end-labelling of the restriction fragment with [γ-^{32}P]ATP (NEN; specific activity 3,000 Ci m-mol^{-1}) using T_4 polynucleotide kinase (Boehringer Mannheim). The 364-base pair long 5′-most *Pst*I/*Pst*I restriction fragment from the smooth muscle cDNA clone (Fig. 1*a*), labelled at the 5′ end with [γ-^{32}P], was used in the S_1 nuclease mapping studies. This probe contains the 16 nucleotides of the 5′ untranslated region of the smooth muscle cDNA down to codon 116, thus including the sequence coding for the amino-proximal isotype-switch peptide (smooth muscle sequence residues 41–76). The *Pst*I site at codon 116 lies in a region which is common to both types of cDNAs. Therefore, protection from S_1 nuclease digestion of the full-length probe (364 bases) indicated hybridization to homologous smooth muscle α-tropomyosin mRNA, whereas protection of a 120-base-long fragment represents hybridization to the striated muscle α-tropomyosin mRNA. Protection of subfragments other than 120 bases long would indicate the existence of new α-tropomyosin isoforms that diverge from the smooth muscle sequence at positions different from the striated muscle isoform.

single nucleotide substitution (even at the wobble position) strongly suggests that a single gene specifies the expression of these two tissue-specific α-tropomyosin isoforms by a complex process of alternative splicing. This possibility has been confirmed recently by the isolation of a single α-tropomyosin gene that contains all the common and isotype-specific sequences of the cDNA clones (N.R.-O. and B.N.-G., in preparation).

To study the developmental and tissue-specific expression of the α-tropomyosin mRNAs, S_1-nuclease mapping analysis of RNA isolated from different rat tissues was performed as described in Fig. 3 legend. Three distinct fragments are protected from S_1-nuclease digestion by skeletal and ventricular adult muscle RNA. Two of these fragments, 120- and 364-bases long, are consistent with the sizes predicted to be protected by striated (α_1 tropomyosin) and smooth muscle (α-tropomyosin) mRNAs, respectively. The third band, 228-bases long, demonstrates the existence of a new α-tropomyosin mRNA (striated muscle α_2 tropomyosin) that diverges from the smooth muscle cDNA probe at the region corresponding to codon 41, which is the first residue of the amino-proximal isotype-switch peptide (smooth muscle residues 41–76). Identity between the striated muscle α_2- and smooth muscle α-tropomyosin mRNAs spans the region encoding residues 41–116 and, therefore, includes the amino-proximal isotype-switch peptide (Fig. 3).

The three different α-tropomyosin mRNAs are tissue-specific and developmentally regulated. The α_1- and α_2-tropomyosin mRNAs are produced only in striated muscle, whereas the α-form seems to be expressed predominantly in smooth muscle. The presence of low levels of the smooth muscle α-tropomyosin isoform in striated muscle indicates either tissue contamination from the vascular smooth muscle or a low level of expression of the smooth muscle isoform in the striated muscle cells. The fact that L_6E_9 myotubes produce both α_1- and α-tropomyosin mRNA favours the second possibility and demonstrates that striated and smooth muscle isoforms can be synthesized in the same cell. The striated muscle α-tropomyosin isoforms are also developmentally regulated. The α_1-tropomyosin mRNA is induced early in muscle development and accumulates to high levels in the adult, whereas α_2-tropomyosin mRNA can only be detected in adult skeletal and ventricular tissues (Fig. 3). These results also demonstrate that identical α-tropomyosin isoforms are expressed in skeletal and cardiac muscles, despite the fact that the two muscle types express different myosin heavy chains[31–33], light chains[34,35], actin[36] and troponins[37–39].

The results presented here shown the existence of three distinct α-tropomyosin mRNAs, all three of which share regions of 100% homology and isotype specific sequences. Such an arrangement suggests that the α-tropomyosin gene has an unusual organization with common and exchangeable exons that can be spliced together according to different combinatorial possibilities.

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Most classical *Mus musculus domesticus* laboratory mouse strains carry a *Mus musculus musculus* Y chromosome

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Restriction fragment length polymorphisms (RFLPs) of maternally inherited mitochondrial DNA have revealed that genetic exchanges can occur between populations, subspecies or even species[1-4]. From the point of view of population genetics the mammalian Y chromosome represents a genomic analogue of the mitochondrion; it is inherited only paternally and remains perpetually monosomic, showing little meiotic recombination with other chromosomes. Using a Y-specific genomic DNA probe obtained from a flow-sorted mouse Y-chromosome library, we have examined the RFLPs in 10 newly established mouse lines of the European semispecies *Mus musculus domesticus* and *Mus musculus musculus*[5], and identified two variant forms of the Y chromosome, each of which is characteristic of one of the semispecies. As reported here, probing the DNA from nine established inbred laboratory strains reveals that the strain SJL carries a *M.m. domesticus* type Y whereas, surprisingly, the Y chromosomes of the other eight strains are of *M.m. musculus* origin. Hence, these strains cannot be regarded as archetypes of *M.m. domesticus* as suggested by protein polymorphisms and mitochrondrial DNA sequence data, but rather as genetic hybrids between the two semispecies.

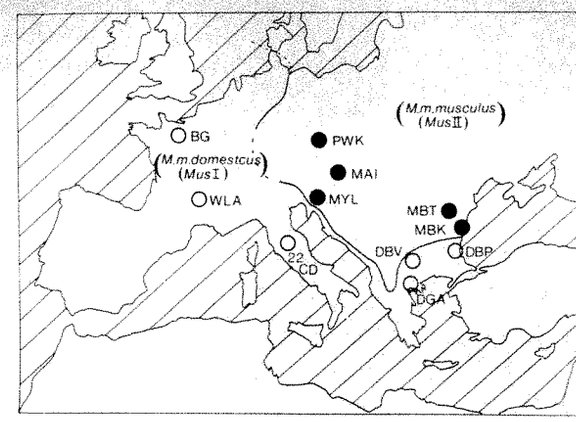


Fig. 1 Map of Europe showing the geographical origin of the 10 wild-derived strains of mouse studied. The two semispecies meet and hybridize along a narrow zone, indicated by the thick line, extending from Denmark, through central Europe to the Black Sea[2,4,5]. *M.m. domesticus* is found in western and southern Europe, *M.m. musculus* in central and eastern Europe. These semispecies have also been termed *Mus* 1 and *Mus* 2 (see ref. 5). The distribution of the two Y-chromosome haplotypes of these strains (indicated by solid and open circles) shows that each is specific to one of the semispecies.

Analysis of genetic variation in wild mouse populations affecting either nuclear genes (measured by protein polymorphisms) or maternally inherited cytoplasmic DNA (that is, mitochondrial DNA RFLPs) has revealed the existence of extensive polymorphism in most systems. These differential patterns allow a clear biochemical distinction to be made between different taxonomical units and show that a variety of interrelationships exist between the different groups[5]. In contrast, an analysis of the RFLPs of mitochondrial DNA in common inbred laboratory strains shows little variation, suggesting that these strains may in fact be derived from a single *M.m. domesticus* female lineage[6]. This implies that the extensive nuclear variation which exists, as evidenced by protein polymorphisms, could have been introduced through the males. One way to assess this contribution is to examine RFLPs of the mouse Y chromosome; this chromosome is unique in that normally it can be transmitted only via the male, it remains haploid in the genome and shows only a very limited amount of meiotic recombination with other chromosomes[7]. Polymorphisms characteristic of one population would therefore tend to become fixed on the Y chromosome.

We have shown recently that Y-specific DNA probes can be obtained from Y chromosome-enriched DNA libraries[8-10]. We have cloned two independent complementary DNA libraries from mouse metaphase chromosomes enriched for the Y chromosome by flow sorting according to the methods described by Baron *et al.*[11]. A random probe (pY353/B) consisting of a 1.5-kilobase (kb) fragment was isolated from one of these libraries; when used to probe *Eco*RI-restricted genomic blots from inbred male and female C57BL/6 mice under either stringent or non-stringent conditions, pY353/B hybridized with the male alone. In addition to the cognate 1.5-kb band, the probe detects four homologous bands of ~5, 7, 9.5 and 15 kb and therefore forms part of a small multi-sequence family. pY353/B reacts with the male teratocarcinoma cell line PCC7 but not with the male teratocarcinoma line PCC4 which has lost the Y chromosome during culture (data not shown). Therefore, we consider this probe to be Y chromosome-specific.

To assess the Y-chromosome polymorphisms that exist in the wild, we used a panel of DNAs from 10 newly established incipient inbred strains; these lines were derived from mice caught at the locations shown in Fig. 1 and subsequently inbred for a minimum of seven generations. The mice fall into the two well established European groups *M.m. domesticus* and *M.m. musculus*. These groups can be distinguished taxonomically or

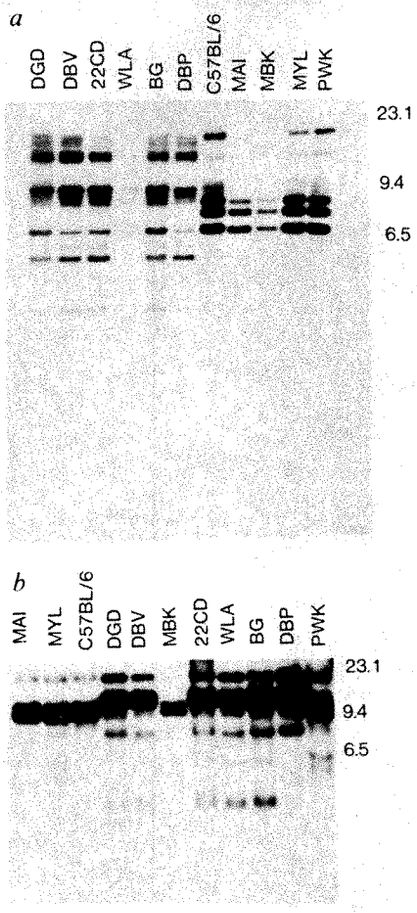


Fig. 2 Hybridization of pY353/B to DNAs from male mice. DNA was extracted from individual male mice and digested with either *Msp*I or *Hin*dIII. DNA (10 μg) was then separated on either 0.7% (*a*) or 0.8% (*b*) agarose gels and transfered to a Zetapore filter membrane. pY353/B was labelled to an approximate specific activity of 2×10^8 c.p.m. μg^{-1} with ^{32}P by nick-translation and used to probe the blots. Hybridization was at 42 °C for 16 h in 50% formamide, 5 × SSC. Blots were washed three times for 30 min each at 68 °C in 2 × SSC, 0.1% SDS, followed by three times for 30 min each at 68 °C in 0.1 × SSC, 0.1% SDS. Filters were then exposed to Kodak X-AR5 film for 16–20 h at −70 °C. Approximate sizes in kilobase (kb) are shown at the right. Mouse lines DGD, DBV, 22CD, WLA, BG and DBP are of *M.m. domesticus* type; lines MAI, MBK, MYL and PWK of *M.m. musculus* type. *a* (*Msp*I digestion) shows that laboratory strain C57BL/6 exhibits three bands of ~6.6, 7.0 and 8.0 kb characteristic of *M.m. musculus*. *b* (*Hin*dIII digest) again shows that C57BL/6 exhibits the *M.m. musculus* characteristic 10- and 12-kb bands. Re-probing the blots, after dehybridization, with a cloned mouse *H-2* cDNA probe showed that the lanes do not contain exactly equal amounts of DNA. The weaker signal seen with WLA results from underloading.

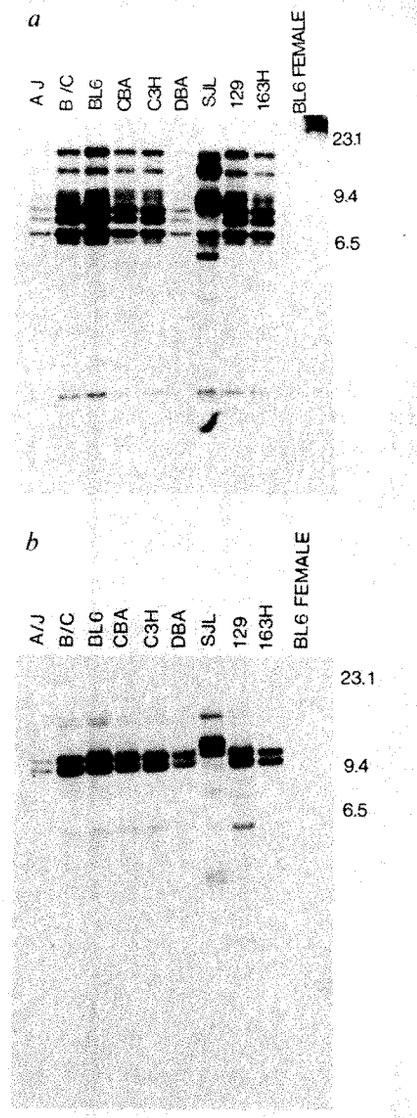


Fig. 3 Hybridization of pY353/B to genomic blots of nine established inbred laboratory mouse strains. Genomic blots were performed as described in Fig. 1 legend. The laboratory mouse strains are all maintained at the Institut Pasteur. Strains are (from left to right) A/J, BALB/c, C57BL/6, CBA/H, C3H, DBA/2, SJL, 129/sv, 163H and C57BL/6 female. Strain 163H is included as male fibroblasts from this line were used to flow-sort the Y (manuscript in preparation). *a*, The result of probing an *Msp*I genomic blot (0.8% agarose) with pY353/B. All strains tested show the characteristic *M.m. musculus* banding pattern except SJL, which shows the *M.m. domesticus* pattern. The result is confirmed by *b* (*Hin*dIII digest, 0.7% agarose) in which all strains show the typical *M.m. musculus* pattern except SJL, which shows the characteristic *M.m. domesticus* pattern of hybridization. Re-probing the blots with a cloned mouse *H-2* cDNA probe showed that the lanes contained equivalent amounts of DNA. Therefore, the weaker hybridization intensity seen in the A/J and DBA/2 tracts is not due to loading differences but probably represents variations in copy number.

more recently on the basis of multiple biochemical markers[5]; they are parapatric (*M.m. domesticus* is found in western and southern Europe, *M.m. musculus* in central and eastern Europe) and meet along a narrow hybridization zone[2,4]. We believe the two groups represent semispecies within the complex *Mus musculus* rather than subspecies, as important restrictions to genetic introgression reduce the hybrid zone to a narrow geographical area; this prevents the formation of a genetic continuum characteristic of subspecies interaction.

DNAs from single male and female mice were digested with *Eco*RI, *Taq*I, *Msp*I or *Hin*dIII, separated on agarose gels and transferred to a Zetapore membrane. pY353/B was labelled with ^{32}P by nick-translation and used to probe the genomic blots. After washing at 68 °C in 0.1 × SSC we detected no hybridization with any of the female DNAs (data not shown) whereas pY353/B hybridized to all male DNAs. No differences could be detected between any of the mice on the *Eco*RI or *Taq*I blots but a clear dimorphism was seen on either the *Msp*I or *Hin*dIII digests (Fig. 2*a*,*b*). Neither *Taq*I nor *Hin*dIII cuts within the probe whereas *Msp*I does. The polymorphic forms coincided exactly with the semispecies designation. All *M.m. domesticus* mice carried one form, *M.m. musculus* the alternative form. There was no significant variation within the two groups using either enzyme. Thus, this probe can be used to discriminate

between the Y chromosomes of *M.m. domesticus* and *M.m. musculus*. Surprisingly, the Y chromosome carried by the laboratory reference strain C57BL/6 showed the characteristic patterns of *M.m. musculus* on both the *Msp*I and *Hin*dIII digests. This finding was unexpected as it is generally assumed (and mitochondrial data support this assumption) that the laboratory strains were derived from *M.m. domesticus*.

To determine whether C57BL/6 is exceptional in this respect, we probed both *Hin*dIII and *Msp*I genomic blots of nine commonly used established inbred laboratory mouse strains with pY353/B. The polymorphic patterns obtained (Fig. 3*a*,*b*) show that the Y carried by SJL is of the *M.m. domesticus* type whereas all the other strains tested (A/J, BALB/c, C57BL/6, CBA/HeJ, C3H, DBA/2, 129/sv and 163H) are clearly *M.m. musculus*-derived. The latter strains of mice must therefore be considered as genetic hybrids between two semispecies, with the maternal contribution derived from *M.m. domesticus* and the paternal genetic information derived from *M.m. musculus*. Further genetic studies (which are in progress) should reveal the extent of the hybrid characteristics and show whether they are restricted to the Y chromosome or not. These data suggest that the Y-chromosome polymorphism involving SJL reported recently[12] may result from the semispecies origin of the Y and not strict intraspecies polymorphism. In fact, no Y-chromosome RFLPs were detected in the new wild-derived *M.m. domesticus* and *M.m. musculus* lines; this could be related to the nature of the probe as it has been shown that the highly repeated cloned Bkm probe[13] is extensively polymorphic both in wild and laboratory mouse strains (K. Jones, personal communication).

We consistently found that the intensity of hybridization of pY353/B to the A/J and DBA/2 inbred strains was 2–4 times less than that to the other strains, despite the blots containing approximately equal amounts of DNA from each mouse. As the hybridizations were performed under stringent washing conditions (0.1 × SSC, 68 °C), this difference probably represents a copy-number polymorphism rather than sequence divergence. Preliminary data using DNA from a mouse carrying a partially deleted Y chromosome (supplied by P. Burgoyne and E. Simpson) suggest that large blocks of DNA carrying this sequence are dispersed on the mouse Y chromosome (C.E.B., unpublished observations). It remains to be determined whether these copy-number polymorphisms arose during the establishment of the strains or whether they were present in the males which started the lines.

Historically, most of the laboratory mouse strains studied here (with the notable exception of SJL) are known to have originated from domesticated mice of North European and North American pet dealers and mouse fanciers. These mice were a mixture of locally caught North European (presumed *M.m. domesticus* origin) mice, subsequently carried across the Atlantic to North America, and imported 'fancy' Chinese and Japanese mice[14,15]. If the *M.m. musculus* type Y chromosome described here originated from the European mice it would imply that some *M.m. domesticus* wild mice carry the *M.m. musculus* Y chromosome. Although we have been unable to show any such exchanges in the wild mice studied so far, they mainly originate from South-East Europe where such exchanges might not occur. It is known that *M.m. domesticus* and *M.m. musculus* mice hybridize in Europe (Fig. 1) and that in Scandinavia and Denmark there is a considerable one-way mitochrondrial gene flow northeastwards from *M.m. domesticus* into *M.m. musculus*[2]. In the southeastern part of the hybrid zone this flow is reversed, occurring in the *M.m. musculus* to *M.m. domesticus* direction[4]. We are at present surveying a very large European mouse population to determine whether there is a Y-chromosome gene flow between the semispecies.

Alternatively, the *M.m. musculus* type Y could have been introduced through the Chinese or Japanese mice. In this regard, Bonhomme *et al.* have recently shown, using numerous biochemical markers, that the Asiatic subspecies *Mus musculus molossinus* (found in China and Japan) is closely related to *M.m. musculus*[5]. Mitochondrial DNA polymorphisms also support this view[6,16]. If this similarity extended to the Y chromosome, it could provide a simple explanation for the presence of a *M.m. musculus* type Y in the laboratory mouse strains, that is, being derived from the subspecies *M.m. molossinus*.

Whatever the origins of the Y chromosome, these data are of particular importance in studies involving hybrid genomes. Eicher *et al.* have reported that C57BL/6 differs from *M.m. domesticus* at an autosomal locus involved in gonadal determination and development (*tda*-1) as well as at the Y-localized testis-determining locus (*Tdy*)[17]. On the basis of the findings reported here, it seems probable that both *Tdy* and *tda*-1 in C57BL/6 have co-evolved from *M.m. musculus*. The partial or complete sex reversal reported can be interpreted as a result of the inability of a *M.m. domesticus* Y chromosome to interact with the *M.m. musculus tda*-1 locus. If this were true it would predict that the introduction of a Y from *M.m. musculus* into the C57BL/6 genome would not result in sex reversal, but the introduction of the SJL Y into the C57BL/6 genome would. Experiments are in progress to test this.

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Simian virus 40 enhancer increases number of RNA polymerase II molecules on linked DNA

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Since the discovery of transcription enhancer sequences in the genomes of the DNA viruses simian virus 40 (SV40)[1–3] and polyoma virus[4], these elements have been shown to play an important part in the control of both viral and cellular gene expression[5–10]. Enhancer elements act in *cis* to increase the amount of RNA produced from linked genes in a manner largely independent of distance and orientation[1–3,11,12]. The mechanisms by which enhancers act are not understood; in particular, it is not known whether the enhancer-dependent increase in the level of stable RNA reflects an increase in the rate of transcription. To address this question, we have used an *in vitro* nuclear transcription assay[13,14] to examine the effect of the SV40 enhancer on transcription of cloned human β-globin genes transiently introduced into HeLa cells[15]. We show here that the SV40 enhancer acts at least in part to increase the number of RNA polymerase II molecules transcribing the linked gene.

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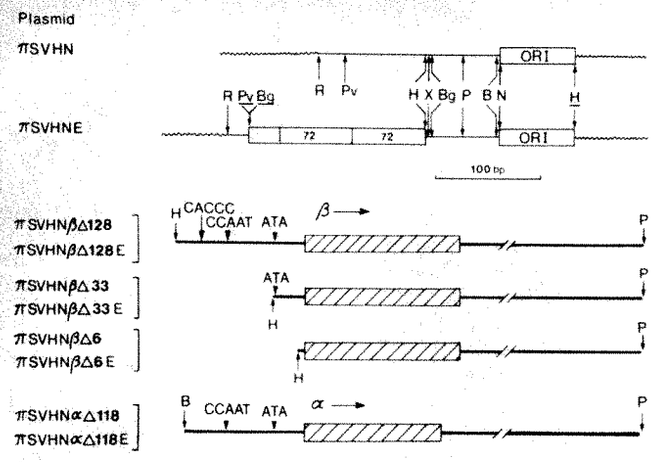


Fig. 1 Maps of the α- and β-globin gene plasmids used in HeLa cell transfection studies. Plasmids were constructed by standard procedures using the πVX miniplasmid as the parental plasmid[16,25]. The two miniplasmids πSVHN (no enhancer) and πSVHNE (with enhancer) are shown at the top of the figure. Open boxes represent SV40 sequences, with the origin of DNA replication (ORI) and the enhancer sequence (72-bp repeat) indicated. The enhancer fragment contains SV40 nucleotides 272 (*Pvu*II site converted by *Bgl*II linker) to 100 (*Xho*I linker converted to *Hin*dIII). The πVX plasmid vector sequences are shown as wavy lines, the polylinker as thin lines. The human β- and α-globin genes used are shown at the bottom, with the exons indicated by the shaded boxes, and introns and flanking sequences by thick lines. The β-globin promoter elements CACCC, CCAAT and ATA[18,19] are indicated. The β-globin plasmids lacking the SV40 enhancer, πSVHN$\beta\Delta$128, πSVHN$\beta\Delta$33 and πSVHN$\beta\Delta$6, and the enhancer-containing β-globin plasmids πSVHN$\beta\Delta$128E, πSVHN$\beta\Delta$33E and πSVHN$\beta\Delta$6E contain the β-globin genes on a *Hin*dIII-*Pst*I fragment inserted between the corresponding sites in the polylinker sequences of πSVHN and πSVHNE. The β-globin deletion mutants were constructed as described previously[22,26], and contain 128, 33 and 6 bp of 5′ flanking sequence of the β-globin gene. The α-globin plasmids πSVHN$\alpha\Delta$118 and πSVHN$\alpha\Delta$118E were constructed by inserting the *Bam*HI (artificially introduced at position −118) to *Pst*I fragment of the human α_1-globin gene between the *Bgl*II and *Pst*I sites of the two miniplasmids. The locations of relevant restriction enzyme cleavage sites are shown: R, *Eco*RI; Pv, *Pvu*II; H, *Hin*dIII; X, *Xba*I; Bg, *Bgl*II; P, *Pst*I; B, *Bam*HI; N, *Nco*I; sites underlined were destroyed during plasmid construction.

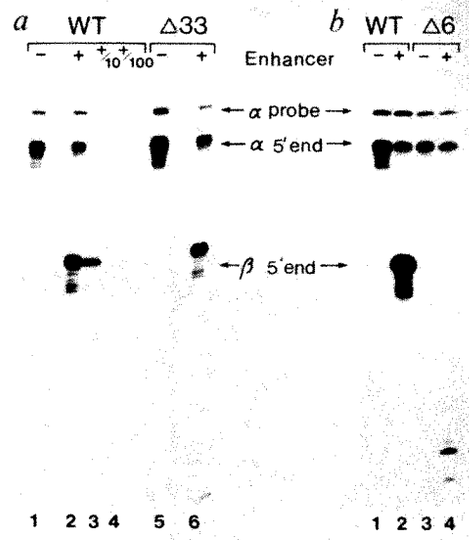


Fig. 2 Effect of the SV40 enhancer on the amount of accurately transcribed β-globin RNA in transfected HeLa cells. HeLa cells were transfected with 20 μg of plasmid DNA per 9-cm dish and the RNA was analysed by S_1 nuclease mapping procedures[27]. Each transfection included an equal mass of a human α-globin gene plasmid to serve as an internal reference. Unless otherwise stated, 40 μg of total cell RNA was used for each assay. The positions of nuclease-resistant DNA fragments mapping the 5′ ends of correctly initiated globin transcripts are indicated; − or + indicates the absence or presence, respectively, of the SV40 enhancer on the β-globin plasmid. The plasmids used contain β-globin genes with 128 (WT), 33 (Δ33) and 6 (Δ6) bp of 5′ flanking sequence. ***a***, Effect of the SV40 enhancer on the $\beta\Delta$128 (intact promoter) and Δ33 (deleted upstream element) β-globin genes. The α-globin plasmid πSVHSα1 (ref. 15), which does not contain an SV40 enhancer, was used as a reference. Lane 1, plasmid πSVHN$\beta\Delta$128; lane 2, plasmid πSVHN$\beta\Delta$128E; lane 3, plasmid πSVHN$\beta\Delta$128E, but the hybridization included only 4 μg of RNA; lane 4, 1/10 of the S_1 nuclease-resistant counts loaded in lane 3; lane 5, plasmid πSVHN$\beta\Delta$33; lane 6, plasmid πSVHN$\beta\Delta$33E. In lanes 3 and 5, the α-globin probe was omitted from the hybridization reaction. ***b***, Effect of the SV40 enhancer on the $\beta\Delta$128 (intact promoter) and $\beta\Delta$6 (no promoter) globin genes. The α-globin gene plasmid πSVHPα2 (ref. 26), which contains an SV40 enhancer, was used as an internal reference. Lane 1, plasmid πSVHN$\beta\Delta$128; lane 2, plasmid πSVHN$\beta\Delta$128E; lane 3, plasmid πSVHN$\beta\Delta$6; lane 4, plasmid πSVHN$\beta\Delta$6E. RNA which apparently initiates within the 5′-noncoding region of the gene is indicated by the arrows to the right of lane 4. Cell culture, DNA transfection and S_1 nuclease mapping procedures have been described previously[15,28]. The single-stranded [5′-^{32}P]-labelled probes were: β-globin, *Hae*III-*Mst*II*, nucleotides −76 to +70; α-globin, *Hha*I (artificially constructed; R.T., unpublished) to *Bss*HII*, nucleotides −11 to +97.

We first evaluated the effect of the SV40 enhancer on the accumulation of β-globin RNA using the S_1 nuclease mapping procedure. HeLa cells were transfected with plasmids carrying a human β-globin gene[15,16] with or without the SV40 enhancer (Fig. 1), and stable RNA was analysed 48 h after transfection. The DNA fragment containing the enhancer does not include sequences sufficient for SV40 early or late promoter function. As an internal reference, a plasmid containing the human α-globin gene was also included in the transfection mix. Figure 2*a* shows that the presence of the SV40 enhancer next to the intact β-globin promoter increases the amount of correctly initiated RNA produced by two to three orders of magnitude[1,16] (compare lane 1 with lanes 2–4).

We next isolated nuclei from transfected cells and incubated them with ^{32}P-labelled ribonucleoside triphosphates under conditions in which only limited elongation of previously initiated RNA chains occurs[13] (see Fig. 3 legend). The amount of *in vitro*-synthesized, labelled RNA specific for the α- or β-globin genes or vector DNA was determined by hybridization of partially fragmented RNA to a large excess of double-stranded DNA fragments immobilized on nitrocellulose[14,17]. The amount of radioactive RNA that hybridizes to a given fragment reflects the number of previously initiated RNA polymerase molecules present on the corresponding region of the transfected plasmid DNA[14]. The results of such a nuclear transcription experiment are shown in Fig. 3; in this experiment HeLa cells were transfected with β-globin plasmids that differ by the presence or absence of the SV40 enhancer sequence, together with a plasmid containing the human α-globin gene as an internal reference. Both globin and vector-specific RNAs were readily detectable after *in vitro* elongation of preinitiated RNA, and a substantial increase in the amount of β-globin specific RNA was observed when the gene was linked to the enhancer (Fig. 3*a*, compare lanes 1 and 2). A similar effect was observed with the α-globin gene using the β-globin plasmid as an internal reference (Fig. 3*a*, compare lanes 2 and 3). Examination of stable RNA showed that in the absence of a linked enhancer, the α-globin gene produced readily detectable levels of correctly initiated RNA, and that the presence of the enhancer at the 5′ side of the gene resulted in a 30–50-fold increase in the amount of RNA produced (data not shown). When 2 μg ml^{-1} α-amanitin was included in the nuclear transcription reaction, no globin- or vector-specific transcripts were synthesized (Fig. 3*b*, compare

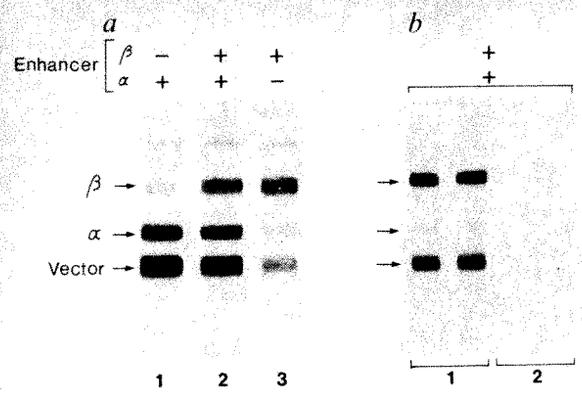


Fig. 3 Effect of the SV40 enhancer on nuclear transcription of transfected α- and β-globin plasmids. Each 'lane' is an autoradiogram of a nitrocellulose strip carrying fragments of β-globin, α-globin or vector DNA, after hybridization to RNA synthesized *in vitro*. The identities of the fragments and the presence (+) or absence (−) of the SV40 enhancer on each of the transfected plasmids are indicated. *a*, Effect of the SV40 enhancer on α- and β-globin nuclear transcription. HeLa cells were transfected with the following plasmids: lane 1, πSVHNβΔ128 + πSVHNαΔ118E; lane 2, πSVHNβΔ128E + πSVHNαΔ118E; lane 3, πSVHNβΔ128E + πSVHNαΔ118. *b*, Effect of α-amanitin on nuclear transcription of transfected plasmids. HeLa cells were transfected with πSVHNβΔ128E and πSVHPα2 (ref. 26), and nuclear transcription reactions performed either in the absence (lane 1) or presence (lane 2) of 2 μg ml^{-1} α-amanitin. Duplicate strips are shown.

Methods. Nuclei were isolated by hypotonic lysis using a Dounce homogenizer (type A pestle) as described previously[13]. Cell lysis was evaluated by Trypan blue staining. Nuclei from two 9-cm plates of transfected cells were used for each reaction. Isolated nuclei were resuspended at 0 °C in a total volume of 100 μl, comprising 50 μl 2 × transcription buffer[13] (50 mM dithiothreitol, 180 mM KCl, 10 mM $MgCl_2$, 20 mM Tris-HCl, 50% glycerol, *p*H 7.8), brought to 0.25 mM ATP, 0.125 mM GTP, 0.125 mM CTP. 100 μCi [α-^{32}P]UTP (410 Ci mmol^{-1}; Amersham) was added to each reaction. The nuclei were incubated at 37 °C for 5 min; no incorporation occurred after this time. Under these conditions, pre-initiated RNA chains are elongated by 300–500 nucleotides[13]. RNA was isolated by protease K treatment, followed by two extractions with phenol/chloroform as described previously[27]; residual DNA was removed by treatment with RNase-free DNase. Approximately 5×10^6 to 10^7 c.p.m. RNA were routinely obtained. Fragmentation of RNA by partial alkali hydrolysis was as described elsewhere[29]. For analysis of the *in vitro*-synthesized RNA by strip blot hybridization[14], plasmids πSVHNβΔ128 and πSVHNαΔ118 (25 μg each) were digested with *Xba*I and *Pst*I and the fragments resolved on a 10 cm-wide 1.2% agarose gel, then transferred to nitrocellulose[30]. Duplicate 1 cm-wide strips of nitrocellulose were then hybridized to equal numbers of counts as described[40], washed, and exposed to Kodak X-AR5 film.

lanes 1 and 2), indicating that the *in vitro* transcription is due to RNA polymerase II. We also verified that reinitiation does not occur in the system by performing nuclear incubations in the presence of 0.5% sarkosyl or 1 mg ml^{-1} heparin. Although these additions slightly diminished the total *in vitro* RNA synthesis, little effect on *in vitro* synthesis of α- and β-globin specific RNA was observed (data not shown).

These results demonstrate that the SV40 enhancer causes a substantial increase in the number of RNA polymerase II molecules present on linked DNA. However, the experiment does not distinguish between *in vitro* elongation of transcripts initiated at the β-globin promoter and the elongation of nascent transcripts initiated at other sites in the plasmid DNA. To address this problem, we examined the effect of promoter deletions on β-globin gene specific transcription in isolated nuclei. Plasmids were constructed in which the enhancer is located at the 5′ side of truncated β-globin genes containing either 33 base pairs (bp) of 5′ flanking sequence (which includes the ATA box, but lacks the upstream promoter elements CACCC and CCAAT[18,19]), (plasmids πSVHNβΔ33 and πSVHNβΔ33E; Fig. 1) or only 6 bp of 5′ flanking sequence (which lacks all promoter elements), (plasmids πSVHNβΔ6 and πSVHNβΔ6E; Fig. 1). The transcription of these plasmids in transfected HeLa cells was then analysed by S_1 nuclease mapping and by the nuclear transcription assay. The S_1 analysis (Fig. 2) shows that (1) neither of the truncated β-globin genes directs the synthesis of detectable levels of correctly initiated β-globin RNA in the absence of a linked SV40 enhancer sequence (Fig. 2*a*, lane 5; Fig. 2*b*, lane 1); (2) deletion of sequences 5′ to the ATA box has essentially no effect on the level of correctly initiated RNA produced when the enhancer is in close proximity to the ATA box, indicating that the enhancer can compensate for the deletion of upstream promoter sequences as reported previously for other promoters[20,21] (Fig. 2*a*, lane 6). (We note, however, that when the SV40 enhancer is located farther upstream from the βΔ33 gene, or on its 3′ side, virtually no transcription is observed[22].) (3) Deletion of all promoter elements results in the loss of correctly initiated β-globin RNA and the concomitant appearance of a small amount of RNA that is apparently initiated at, or spliced to, sites in the 5′-untranslated region of the gene (Fig. 2*b*, lane 4).

Figure 4 shows a nuclear transcription analysis of the β-globin promoter deletions. The RNA synthesized *in vitro* was hybridized to the separate strands of the β- and α-globin genes cloned into M13mp8. The linkage of the SV40 enhancer to the intact β-globin gene increases specifically the amount of transcription of the coding (or 'anti-sense') DNA strand (Fig. 4*a*, compare strips 1, 2 with 3, 4). A small amount of transcription of the noncoding strands of both β- and α-globin genes was detected, even in the absence of a linked SV40 enhancer. We also observed a decrease in the number of RNA polymerase molecules on the α-globin reference gene when the test β-globin gene was linked

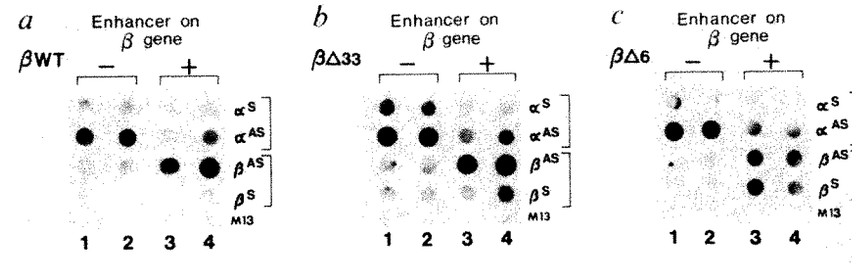


Fig. 4 Effect of the SV40 enhancer on nuclear transcription of β-globin promoter deletion mutants. Each panel contains pairs of duplicate strips of dot blots on which are spotted recombinant M13 phage carrying the sense (S) and anti-sense (AS) strands of the α- and β-globin genes. Bona fide transcripts hybridize with the anti-sense strand. HeLa cells were transfected with πSVHPα2 (ref. 26) and *a*, plasmid πSVHNβΔ128 (strips 1, 2) or πSVHNβΔ128E (strips 3, 4); *b*, πSVHNβΔ33 (strips 1, 2) or πSVHNβΔ33E (strips 3, 4); *c*, πSVHNβΔ6 (strips 1, 2) or πSVHNβΔ6E (strips 3, 4).

Methods. Nuclear transcriptions, RNA preparation and hybridization conditions were as described in Fig. 3 legend. Recombinant M13 phage were constructed by insertion of the 1.5-kb *Pst*I fragment of the human α_1-globin gene, or a 2.2-kb *Pst*I fragment of the human β-globin gene (from a *Pst*I linker at the endpoint of the −33 deletion to the *Pst*I site 500 bp on the 3′ side of the gene) into M13mp8 in either orientation, by standard procedures. The M13 DNAs (5 μg) were spotted onto nitrocellulose in 10 × SSC with a 'Manifold' apparatus (Schleicher & Schuell).

to the enhancer, suggesting that co-transfected enhancers can compete for limiting factors in the *in vitro* transcription assay. We do not observe this effect at the level of stable RNA under our conditions, indicating that factors that may be limiting *in vitro* are not limiting *in vivo*. Others have reported enhancer competition *in vivo* using different transcription units and transfection conditions[23]. In agreement with the observation that deletion of the β-globin upstream elements in plasmid πSVHNβΔ33E does not affect the accumulation of stable RNA, we find that the behaviour of this plasmid in the nuclear transcription assay is identical to that observed with the plasmid carrying the intact β-globin gene (Fig. 4, compare *a* and *b*). A strikingly different result was obtained when all the β-globin promoter elements were removed (Fig. 4*c*). Again, a small amount of *in vitro* transcription of both strands of the truncated gene was detected even in the absence of a linked enhancer sequence. However, linkage of the SV40 enhancer sequence to the gene lacking promoter elements resulted in a small increase in transcription of both DNA strands (Fig. 4*c*, compare strips 1, 2 with 3, 4); this increase may result from the activation of normally silent 'cryptic' start sites such as that observed in the 5′-untranslated region of the β-globin gene (Fig. 2*b*, lane 4). We conclude that enhancer effects seen in the nuclear transcription assays do depend on known promoter elements of the β-globin gene, and therefore relate to the production of bona fide β-globin RNA.

We have demonstrated here that the SV40 enhancer causes an increase in the number of RNA polymerase II molecules engaged in the transcription of linked DNA. The increase is dependent on known components of the β-globin gene promoter[18,19]. It was reported previously that deletion of the adenovirus 2 E1A enhancer causes a reduction in synthesis of E1A RNA measured in pulse labelling experiments[24]. Our observations exclude the possibility that the enhancer acts solely to allow stabilization of nascent transcripts. The magnitude of the enhancer effect observed in the nuclear transcription assay appears to be smaller than the two to three orders of magnitude effect on the level of stable RNA (compare Figs 2 and 3). Moreover, the 10-20-fold difference in the effect of the SV40 enhancer on the production of stable α- and β-globin RNA[15] is apparently not observed in the nuclear transcription assay. However, accurate quantitation of enhancer effects in the *in vitro* nuclear transcription assay is difficult. At least some of the low level of α-globin nuclear transcription observed in the absence of a linked enhancer sequence (Fig. 3*a*) must be due to elongation of correctly initiated RNA, as this plasmid does produce easily detectable levels of stable α-globin RNA (data not shown; see ref. 15). It is possible, however, that the low level of β-globin nuclear transcription observed in the absence of a linked enhancer does not represent elongation of RNA initiated at the correct β-globin cap site. This possibility is suggested by the observation that nuclear transcription in the absence of the enhancer is not significantly dependent on the β-globin promoter (Fig. 4, compare *a* and *c*). The magnitude of the effect we observe therefore provides a minimum estimate of the actual effect of the enhancer on polymerase loading. We cannot exclude the possibility that the enhancer also acts at levels other than transcription initiation to increase the production of stable RNA.

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Simian virus 40 enhancer increases RNA polymerase density within the linked gene

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Enhancers are regulatory DNA elements, usually about 200 base pairs (bp) long, which are able to stimulate transcription of linked genes in eukaryotic cells[1,2]. This activation can be exerted over large distances, and from a position 5′ or 3′ to the gene[1]. Enhancers have been identified in viral genomes[1-9] and cellular genes[10-15]. Using a transient expression assay, we have analysed transcription of the rabbit β-globin gene and the thymidine kinase gene from herpes simplex virus with and without a simian virus 40 (SV40) enhancer. S_1 nuclease mapping shows a high level of specific transcripts when the genes are linked to the enhancer. To determine whether this increased number of transcripts is due to a higher transcription rate, or perhaps to a shift from nonspecific to specific initiation, we have performed run-on transcription assays with isolated nuclei. Our results, presented here, demonstrate that the SV40 enhancer increases the RNA polymerase density within the linked gene. Therefore, enhancers apparently increase the rate of transcription initiation without influencing the specificity of initiation.

To study the enhancer effect on polymerase II transcription units, we constructed clones containing the rabbit β-globin gene and the herpes simplex virus (HSV) thymidine kinase (*tk*) gene with and without the SV40 enhancer (see Fig. 1).

The rabbit β-globin and the HSV *tk* genes, which were previously shown to respond to an enhancer[1,8], were cloned as 2-kilobase (kb) fragments into the plasmid pUC9 (ref. 16). The SV40 72-bp repeat enhancer was cloned as a 195-bp fragment[17,18] ~1.5 kb downstream of the respective promoters.

To demonstrate enhancer-dependent expression of the two genes, we transfected the clones into human HeLa cells, using the calcium phosphate co-precipitation method[18,19]. RNA was extracted after 2 days and analysed by S_1 nuclease mapping. The S_1 analysis clearly shows that the enhancer stimulates the level of transcription of the β-globin and *tk* genes ~40-fold and 15-fold, respectively (Fig. 2). The effect on β-globin transcription, however, is less pronounced than in previous experiments in which stimulation by the SV40 enhancer was ~200-fold[1]. This difference results mainly from an increased level of transcripts in the absence of an enhancer when using the pUC vector instead of the previously used pBR322 (data not shown), and was not analysed further.

An analysis of both the 5′ and 3′ ends of the rabbit β-globin transcripts demonstrates correct initiation, 3′ processing and

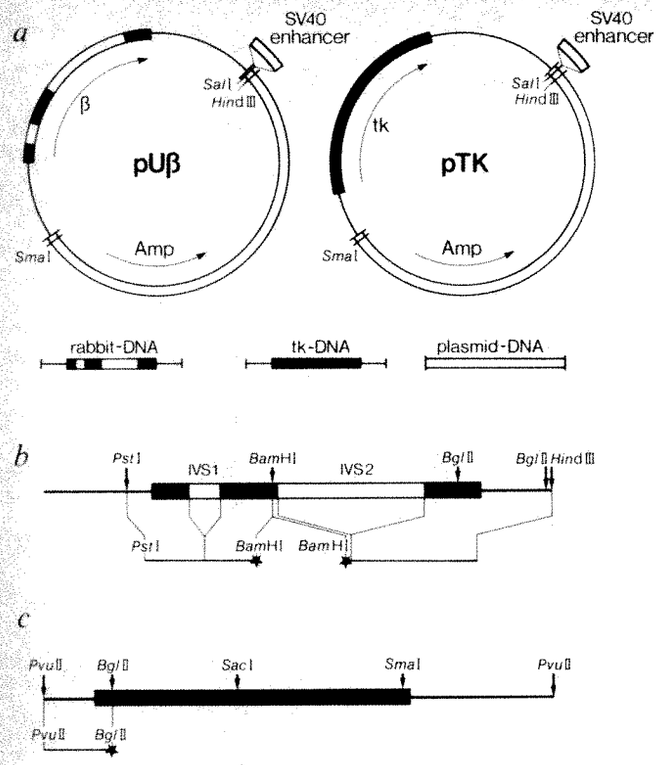


Fig. 1 Maps of recombinant DNAs and schematic representation of the S_1 nuclease mapping probes used. *a*, pUβ: the 2-kb *Bgl*II fragment of the rabbit β_1-globin gene[29] was cloned into the *Bam*HI site of pUC9 (ref. 16) so that the direction of transcription of the globin gene was opposite to that of the *lacZ* gene contained in the plasmid. pTK: the 2-kb *Pvu*II fragment of the HSV *tk* gene[30] was first subcloned into the polylinker of pUC8 and thereafter excised as a *Bam*HI fragment for insertion into pUC9. The clones containing a 195-bp insert of the SV40 enhancer[17,18] were called pUβE and pTKE, respectively. *b*, A β-globin gene lacking both intervening sequences was used to prepare the radioactive probes. To map β-globin transcripts, DNA was end-labelled at the *Bam*HI site using either T4 polynucleotide kinase (for 5′-specific probe) or the Klenow fragment of DNA polymerase I (3′-specific probe). *c*, DNA was labelled at the *Bgl*II site to obtain a 5′-specific *tk* gene probe.

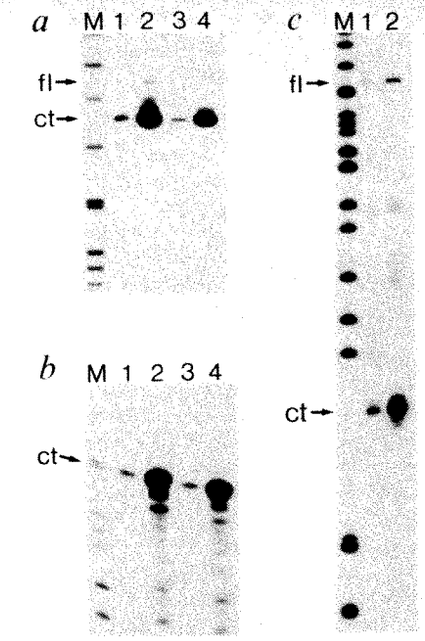


Fig. 2 Enhanced β-globin and thymidine kinase transcription. *a*, Hybridization of a 5′-specific β-globin probe (Fig. 1*b*) to 20 μg of RNA from HeLa cells transfected with: recombinant pUβ (lane 1); pUβE (lane 2); pUβ, linearized with *Hin*dIII (lane 3); pUβE, linearized with *Hin*dIII (lane 4). M, End-labelled marker DNA fragments (pBR322 cleaved with *Hpa*II). fl, Full-length probe (453 nucleotides); ct, correct terminus (354 nucleotides). *b*, Hybridization of a 3′-specific β-globin probe to 20 μg RNAs of the same transfections as in *a*. ct, 240 nucleotides. *c*, Hybridization of a 5′-specific *tk* gene probe (Fig. 1*c*) to 20 μg RNA from HeLa cells transfected with recombinant pTK (lane 1) and pTKE (lane 2). fl, 270 nucleotides; ct, 54 nucleotides.

Methods. Tissue culture cell lines were grown and transfected as described previously[18]. As a control for transfection efficiency, a 'tracer' gene was added to one of two duplicate plates: (1) A clone containing the SV40 T-antigen gene under the control of the adenovirus major late promoter and an SV40 enhancer was mixed 1 : 100 with the appropriate β-globin clone. Two days post-transfection, the cells in the plate co-transfected with tracer were fixed and stained for T-antigen by immunofluorescence. (2) The cells in the plate without tracer were collected for RNA extraction. Similar numbers of stained nuclei were observed independent of the globin construct used (523 T-antigen-positive per 14,000 cells and 509 positive per 13,000 cells with pUβ and pUβE, respectively). This finding was corroborated by Southern blot analysis of nuclear DNA from transfected HeLa cells, which yielded equal amounts of cloned DNA present within the nuclei (not shown). RNA analysis was performed as described elsewhere[31,32]. End-labelled single-stranded DNA (see Fig. 1*b*, *c*) was hybridized to unlabelled RNA, treated with S_1 nuclease, denatured, fractionated by gel electrophoresis and autoradiographed.

splicing of the two introns during maturation of messenger RNA, independent of whether an enhancer was present or not (Fig. 2*a*, *b*). Also, the *tk* mRNA expressed from pTK and pTKE has a correct 5′ end (Fig. 2*c*). The enhancer, therefore, does not apparently influence the specificity of initiation or the processing of the pre-mRNAs.

However, S_1 nuclease analysis measures only the steady-state level of stable transcripts. In particular, the presence of a clean S_1-protected band does not rule out the possible existence of a wealth of nonspecific unstable transcripts from the same gene.

To determine whether an enhancer shifts the equilibrium from nonspecific to specific transcripts[20], or whether it increases the rate of gene transcription, we measured the RNA polymerase II density. To this end, we have determined the enhancer effect by a run-on transcription experiment in isolated nuclei[21,22]; this technique is widely used to determine the polymerase density on an endogenous gene of defined copy number.

It is known that the enhancer effect is not due to differential uptake of the DNA[1]; in particular, the effect is also seen when defined numbers of molecules are injected into cells[2,8] and when template molecules with and without enhancer are replicated to high copy numbers using COS cells (refs 20, 23, 24 and T. Gerster and W.S., unpublished). Therefore, the run-on technique is also applicable to the study of transcription in a transient assay[25]. The combination of the transient expression system with a nuclear run-on assay offers several advantages: for example, the rate of transcription within different cloned and mutagenized genes can be compared, which is particularly useful with mutants possibly affecting mRNA stability or transcription termination of mRNA (for the latter case see below and Fig. 3).

Nuclei of HeLa cells transfected with our β-globin and *tk* clones were isolated and digested with RNase A. Pre-initiated RNA was then elongated *in vitro* for 10 min in the presence of [α-^{32}P]rGTP, giving rise to labelled RNA molecules ~200–300 nucleotides long (Fig. 3*a*). As RNA polymerase II appears to protect ~60 nucleotides from nuclease digestion, the *in vivo* elongation rate is ~15–20 nucleotides per min in HeLa nuclei; this rate is much lower than that found *in vivo* but is in good agreement with previous *in vitro* studies[22,26].

The labelled RNA was then hybridized to dot-blots of cloned DNA. The dot hybridization signals in the run-on assays are

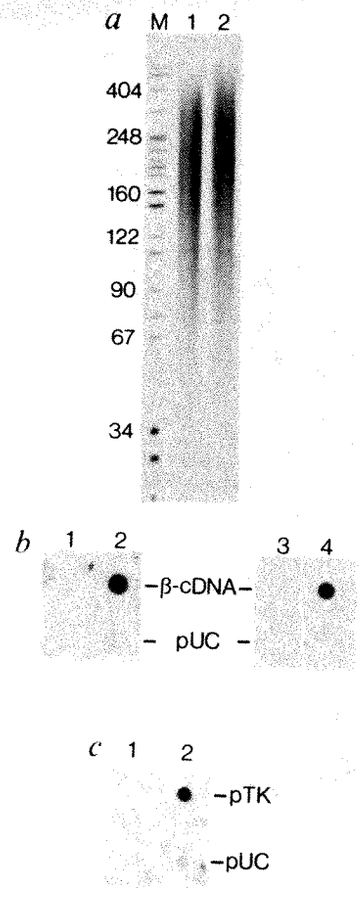


Fig. 3 Run-on assays also reveal the enhancer effect. *a*, RNA from nuclei which had been treated with RNase A and labelled *in vitro* was isolated and subjected to denaturing polyacrylamide (6%) gel electrophoresis. Cells transfected with pUβ (lane 1) and pUβE (lane 2) show a similar size distribution of *in vitro* elongated nuclear RNA; M, end-labelled marker DNA fragments (pBR322 cleaved with *Hpa*II). *b*, Unlabelled DNA of pUC9 and pcβ (rabbit β-globin cDNA) was denatured, spotted onto nitrocellulose membrane and hybridized to 4×10^6 c.p.m. (lanes 1 and 2) or 1.2×10^6 c.p.m. (lanes 3 and 4) of nuclear RNA from cells transfected with: pUβ (lane 1); pUβE (lane 2); pUβ linearized with *Hin*dIII (lane 3); pUβE linearized with *Hin*dIII (lane 4). *c*, Hybridization of pUC and pTK DNA to 6×10^6 c.p.m. of *in vitro* elongated nuclear RNA from cells transfected with pTK (lane 1) and pTKE (lane 2). HeLa cells were transfected as described previously[18]. The nuclei were isolated 36 h post-transfection as described[21], except that divalent cations were omitted from the buffers. Run-on assays were performed as published elsewhere[22].

expected to be proportional to the polymerase densities as RNase A treatment of the nuclei before *in vitro* elongation removes the pre-existing different amounts of unlabelled RNA and thus avoids competition of the mature mRNA with nascent transcripts[22]. We found that nuclear RNA from cells transfected with pUβE or pTKE hybridizes strongly to rabbit β-globin complementary DNA or *tk* DNA, respectively, whereas nuclear RNA from pUβ- or pTK-transfected cells hybridizes only weakly (Fig. 3*b*, *c*), in agreement with the S_1 nuclease mapping assays shown in Fig. 2.

The nuclear RNAs hybridize ~10% as strongly to plasmid vector DNA as β-globin or *tk* gene sequences (Fig. 3*b*, *c*), indicating (1) that the enhancer primarily stimulates transcription from the β-globin or *tk* promoter rather than from pseudopromoters within plasmid sequences, and (2) that there is efficient transcription termination preventing readthrough into plasmid DNA.

In agreement with our S_1 nuclease mapping data (Fig. 2), similar results were obtained whether transfection was performed using circular or linear DNA (Fig. 3*a*, *b*; the latter is probably ligated within the transfected cells[18]).

Thus, our results indicate strongly that an enhancer increases the RNA polymerase II density on a gene to which it is linked, that is, the enhancer facilitates transcription by increasing the frequency of initiation. In particular, the enhancer does not seem to shift the equilibrium from nonspecific to specific transcripts, as could have been inferred from the work of Humphries *et al.*[20]. These authors studied the expression of human α-, β- and δ-globin genes using vectors containing an SV40 replicon[27] and were struck by the high levels of nonspecific transcripts in the absence of an enhancer. However, we consider it likely that the high level of nonspecific β-globin transcripts is a peculiarity of their assay conditions. With replicating SV40 templates, there can be a considerable fraction of nonspecific transcripts from multiple scattered initiation points[24] which are perhaps related to the multiple initiation sites of SV40 late transcripts[28].

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Erratum

Functions of the canal system in the rotaliid foraminifer, *Heterostegina depressa*

R. Röttger, M. Spindler, R. Schmaljohann, M. Richwien & M. Fladung

Nature **309**, 789–791 (1984)

ON page 789, the last sentence of the second paragraph should read "We have examined the symbiont-bearing Recent nummulitid, *H. depressa*, in which the canal system within the chamber walls and within the marginal cord replaces the primary aperture seen in other foraminifera."

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General

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Unlearned Language: New Studies in Xenoglossy. By IAN STEVENSON. *University of Virginia Press: 1984. Pp.223. ISBN 0-8139-0994-5. $17.50.*

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Tests of influence

P.E. Bryant

Beyond IQ: A Triarchic Theory of Human Intelligence.
By Robert J. Sternberg.
Cambridge University Press: 1985. Pp.411. Hbk £25, $39.50; pbk £8.95, $14.95.

A PARADOX touches the study of intelligence. The subject is controversial — so controversial that it would be difficult to find even two psychologists who completely agree with each other on the nature of intelligence. Yet the psychologists' work in this area has had more impact outside the laboratory than anything else that has interested them, success which has merely increased the controversy. My own suspicion is that, if it were not for the obvious practical uses of these omnipresent tests, psychologists would have given up asking themselves about the underlying nature of intelligence as a far too abtruse and unrewarding question many years ago. But the very fact that the tests work, and that they seem to measure something which could reasonably be called intelligence, has posed a question which many have found impossible to resist investigating and trying to answer.

Robert Sternberg is the latest and almost certainly the most energetic in a long line of people who have devoted their time to attempting to say what intelligence means and what is responsible for intelligent behaviour. His approach is inclusive, and has become more so over the years. In this book, which is not his first on the subject, he sets out to combine three different approaches in what he calls a "triarchic" theory of intelligence. In order to understand why these three approaches have, on the whole, not been combined in the past, one needs to know something of the history of the study of intelligence.

It goes back to the first two pioneers of the intelligence test: Galton, whose tests were not a success, and Binet who, in contrast, produced the first effective intelligence test which has influenced every such test devised since then. The reasons for Galton's failure and Binet's success are important. Galton adopted a theoretical approach: he decided that intelligence consists of this and that ability and then produced tests to measure them. One test, for example, measured the speed of people's movements, another how well they named colours. These tests were certainly measures in the sense that different people produced different scores on them, but they did not measure intelligence. It soon turned out that they bore no relation to educational attainment, and that made it difficult to believe that they had anything to do with intellectual capacity.

Binet, too, had his theories, as Sternberg notes, but he did not let them play much part in his tests. His approach was pragmatic, and he had one clear guideline. Whatever intelligence is, he argued, it increases during childhood. So he only accepted tests which older children were more likely to answer correctly than were younger ones. This approach worked. Binet's test did predict reasonably well how children would manage at school, and every other intelligence test since then has had a strong, though never perfect, relation to educational achievements.

But this practical success left an arresting theoretical vacuum. The tests themselves did little to reveal the underlying nature of intelligence. In the following 50 years or so the main attempts to fill this gap were made by people who used factor analysis as their main tool and their answers were distinctly uninspiring. They looked rather like the first part of a cooking recipe; intelligence was held to consist of this factor and this one and so on, and there was no sense of a system that actually worked. Sternberg gives reasonably short shrift to these theories. He is concerned with the workings of intelligence.

This is not at all surprising since Sternberg comes from a tradition in psychology which developed quite separately from the intelligence testing movement and which originally had nothing to do with individual differences. The study of information processing is concerned with the underlying processes involved in taking in, remembering and coordinating information about the environment, and its subject matter goes from the simplest kind of remembering or attention through to complex logical processes such as making sophisticated analogies or drawing inferences. Sternberg has always argued that this is the stuff of intelligence, and most of his work has taken the form of forging links between studies of information processing and studies of differences in intelligence.

But now he thinks that this is not enough. His "triarchic" model is really a model of how to approach the question of intelligence rather than a fully fledged theory about intelligence. He argues that we need to know not only about the components of intelligence (the information processing involved) but also about the role of experience (particularly about the way that skills become automatic after a while) and, as well, about the context in which intelligent behaviour takes place. These three kinds of question are Sternberg's triarchy and the first half of the book is a convincing argument that all three are needed. In the second half he deals with particular problems which have always been closely associated with the study of intelligence, many of which — such as syllogisms and analogies — crop up a great deal in intelligence tests. His aim is to subject them to his triarchic approach, but here the analysis is often uneven though it is always very detailed. Sternberg's "componential" leanings take over. There is little in this part of the book about either context or the effects of experience. Sternberg has more research to do on these two questions, but given his phenomenal productivity there seems little doubt that he will manage it.

In the end the proof of Sternberg's pudding will be the same as it was with Galton's and Binet's. His scheme will only influence people outside the world of psychology, and to a great extent within it too, if it manages to produce good, new tests. Only then can we be sure that his scheme transcends the laboratory in a way that Galton's so signally did not. Sternberg seems to recognize this when at the end of the book he suggests several ways of improving tests of intelligence. He should try these out. In the meantime he has written an attractive and valuable account of the long and successful enterprise which began so well with Binet and still seems to be in very good hands. □

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Upright education — the Geradhalter, *an invention of a nineteenth-century educator, one Dr Schreber, intended to improve children's posture by discouraging them from slumping forward Schreber used this and similar devices on his own children, one of whom was mentally deranged for most of his adult life. The illustration is taken from Robert Trivers's* Social Evolution, *published by Benjamin/Cummings.*

On the rocks down under

Michael Audley-Charles

Phanerozoic Earth History of Australia.
Edited by J.J. Veevers.
Clarendon: 1985. Pp.418. £55, $75.

REGIONAL geology is concerned with how particular areas of the outer part of the Earth have evolved. It attempts to account for all aspects of the rocks that can be mapped at and below the surface. The history of vertical and lateral movements, changes in climate, environments of deposition, rates and modes of erosion have to be interpreted from the great variety of observations, many of which are qualitative or descriptive. Magmatic, metamorphic, sedimentary and deformation processes have to be related in space and time to identifiable regional tectonic events that are often diachronous.

The elephant trap for writers of regional geology is to smother the reader in a snowstorm of detailed descriptions that fail to discriminate between the trivial and the vital. Readers thus afflicted, especially perhaps undergraduates, are led to associate regional geology with tedium. For this book, John Veevers has gathered together a team of writers who cover 575 million years of evolution of a region which represents a fortieth of the Earth's surface and a sixteenth of the continental lithosphere. Australia has no elephants and these authors eschew snow.

The apparent polar wander paths reveal that after some dalliance in the northern tropics during the early Palaeozoic, Australia has stayed in the Southern Hemisphere for most of the Phanerozoic. Records of oceanic palaeomagnetism for the last 160 Myr show how Australia's continental margins have been formed by a "can opener" type of rifting sequence which progressed from the north about 160 Myr anticlockwise around the margins to reach the Coral Sea about 60 Myr. These oceanic data do not reveal where or how these events began in what is now central New Guinea about 200 Myr. The origin of Australia's northern rifted margin before its late Cenozoic collision is not made explicit.

The evolving sequences of palaeoclimates and palaeoenvironments related to continental drift are dealt with deftly, the authors citing what they judge to be the three or four most sensitive and reliable diagnostic pieces of evidence for each Phanerozoic System of rocks, while a 25-page discussion of Australia's biogeography provides a glimpse of many unsolved problems and some of their seemingly more important implications. For example the tantalizingly uncertain data for Australian angiosperms leave us not sure whether some flowering plants were able to enter northern Australia from Asia during the early Cenozoic nor whether the flowering plants were able to migrate from northern Australia into Asia in the late Mesozoic and early Cenozoic.

The main part of the text presents Australia's structural evolution through the Phanerozoic by recognizing only three morphotectonic elements: the northern convergent margin, the huge area of platform and the various divergent (rifted) margins on the west, south and east. The stratigraphic evolution is dealt with by the convincing device of recognizing three stratigraphic regimes: the Portoroo (Late Cretaceous and Cenozoic), Innamincka (Mid Carboniferous to Mid Cretaceous) and the Uluru (Cambrian to Mid Carboniferous).

This is not an encyclopaedic account of Australia's Phanerozoic geology. The reader should not expect to turn to the book for detailed description of particular successions nor for a biostratigraphical discussion of the faunas and floras; the stratigraphy tends to be underlain by tectonic rather than biostratigraphic concepts. Sedimentological information is used throughout, but much of the text allows the fossils to sleep quietly (unmentioned) in their graves, so much so that some palaeontologists may turn in theirs. Perhaps that highlights the distance between stratigraphy and regional geology — a divorce not to be encouraged.

This book takes a refreshing approach to the synthesis of stratigraphy and tectonics, helped by writing which is concise without being over-condensed. Not only have Veevers and his colleagues produced an attractive and readable account of Australian geology, but more than in any other text I have seen succeed in conveying the excitement of regional geology. □

Michael Audley-Charles is Professor of Geology at University College London.

Chemists' enzymes

Richard Perham

Enzyme Chemistry: Impact and Applications.
Edited by Colin J. Suckling.
Chapman & Hall: 1984. Pp.255. £17.65, $36.
The Chemistry of Enzyme Action.
Edited by M. I. Page.
Elsevier: 1984. Pp.568. $66.50, Dfl. 179.

CHEMISTS have much to learn from, as well as contribute to, the study of enzyme catalysis. Although we no longer stand in awe at the catalytic power of enzymes, it is plain that there are still formidable problems to grapple with before we can claim a satisfactory understanding of their mechanisms. It is equally evident that we have not yet managed to apply all the lessons we have learned to, say, the industrial use of existing enzymes or the design of other catalysts.

Enzyme Chemistry is avowedly a didactic book, which sets out to document some of the past and to illuminate some of the future in the chemical study of enzymes and their products. As Kluger observes in an opening chapter which skilfully reviews the underlying physical organic chemistry of enzymes, if we were given the detailed three-dimensional structure of a protein of undisclosed catalytic specificity, it is unlikely that we could deduce the reaction catalysed. The goal of biomimetic chemistry — the design and construction of molecules that rival enzymes as catalysts — is thereby put in perspective. Shinkai concentrates on an encouraging approach to this goal that begins with a thorough study of the chemistry of coenzymes, whereas Colin Suckling addresses the question of selectivity in chemical and enzyme synthesis. The fastidious selectivity and exquisite stereochemical preferences of enzymes are, of course, now well known but — as Suckling gently reminds us — their use has sometimes been treated, a little scornfully, as unacceptable in the perfect total synthesis. He cites some splendid examples of a more robust attitude and of its beneficial effect on biomimetic chemistry.

Industrial chemists have never shunned an enzyme or even whole cells as a means to an end. This theme is taken up by Hesp and Willard in a lively and optimistic discussion of enzyme chemistry and drug design. A rational approach to chemotherapy has seemed just around the corner for almost 50 years, ever since the discovery of the biochemical basis of sulphonamide antibiotics. That prospect still tantalizes but one perceives a quickening of step as the new sophistication of enzyme and protein chemistry and of molecular modelling begins to impinge. Similar optimism is advanced by Cane in a neat chapter on the enzymes responsible for the biosynthesis of natural products. Those chemists who still resist the use of enzymes in total synthesis will be shocked to find his advocacy of the use of recombinant DNA techniques to facilitate a study of the enzymes involved. Bornyl pyrophosphate synthetase, an enzyme of monoterpene biosynthesis, has an estimated turnover number of 10 mol of substrate per mol of enzyme per hour. Cane flatters this enzyme (he is obviously but justifiably fond of the pathway) by referring to its catalytic power as extremely modest. Given that this may be typical of secondary metabolic enzymes, chemists would be foolish indeed to reject the help that molecular biology can offer them. They might even indulge in the delights of site-directed mutagenesis.

The book is completed by an article (Brown and Smith) on metal ions in biological systems—inorganic chemistry is not neglected—and an assessment by Keith Suckling of the effect of enzymological ideas on seemingly unrelated areas of biochemistry.

For a multi-authored book, the style and standard of presentation is unusually even. The authors communicate their affection for the subject and display a refreshing awareness of the benefits to chemistry that flow from enthusiastically embracing the study of enzymes, as well as the metabolites they convert. A significant criticism is that the references terminate in mid 1982 so that, for example, much of the current achievement of the molecular biological approach, let alone its potential, goes unrecorded. Biology is without doubt well placed to repay chemistry.

The Chemistry of Enzyme Action is part of the series entitled *New Comprehensive Biochemistry*. It is a large book (15 chapters) and includes most of the ground covered by *Enzyme Chemistry*, but goes well beyond it both in the range of topics and the depth in which they are treated. The principal aim of the book is to describe the physical and organic basis of enzyme action. Because the approach is mechanistic, the opening chapters concentrate on the energetics and substrate interactions of enzymes (Page, Kollman, Williams) and the use of kinetic (Engel, Douglas and Wilson) and isotope (Williams) methods in exploring mechanisms. Several systems are then selected for a detailed chemical scrutiny: enzyme reactions that utilize imine formation (Hupe), enzymes that require pyridoxal phosphate (Akhtar, Emery and Robinson), folate and biopterin cofactors (Benkovic and Lazarus), glycosyl transfer (Sinnott) and vitamin B_{12} (Brown). Biomimetic systems are represented in the concluding chapters on micelles and similar aggregates (Brunton), cyclodextrins (Komiyama and Bender) and crown ethers (Stoddart).

Despite its size, the book therefore remains selective in its coverage. The complementary part of this story, the participation of the enzyme protein and its structure in the enzymic reaction, is consciously left very much on one side. Thus, individual chapters will be read with profit by specialists but I fancy that the average undergraduate would find the rigour and detail too demanding. The logical place for such a book is the departmental library and the shelves of enzymologists with relevant interests. Typographical errors, while they do not abound, pop up from time to time, but the overall presentation is not unpleasing. All the authors can be congratulated on the comprehensive reference lists they have compiled. □

Richard Perham is a Reader in the Department of Biochemistry, University of Cambridge, and President of St John's College, Cambridge.

An independent view

John H. Lawton

The Ecological Web: More on the Distribution and Abundance of Animals.
By H.G. Andrewartha and L.C. Birch.
University of Chicago Press: 1985. Pp.506. $40.25, £33.25.

SCIENCE is most exciting when two alternative views of the world clash. The battle in population dynamics is between those who believe that density-dependent processes play a significant part in determining the distribution and abundance of animals, and those who do not. Andrewartha and Birch have led the density-independent school, ever since their book *The Distribution and Abundance of Animals* appeared in 1954. Their views have not changed significantly over the past 30 years, although the arguments have become more sophisticated and the controversy has recently enjoyed new vigour.

The central theme of *The Ecological Web* is a "theory of environment" based on "envirograms" — dendrograms whose branches trace pathways of influence impinging upon study-species. There are four directly acting components, resources, mates, malentities (that is, hazards) and predators, all of whose actions can be influenced by a cascade of steps in other parts of the envirogram. Andrewartha and Birch's views of population dynamics are therefore particular and detailed. They dismiss general, analytical models in two sentences: "We cannot see many testable hypotheses emerging from such abstract models.... So we say no more about them" (p.187). Approximately the first two-thirds of the book is devoted to describing and establishing their theory of environment; there follow in the final third several case-histories, on rabbits, two birds, marine fish (briefly), three species of insects and man. Only the spruce budworm, fish and man are not Australian examples.

I ought now to make my own position clear. Unlike Andrewartha and Birch, I find abstract general models of ecological processes useful, and I do think that density-dependent processes are largely irrelevant in understanding population dynamics. However I set out to read *The Ecological Web* with an open mind, but in the end found it lacking in many ways.

The theory of environment is largely verbal and pictorial, but is used to attack more formal, mathematical theory. The result is rather like comparing apples and oranges. The weakness of predominantly verbal arguments is best illustrated by an example. In the section "Competitive Coexistence and Displacement" the authors conclude, after reviewing several case-histories, that species can live together "because they are alike — not identical . . Conversely, when two species fail to coexist it is usually because they differ with respect to their success in professing a niche that they have in common". They seem not to notice that this conclusion is tautological; nor does it take us any nearer to a general understanding of competitive coexistence or exclusion.

Perhaps more seriously, Andrewartha and Birch often do not seem to understand many of the theories they either attack, or so lightly dismiss as futile. Moreover, they too often pass over evidence that contradicts their view of the world. Consider, for example, their efforts to explain population cycles in animals such as snowshoe hares or lemmings. They use neutrally stable Lotka-Volterra models (p.255) yet claim that "oscillations decline and finally disappear"; they do not. More interesting and realistic general models are ignored, as are efforts to test them[1,2]. Alternatively, take cases of "apparent competition" via shared enemies, or the role of spatial heterogeneity in population dynamics. They have much to say about both, but all their points are covered in more rigorous ways by existing general theoretical models. A small number of other examples will suffice to make my point. Great tit populations[3] are not limited by nest sites, as claimed. Population density does have important effects on the dynamics of tsetse flies[4], spruce budworms[5] and carabid beetles[6,7]. Sadly, I have to conclude that Andrewartha and Birch often develop their arguments by ignoring counter-evidence.

It appears that most of the confusion in this area of ecology centres on whether or not people are more impressed by population fluctuations, or by the fact that, despite such fluctuations, numbers of many species remain within comparatively narrow limits. Clearly not all populations are well regulated, but many are. It is important to know whether there are systematic patterns in the relative contributions of density-dependent and density-independent processes to the population dynamics of species from different taxa and habitats; but you will not find the answer to such questions in this book. Much has happened in population biology in the 30 years since the publication of *The Distribution and Abundance of Animals*. Because its treatment of these developments is so inadequate, *The Ecological Web* does little to advance the case for density independence. □

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Classified material

V.H. Heywood

Guide to Standard Floras of the World.
By D.G. Frodin.
Cambridge University Press: 1985. Pp.619. £95, $175.

THE best description of this remarkable compendium is given by the subtitle, "An annotated, geographically arranged systematic bibliography of the principal floras, enumerations, checklists, and chorological atlases of different areas". The second part of the book, some 500 pages, comprises just that. There are, however, really two separate elements in this enterprise — a thoughtful analysis of *Floras* and floristics, and the application of this analysis to the geographically arranged guide to the most useful *Floras* and checklists of vascular plants of the world, selected according to the concept of "standard" works. The idea for this compilation dates back to 1962, and we are told that the main catalyst was the publication of the so-called "Green Book" produced by the *Flora Europaea* editorial committee for the guidance of contributors, in which a list of "standard" *Floras* of Europe was given.

Preparation of the *Guide* began in 1970 and was completed in 1975. It is a tribute to the author that he was able to contemplate such a work based in Papua New Guinea, scarcely one of the world's leading bibliographical centres (in a remarkable piece of understatement he comments that the "remoteness of Port Moresby was ... a handicap"!). Publication has been delayed for nearly six years after the submission of the manuscript to the publishers, and with a cut-off year of 1980 the text is already seriously out of date in many places.

Floristic projects are advancing so rapidly today that the whole question of maintaining an up-to-date database needs careful consideration. It is not at all clear to me that a hard-copy guide such as this, however selective it is, is the most effective instrument for bibliographical purposes. The author explains, however, that his aim was not just to present a classified bibliography but, by being analytical and interpretative, to act as a mirror on the progress of the subject. His ideas are expanded in the first section of the book. This includes essays on the style of *Floras* which are an important contribution to the literature on the subject. Frodin believes that the basic role of a *Flora* should still be a utilitarian and practical one — "inventory, identification and essential related data". He further echoes the increasingly expressed view that, for tropical countries in particular, floristic works should be written in such a way as to have a wide public appeal.

A disappointing feature of this introductory section is that although the author looks to the future, and emphasizes the need for a reappraisal of the ways in which floristic data are stored and presented, he does not put forward any very concrete suggestions. It is unfortunate that the book was completed just at the time when several new approaches to floristics were being implemented, most of them related to electronic data-handling. Thus a whole series of developments in the use of databases in systematics are omitted or only touched upon, such as the Vicieae database at Southampton, the revived Flora North America project (which will be heavily dependent on database technology) and the European Science Foundation Taxonomic Information System and database at Reading, not to mention the substantial reorganization of taxonomic services, involving electronic data processing, that several major taxonomic institutions are currently engaged upon.

In the main part of the *Guide*, Frodin's essays and annotations make interesting reading and present a very idiosyncratic interpretation of the facts reported. This material would have been better published separately. Indeed, altogether it is highly questionable whether it was wise for a world bibliography of *Floras* to have been undertaken as a one-man operation. One cannot but admire the author's enormous achievement in compiling this unique, expensive and fascinating work, but, at the same time, one cannot help feeling that the time for personal statements in this field has been bypassed by events, as Frodin himself hints. □

V.H. Heywood is Professor in the Department of Botany, University of Reading.

Past futures

D.Q. Bowen

Late Quaternary Environments of the Soviet Union.
Edited by A.A. Velichko.
University of Minnesota Press/Longman: 1985. Pp.327. $45, £50.

This is a third, companion volume to *Late Quaternary Environments of the United States* (reviewed in *Nature* **311**, 488; 1984), and similarly arises from the USA–USSR bilateral agreement on the environment and meetings of the scientists sponsored by that agreement. This English language edition brings to the West a whole new range of data, new not least because much of the research summarized here is the product of recent years only.

As such, the book is self-evidently a publication of some significance. Given the vast area of the Soviet Union (exceeding that of the United States and Canada combined), and the diversity of the environments it encompasses, it is inevitable that the quality of coverage will be uneven. But slowly the long-standing, mostly geomorphologically based work is being supplemented by sedimentological, chronological and biostratigraphical studies. Appropriately enough, nearly all those Soviet scientists who have worked so hard to transform the situation figure in the book.

There are 30 chapters, many of which cover topics of international interest: the Late Pleistocene glaciation of the northern Soviet Union (in which a carefully argued case that large ice-sheets *did not* occupy the sites of the Barents and Kara Seas is prominent); mountain glaciation; permafrost in the Late Pleistocene and Holocene; and loesses, with a not unexpected demonstration, which nevertheless fills another gap in global correlation, that nine soil complexes are found above the Matuyma–Brunhes reversal in Central Asia. Furthermore, in the Tadzhik Depression the Olduvai reversal is identified; thus parallels emerge between Europe and points farther east, and ultimately with the global deep-sea isotope signal. An account of vegetational history gives a welcome insight into a greatly expanded field of endeavour, even though the data are regionally uneven and of varying quality, while a fascinating chapter on inland sea basins shows how sea-level change varies according to global as well as regional controls: thus 60,000 years ago the Caspian Sea rose to 76m above its present level, an event attributed to reduced evaporation and increased input from the Volga.

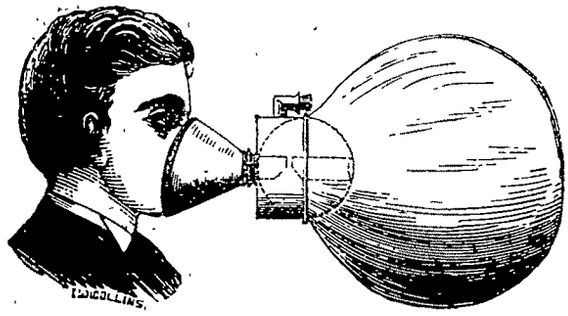
Palaeoclimatic reconstruction has long been a strength of Soviet scientists and included are convincing reconstructions of the principal stages along with inferences on their atmospheric circulation. It is shown that the "glacial monsoon" of the western Soviet Union, created by juxtaposition of cold high-pressure zones over the ice sheet and warmer low-pressure systems farther south, played a vital role in the transport and deposition of the loess deposits so critical for correlation.

Many readers will repair eagerly to the section on the dispersal of primitive cultures, not least in the hope that evidence from Siberia will throw light on the timing of the entry of man into the New World via the Bering Strait and on the attendant possible extinction of the megafauna. Unfortunately, however, the evidence from many late Palaeolithic sites in the Soviet Union is equivocal and contributes little to resolution of this problem.

The introduction to the English edition by H.E. Wright and C.W. Barnosky pulls the wide range of chapters together in an integrative and informative manner. Given the continuing exploration and development of the natural resources of the Soviet Union, it seems likely that much of the Late Quaternary framework evident there will be further elaborated and be of still greater benefit to Quaternary scientists elsewhere.

D.Q. Bowen is Professor of Physical Geography at The University College of Wales, Aberystwyth.

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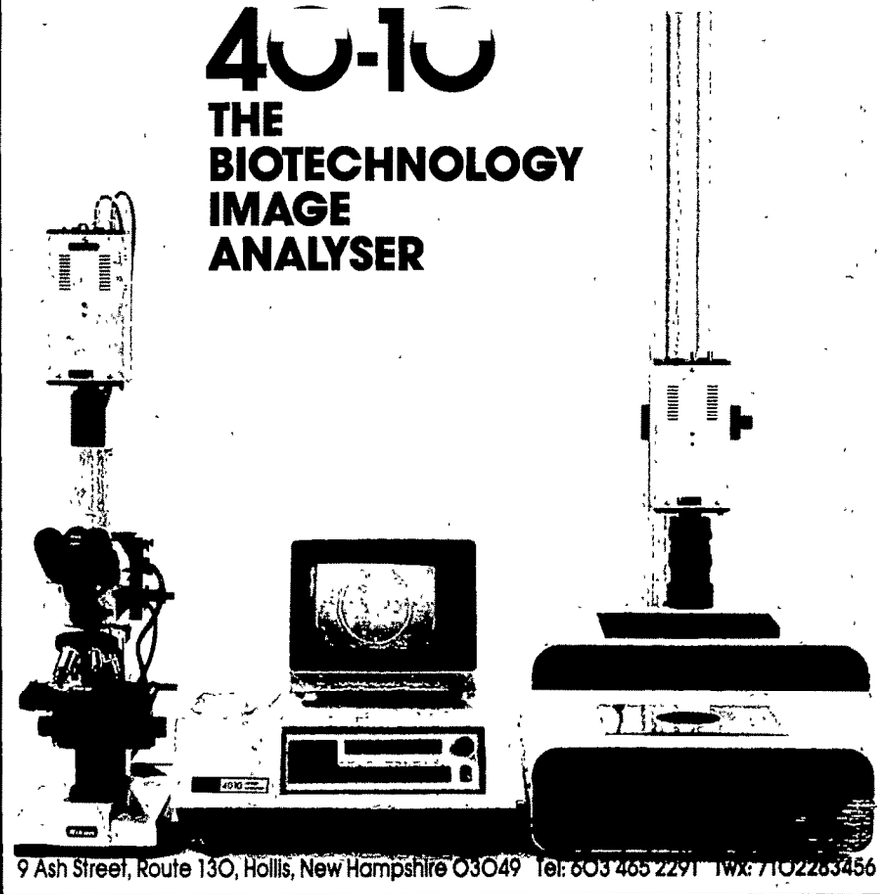
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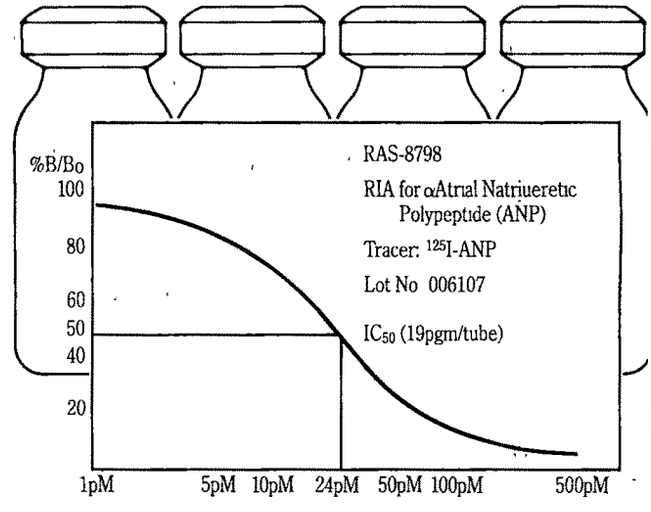
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rat Atriopeptin II	27
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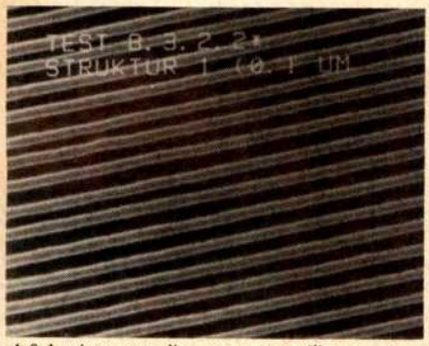
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Reader Service No.02

Eye movements recorded

This month's sampling of new products includes a sensitive system for recording human eye movements and a bench-top dry ice machine.

● Microtome manufacturers Temtool Ltd claim that a few years ago disposable 'razor' blades dominated the market, but now the economics of disposable versus re-usable knives is such that the re-usable knife is again competitive, especially when an automatic knife sharpener is used. The Rotosharp 2 from Temtool is an automatic sharpener that takes a wide variety of knife sizes and types (including tungsten carbide) and produces good edges in a few minutes.
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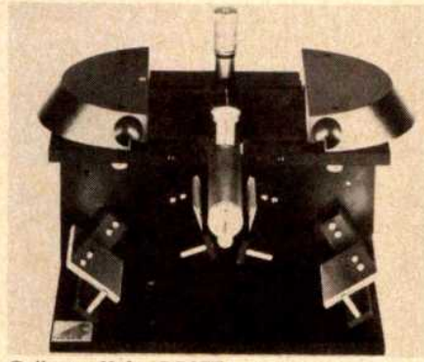
A 0.1-micrometre line pattern on silicon, written on a 0.35-micrometre-thick PMMA resist with the Philips Beamwriter.

● Sophisticated software, operating on a simple question-and-answer basis, has virtually eliminated manual adjustment of the Beamwriter EBPG-4 vector scan lithography system from Philips. Allowing linewidths down to 0.1 micrometres, the EBPG-4 offers true sub-micrometre resolution. Developed from the world's first commercial production-oriented vector scan electron beam pattern generator (EBPG) system, the EBPG-4 Beamwriter has been specially designed for direct writing onto silicon or gallium arsenide wafers but can also be used for making masks and reticles.
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● For applications in the laboratory where only the standard, 13A a.c. mains power supply is available, Lenton Thermal Designs Ltd has created a range of programmable bench-mounted furnaces: the Ultraspeed and the ECF series. The Ultraspeed reaches maximum operating temperature in only 10 min, and is available in two chamber sizes, 1.5 litres and 2.0 litres. ECF furnaces are available as a general purpose laboratory furnace and as an ashing furnace for the ashing of foods prior to microanalysis.
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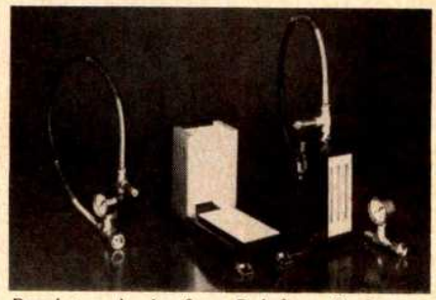
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● Now available from Barnes Analytical/Spectra-Tech is the Collector II, an advanced version of their FTIR accessory for DRIFT (diffuse reflectance infrared Fourier transform) spectroscopy. A major development is the Blocker, a device which 'blocks' and greatly reduces or eliminates the specular reflectance component. The Collector II is especially suited for obtaining spectra from fibres, powders, foams, paint chips, catalysts (zeolites) and coal, as well as LC and HPLC eluents.
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Collector II for DRIFT spectroscopy.

● The SP8773XR absorbance detector, designed for routine operation at low detection limits, complements the Spectra-Physics line of modular, extended-range LC systems. LED display indicates real-time status, and provides self-diagnosis data for easy troubleshooting. Precision flow cells are readily interchanged to accommodate micro to preparative analyses.
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Bench-top dry ice from Polyfoam Packers.

● The Insta Ice Machine, available from Polyfoam Packers Corporation, is a portable bench-top dry ice machine weighing only six pounds. Blocks can be made in 1 lb or 2 lb sizes that do not require any further compacting. No electricity or batteries are needed — all that is required is a cylinder of liquid CO_2 with a syphon.
Reader Service No. 105.



Skalar's device for measuring eye movement.

● Precise recording of human eye movements is possible with a new system from Skalar of Holland. It involves a scleral induction coil, embedded in a flexible annulus of silicone rubber, which adheres tightly to the limbus of adult human eyes, concentric with the cornea, in a magnetic field. Two a.c. magnetic fields of similar frequency but in spatial and phase quadrature are created by field coils. An a.c. potential is induced in a sensor coil mounted on the eye and the magnitude and phase of the signal depend on the orientation of the eye in the field.
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● Anti-tubulin, useful for localizing microtubules in cells, quantitating the amount of tubulin in cells and tissue by radioimmunoassay and for gene cloning and studies of transformed cells, is available from Polysciences, Inc. The antibody can be used for immunocytochemical staining at dilutions of 1:50 to 1:100.
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Reader Service No. 6.

Women in science and engineering

from Richard Pearson

One of the biggest changes in the employment scene in the second half of this century has been the rising participation of women in the labour market.

In the 1930s only one in ten women of working age in the United Kingdom were in employment, now nearly half are in or seeking employment; and women now account for 43 per cent of the labour force.

To date, however, women have been concentrated in the unskilled, lower-paid jobs, often working part-time hours. In terms of paper qualification, far fewer women are qualified than men. The 1981 *Census of Population* shows that while 7 per cent of adult men were graduates, the proportion of women so qualified was less than half this figure, at 3 per cent. However, the proportion of women gaining degrees has been rising fast towards equality, so that in 1983 42 per cent of those in higher education in 1983 were women compared with under 30 per cent twenty years earlier. Although more women are becoming qualified, the range of qualifications they are gaining is still markedly different from their male counterparts.

One of the most critical points for career choice is in the school system, where, by the age of 13, most girls have committed themselves to arts rather than science subjects, which severely constrains their later entry into science-based careers and courses in higher education. In terms of school qualifications, the proportion of girls gaining two or more A level qualifications in science, the usual minimum entry qualification for science or engineering courses in higher education, has barely changed, averaging about one in five.

Thus although the proportion of women going into higher education has risen dramatically over recent years, and they now account for more than half the intake into many medical schools, the changes on science and engineering courses have been far less marked. While in the case of engineering the proportion has more than doubled, women still account for only one in ten students, and the proportion of women on science courses has risen only slightly (Table 1). Of the women graduating last year, nearly two-thirds had art or social science degrees, only 21 per cent had science degrees and a mere 4 per cent had engineering degrees.

Table 1 Women first-year students as % of all students

Medicine	49.0
Engineering and technology	9.5
Science	33.1
Social science	44.4
Language, literature	69.0
Other arts	54.6
All subjects	40.7

Source: UCCA figures for 1982.

Curiously, girls' preferences within engineering do not seem to conform to the expected pattern in relation to individual subjects, thus women represent an above average 12 per cent of the total in chemical engineering, but under 6 per cent in electrical engineering and electronics, a field generally regarded as having a less traditional heavy engineering image. This rather lower representation may be because the competition for places is much harder in these subjects.

Table 2 Percentage of women in main graduate occupations.

Teachers	56.0%
Medics	29.6%
Scientists	16.0%
Accountants	8.1%
Lawyers	15.1%
Architects	4.2%
Engineers	1.2%

Source: *Census of Population*, for 1981.

In terms of jobs, the contrasts with men are even more marked. The most fundamental difference in 1981 was that almost a third of the women graduates (about 200,000) were not working or seeking work, mostly presumably for family reasons, while among men the corresponding figure was only 11 per cent. Interestingly, similar proportions of men and women graduates were unemployed — three per cent. Of the 450,000 working women, almost half were in teaching and indeed in this occupation they outnumbered their male counterparts. In medicine, women are relatively well represented, accounting for nearly one in three of all medical and dental practitioners. However, only about one in six of scientists are women, and fewer than one in eighty of the engineers (Table 2). There are far more graduate women in accountancy and in law than in engineering.

To remedy this low representation of women in science and engineering, for which both women and society generally suffer, the year 1984 was designated as WISE — Women in Science and Engineering — Year. Sponsored by the Engineering Council and the Equal Opportunities Commission, the main aim of the campaign was to educate and encourage girls, through a wide range of promotional activities, to take up careers in science and engineering. Even before WISE Year there have been initiatives aimed at encouraging schoolgirls to consider engineering careers. These have included special "Insight" programmes to allow sixth-formers to have close experience of engineering over a period of a weeks in industry, special scholarships and local school-industry liaison activities. The latter have not always been a success, however, with one girls' school returning a package of engineering careers leaflets with the comment "This must have been sent to us in error, this is a girls' school"

WISE Year included over 500 special conferences and events in schools, colleges and employers' premises, and many activities are continuing into 1985 and beyond. A limited number of new scholarships for girls was awarded during 1984 by the Engineering Training Board and others, but because the year was primarily aimed at raising awareness, it will be several years before any effective evaluation can be made, when any changes in subject choice among girls in schools and higher education will start to become apparent. At any rate, the organizers are already claiming that the year was a success.

An added urgency for women to enter technology-based courses was the fact that 1982 saw the number of 18-year-olds in the population, the traditional entry group into higher education, start to fall. The number will decrease from 1,000,000 to 650,000 in 1995 with most of the fall occurring during the latter part of the period. While the implications of this fall in terms of demand for higher education places have been hotly debated for several years now, in the cases of less popular subjects such as engineering, such a fall could have a significant effect on the numbers graduating. A rising relative demand by women will help mitigate against the overall fall and an increasing representation by women on engineering and technology courses may be the only means of ensuring an adequate future supply of graduates with key skills. Thus meeting the needs of the market and providing equality of opportunity could well become self-supporting policies in the late 1980s. □

Richard Pearson in at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.

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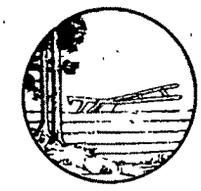
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M Roch Carbonneau, Directeur, Départment de sciences biologiques, Universite de Montréal, C.P 6128, succursale A, Montréal (Québec) H3C 3J7
Conformément aux exigences prescrites en matière d'immigration au Canada, cette annonce s'adresse aux citoyens et aux résidents permanents au Canada. (NW1720)A

The Department of Cell Biology at Baylor College of Medicine currently has two faculty positions open for outstanding scientists, at either a junior or senior level, in the area of contemporary molecular genetics We are particularly interested in candidates that are involved in applying techniques of recombinant DNA research for studying basic problems in cell and development biology. The present well-funded and nationally renowned faculty in this Department reflects a diverse set of research interests, and the existing resources should be an attractive feature for prospective candidates

Send curriculum vitae with names of three references to:

Dr. Jeffrey M. Rosen,
Professor, Depart. of Cell Biology,
Baylor College of Medicine,
1 Baylor Plaza,
Houston, Texas 77030 (NW1623)A

Faculty Position — Biochemistry

The Department of Biochemistry, University of Virginia School of Medicine invites applications for a tenure-track, full-time faculty position at the assistant or associate professor level starting no later than 1 September 1986.

Applicants must exhibit a strong commitment to teaching and to establishing a vigorous, independent research program. The candidate must have a doctoral degree in Biochemistry or a closely related field with postdoctoral experience; however, applications are encouarged from all outstanding candidates irrespective of their current interests.

Please submit letter of application, curriculum vitae, and three reference letters to: Dr. Ching-hsien Huang, Chairman of Search Committee, Department of Biochemistry, Box 440, University of Virginia School of Medicine, Charlottesville, Virginia 22908. Application deadline is 1 August 1985. *An Equal Opportunity/ Affirmative Action Employer* (NW1704)A

THE UNIVERSITY OF TEXAS AT AUSTIN U.S.A.

POSTDOCTORAL ASSOCIATE IN PLANT AND MICROBIAL MOLECULAR GENETICS

A 12-month appointment is available beginning June 1985, with possibility of renewal. Salary commensurate with experience Preference given to applicants with expenence in microbiology, trans poson mutagenesis, Ti-vectors, and recombinant DNA technology Major research emphasis on microbial cellulose gene structure and expression, but other research on the molecular genetics of cellulose gene expression in higher plants and algae will be undertaken Well equipped new laboratory and excellent opportunity to interact with a versatile research team in an internationally recognized Botany Department.

Please send résumé, brief summary of research experience, and list of three referees to Dr. R Malcolm Brown, Jr., Department of Botany, The University of Texas at Austin, Austin, TX 78713-7640, U S A. Telephone (512) 471-3363. (NW1715)A

UNIVERSITY OF VERMONT COLLEGE OF MEDICINE

DEPARTMENT OF MEDICINE

RESEARCH ASSISTANT PROFESSOR

Full time research faculty to conduct independent and collaborative research in fields relating to hemostasis and thrombosis using cultured vascular cells Areas of expertise can include biochemistry, microbiology, cell biology, or cell physiology

Applicants must have a Ph D degree with demonstrated proficiency in growing vascular endothelial cells in culture A research grant/career award providing 50% of annual salary desirable but not essential.

Qualified and interested candidates should send CV, names and addresses of 3 references and brief outline of research interests by June 15 to. John C. Hoak, M.D., Department of Medicine, Fletcher House 311, MCHV, Burlington, Vermont 05401.

An Affirmative Action Employer. Women and minorities encouraged to apply (NW1716)A

DIRECTOR

BIOTECHNOLOGY RESEARCH INSTITUTE MONTREAL

The Organization

The National Research Council of Canada is establishing a new Biotechnology Research Institute in Montreal. This new research institute emphasizes the industrial applications of biological sciences and focuses on the development of new processes and products. It fosters generic research and technology transfer to industry and promotes communication and collaboration amongst industrial, university, federal and provincial government laboratories.

The Research Areas

The main areas of research include:

- Fermentation and biochemical and process engineering
- Genetic engineering
- Cell fusion and molecular immunology
- Enzyme technology and protein engineering

A major large scale fermentation facility will allow pilot scale experimentation with new fermentation methods and economic evaluation. The R & D will focus on agrofood, energy treatment and chemicals, pharmaceuticals, waste product and forestry.

The Position

The Director reports to the Vice-President (Biotechnology and Chemistry) and is an active contributor to the National Biotechnology Program. The Director is responsible for planning, organizing, implementing and controlling the Institute's research and development programs and advises senior management on long term research goals. The Director is also responsible for establishing close ties with universities and industries and undertaking research projects on a cooperative basis.

The Candidate

The person appointed will have an established reputation for outstanding leadership in research and development and the ability to lead, manage and coordinate the activities of the Institute. Candidates will have many years of research experience in a biotechnology related discipline and demonstrated ability in research managment. Knowledge of both the English and French languages is essential.

The Institute

The Biotechnology Research Institute is located in downtown Montreal in a new $60 million, 18,000$m^2$ complex. It will employ 220 people and will host up to 80 researchers from industries, universities and other research organizations. It will have an annual budget of $35 million.

BENEFITS are fully competitive and SALARY will be commensurate with qualifications and experience.

PLEASE SEND A DETAILED RÉSUMÉ IN CONFIDENCE TO THE:
General Manager,
Personnel Branch,
National Research Council of Canada,
Ottawa, CANADA, K1A 0R6.

(NW1706)

National Research Council Canada | Conseil national de recherches Canada

Canada

MOLECULAR BIOLOGY DEPARTMENT

Post Doctoral Scientists

Salary from £9,000

Celltech, a leader in the field of biotechnology, has achieved its present success in the space of four years. Pioneers in several areas, our achievements are based on the foundations of scientific excellence, innovation and commercial expertise

Due to continuing expansion, the Molecular Biology Department requires further Post Doctoral Scientists or graduates about to submit a PhD thesis to join teams cloning and sequencing genes of potential therapeutic importance Experience with the isolation of proteins and/or cloning techniques would be an advantage.

Celltech is an ambitious and forward looking company, which places as much emphasis on its people as its products. You will work in a small friendly team, gaining invaluable broad-based experience, with plenty of opportunity for advancement. Please send a detailed c.v to Jane Walley, Celltech Ltd., 244-250 Bath Road, Slough, Berks SL1 4DY, quoting Ref. 258

CELLTECH

(6331)A

READVERTISEMENT

THE ANIMAL VIRUS RESEARCH INSTITUTE

HEAD OF IMMUNOLOGY AND PATHOLOGY

Applications are invited for a new post of Head of the Division of Immunology and Pathology, the appointment to commence as soon as possible.

Candidates should have a high academic research record in molecular or cellular immunology, with experience, or an interest in, the immunology of virus infection in farm animals, and proven skills of leadership

The Division includes Departments of Immunology, Experimental pathology and Vaccine Research.

Salary in scale £16,229 — £21,834 Grade 6 (pay award pending). Sick leave and non contributory superannuation schemes. 25 days annual holiday. House may be available for suitable married applicant. The successful candidate may qualify for financial assistance with removal expenses.

HIGHER SCIENTIFIC OFFICER

Applications are invited for the post of Higher Scientific Officer Band 1 to work in the Division of Molecular Biology, Department of Biochemistry. The appointment to commence as soon as possible. The successful candidate will assist with work on the structure and function of the blue tongue virus genome

Candidates should have at least 3 years post graduate research experience. Degree in Biochemistry or Microbiology and a PhD

Salary scale £7,435 — £10,039 (pay award pending), sick leave and non-contributory superannuation schemes, 22 days annual holiday. House may be available for suitable married applicant.

For both posts applicants should send a curriculum vitae and the names of two referees by 31 May 1985 to the Secretary, The Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, from whom further particulars may be obtained. The Animal Virus Research Institute is an Equal Opportunities Employer. (6216)A

INSTITUTE OF CANCER RESEARCH

TECHNICIAN/RESEARCH OFFICER

Required for CRC/MRC funded post at the Institute's laboratories at Sutton, Surrey, to work on clonal analysis of formal and neoplastic tissue in mouse aggregation chimaeras The mosaic markers currently used include H2 antigens and lectin polymorphism demonstrated immunocytochemically in tissue sections (cf J Embryol exp Morphol, 76, 83-93; Nature 313, 689-691; Cell, 40, 425-429).

Applicants should have expertise in immunocytochemistry, including cryostat sections. Additional experience in the following would be an advantage: making chimaeras, preparing antibody-enzyme conjugates, microdissection, general animal handling. The post is available now; an early starting date would be ideal so as to provide an overlap to learn techniques from the present incumbent, who is expected to leave during the summer. Salary in scale £5,738 - £8,492 pa, plus London Allowance of £623 pa.

Further details may be obtained from Dr Bruce Ponder, Institute of Cancer Research, Haddow Laboratories, Clifton Avenue, Sutton, Surrey SM2 5PX, telephone 01-643 8901, ext 293. Applications in duplicate should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting ref. 301/B/83. Closing date for applications 17th May, 1985. (6264)A

INSTITUTE OF CANCER RESEARCH

TEAM LEADER

Applications are invited for a 5-yea appointment with career prospects t lead a research team in the Institute' Cell and Molecular Biology Sectio in the Chester Beatty Laboratories Fulham Road, London, SW3. Estab lished groups in the Section ar working on Oncogenes, Chromatin Structure and Function, Develop ment Genetics, Cloning Technolog and Human Retroviruses. The Insti tute is jointly funded by the Cance Research Campaign and the Medica Research Council on a system of si year grants which are reviewed ever fourth year.

Candidates should be experience in any field of research related to th molecular biology of cancer bu applications from those working or the structure and function of trans forming proteins are especiall sought.

The salary, depending on quali fications and experience will be in th range £10,330 — £14,925 pa, plu London Allowance of £1,233 pa

Applications consisting of a CV an outline research proposal, and th names of three referees should b sent, in duplicate, to the Personne Officer, Institute of Cance Research, 17A Onslow Gardens London, SW7 3AL, quoting re 300/G/12.

Applicants are advised that in th majority of the Institute's premise smoking is prohibited. (6274)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

UNIVERSITY LECTURER OR UNIVERSITY DEMONSTRATOR IN VIROLOGY

Applications are invited for a post of either a University Lecturer or a University Demonstrator (the equivalent of University Assistant Lecturer) in the Department of Pathology, appointment to commence on 1 October 1985 or as soon thereafter as possible.

Candidates should hold a PhD degree or equivalent and have several years post-doctoral experience in a relevant area. The successful candidate will join the Division of Virology in the Department of Pathology and will be expected to contribute to both teaching and research in Virology.

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'B', 19 Trumpington Street, Cambridge, CB2 1QA, to whom applications (10 copies) including a curriculum vitae, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 1 June 1985. (6263)A

BRISTOL POLYTECHNIC

SCIENCE DEPARTMENT

LECTURER II/ SENIOR LECTURER IN CLINICAL CHEMISTRY/ BIOCHEMISTRY REF NO L/12

Applications are invited from graduates in a relevant subject an preferably FIMLS who have researcl and/or hospital laboratory experienc in clinical chemistry A particular in terest in the development of new assa methods is desirable.

The successful applicant will teacl clinical chemistry and biochemistry o Degree, BTEC and FIMLS course and will be expected to initiate a active research programme.

The appointment will commence o 1 September 1985. Salary scale; LI £7,548 — £11,175 (bar) — £12,099 pe annum; SL £11,175 — £13,128 (bar — £14,061 per annum.

The appointment will be made o the appropriate scale according t relevant previous service/experience (Progression from the LII scale to th SL scale in in accordance with the pro visions of the Burnham Further Edu cation Report).

For further details and an appli cation form, to be returned by 1 May, 1985, please contact the Per sonnel Office, Bristol Polytechnic Coldharbour Lane, Frenchay, Bristo or ring Bristol 656261 ext 216 or 217 (6247)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF MICROBIOLOGY

JUNIOR RESEARCH ASSOCIATE (FERMENTATION TECHNOLOGY), POSTDOCTORAL RESEARCH ASSOCIATE, JUNIOR RESEARCH ASSOCIATE

The following posts, financed by a grant from Tyne and Wear County Council, within the Microbial Technology Group, are available for two years for the promotion of the industrial exploitation of the Group's research activities.

JUNIOR RESEARCH ASSOCIATE (FERMENTATION TECHNOLOGY)

To take charge of the day to day operations of the Fermentation Unit and to provide support for collaborative projects with local industry. Applicants should have a good honours degree in an appropriate biological subject and experience in fermentation and/or appropriate industrial or research experience in running fermenters. Commencing salary up to £7,520 per annum on the National Range IB salary scale (£6,600 - £8,920 per annum).

POSTDOCTORAL RESEARCH ASSOCIATE

To work on the development of new techniques for the isolation and screening of novel microorganisms for new bioactive compounds. Applicants should have experience in Microbiology or a related biological discipline and preference will be given to those familiar with analytical techniques used in chemosystematics and with computer assisted data handling methods. Commencing salary up to £7,980 per annum on the National Range IA salary scale (£7,520 - £12,150 per annum).

JUNIOR RESEARCH ASSOCIATE

To work with the postdoctoral Research Associate on the isolation of novel microorganisms. Applicants should hold a good honours degree in a biological subject. Commencing salary £6,600 per annum on the National Range IB salary scale (£6,600 - £8,920 per annum).

JUNIOR RESEARCH ASSOCIATE

To work with the postdoctoral Research Associate on the isolation of novel microorganisms. Applicants should hold a good honours degree in a biological subject. Commencing salary £6,600 per annum on the National Range IB salary scale (£6,600 - £8,920 per annum).

Written applications, providing full curriculum vitae and names and addresses of three referees should be submitted to Professor A R Archibald, Microbial Technology Group, Department of Microbiology, Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH. Tel 0632 328511 Ext 3710 not later than 23rd May 1985.

(6302)A

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY

DEPARTMENT OF NEUROPATHOLOGY

"NEW BLOOD" LECTURESHIP IN NEUROIMMUNOLOGY

Applications are invited for a "New Blood" lectureship in Neuroimmunology in the Department Of Neuropathology from 1 October 1985. Applicants should have research interests in the development of antibodies as cell-specific markers to be used for the identification of various types of cells in the brain. The successful candidate will be expected to follow progress in immunology relevant to Neurological and mental illness to assist in the development of the clinical application of recent discoveries and to teach neuroimmunology to postgraduate psychiatrists and neuroscientists.

Salary according to age, qualifications and experience either on the Lecturer scale (£7,520 — £14,925 plus £1,233 London Allowance) or the Clinical Lecturer scale (£10,130 — £17,560 plus £807 London Allowance).

Further information can be obtained from Professor P L Lantos and application forms are available from Miss M E Harries, Department of Neuropathology. Tel 01-703 5411 Ext 338.

Applications with a full curriculum vitae should be returned to the Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF by 12 May 1985.

(6268)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF ASTRONOMY

ACCRETION PROCESSES ON COMPACT OBJECTS

An SERC-supported postdoctoral position in the above research area will be available from October 1985. Present work in this area in the Astronomy Department includes theoretical studies of accretion processes in cataclysmic variables and X-ray binaries, and interpretation of EXOSAT and other results on these systems obtained on collaboration with members of the X-ray Astronomy Group. Applications are invited from both theoretical astrophysicists with backgrounds in relevant subjects (e.g. astrophysical fluid mechanics, solar/stellar physics, or accretion processes in other contexts) and from astrophysicists experienced in the interpretation of data from related systems.

The post is tenable for up to three years, at a starting salary (under review) up to £9,390 plus USS benefits, depending on qualifications and age. Further information can be obtained from Dr A R King, Astronomy Department, University of Leicester, Leicester, LE1 7RH. Applications, including a curriculum vitae and list of publications, must be received by 15 June, 1985, and applicants should themselves request two referees to write to Dr King by the same date.

(6291)A

CSIRO AUSTRALIA

RESEARCH FELLOW

$A25,342 — $A37,274

DIVISION OF MOLECULAR BIOLOGY SYDNEY NSW

FIELD: Molecular Biology/Genetic Engineering.

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes. Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Molecular Biology is a member of the Institute of Animal and Food Sciences

GENERAL: The Division of Molecular Biology conducts scientific research in molecular genetics and cell biology It has a staff of approximately 100 Programs are divided into Animal Cell Growth and Development, DNA Maintenance and Modification, and Gene Technology. It is situated in Sydney, at North Ryde, close to Macquarie University and major biotechnology companies.

DUTIES: The appointee will be located in the Gene Technology program, and will undertake collaborative research in isolation, analysis and expression of genes coding for vaccine antigens, and the development of novel systems for their delivery in vivo. The broader activities of this program include research into the regulation of gene expression in animal, fungal and procaryotic systems.

QUALIFICATIONS: A PhD degree or equivalent qualifications in molecular genetics, biochemistry or related discipline, preferably with relevant post-doctoral training. Experience in recombinant DNA research is essential. Experience in molecular techniques of protein and immunological analysis would be an advantage.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No. A3475, should be directed to:

Dr G.W. Grigg
Chief
CSIRO Division of Molecular Biology
PO Box 184
NORTH RYDE NSW
AUSTRALIA 2113

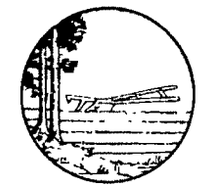
by four weeks from date of application.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1828)A

MACAULAY INSTITUTE FOR SOIL RESEARCH

DEPARTMENT OF MINERAL SOILS



HIGHER SCIENTIFIC OFFICER

Applications are invited for appointment on a 3-year contract, on the Surface Water Acidification Programme (SWAP) under the auspices of The Royal Society, The Norwegian Academy of Science and Letters and the Royal Swedish Academy of Sciences.

A physical chemist/geochemist/soil chemist is required to conduct research into the chemisry of soil/water interactions at three river catchments in Scotland as part of an integrated study of the effects of acid deposition on the acidification of surface waters and on the survival of fish and freshwater biota

Candidates should possess a First or Upper Second Class honours degree in chemistry, geochemistry, or soil science with at least two years' relevant post-graduate experience. Salary £7,435 — £10,039 per annum according to experience Non-contributory superannuation scheme operative. The Macaulay Institute for Soil Research is an Equal Opportunities Employer.

Forms of application and further particulars may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, to whom they should be returned by 20 May 1985.
Quote Ref. SWAP/3/85.

(6286)A

APPLIED BIOSYSTEMS LTD

DNA SYNTHESIS
PEPTIDE SYNTHESIS

The leading company in the field of instrumentation for biotechnology reseach seeks two specialists in the areas of DNA Synthesis and Peptide Synthesis for its newly established U.K. subsidiary. The positions are both in our Technical Support Group and will be based at Birchwood Science Park, Warrington where new laboratories are currently under construction. To be considered, a candidate must have a first class academic background and direct experience of current methods of DNA Synthesis, particularly Amidite Chemistry, and for the Peptide position of Solid Phase Peptide Synthesis.

C.V. and current salary requirements to

Mrs A Thompson
Applied Biostystems Ltd
Kelvin Close
Birchwood Science Park
Warrington, Cheshire Tel. 0925-825650

(6249)A

POSTDOCTORAL TRAINING PROGRAM IN SLOW INFECTIONS

NIH/UCSF Postdoctoral training program in the molecular biology and chemical structure of slow infectious agents, or prions. Program emphasizes studies on experimental scrapie in rodents as a model degenerative neurologic desease Areas of study include: molecular cloning, expression of cloned genes, gene mapping and time structure, immunogenetics, production of monoclonal antibodies to synthetic peptides, ultrastructure and x-ray diffraction of filamentous and membrane proteins, molecular neuropathology and experimental neurology Program is directed by five UCSF faculty members. Candidates should be U.S. citizens or permanent residents committed to a research career and hold a Ph.D., M D. or D.V M. degreé. Experience in molecular biology, biochemistry, biophysics, macromolecular structure, immunology, virology, neurology or neuropathology is encouraged. Appointments, beginning June 1, 1985, are for 3 years with a salary ranging from $15,996 to $30,000.

Send curriculum vitae and 3 letters of reference to Dr. Stanley Prusiner, Department of Neurology, HSE 781, University of California, San Francisco, CA 94143.

The University of California is an affirmative action equal opportunity employer. (NW1710)A

POSITION AVAILABLE AT THE UNIVERSITY OF CALIFORNIA, DAVIS IN HUMAN DEVELOPMENT

12-month (including one month vacation), 70% teaching/30% research, tenure-track position at the Assistant Professor level, beginning January 1, 1986 (negotiable). Salary range. $29,600-$33,600 (range adjustment expected 7/1/85). Applicants must hold a Ph D in relevant discipline with a specialization in human behavioral development. Emphasis in biological bases of behavior or behavioral genetics is highly desirable. Must have demonstrated research expertise in adolescent/adult development. Responsibilities will include teaching and advising in Child/Human Development at the undergraduate and graduate levels.

A vita, a statement of qualifications, list of publications, transcripts of undergraduate and graduate work if within 5 years of receiving doctorate, and names and addresses of at least three references should be sent to: R. H. Kraft, Chair, Human Development Search Committee, Department of Applied Behavioral Sciences, U.C. Davis 95616. Final filing date: To be assured of consideration applicants should submit materials by July 31, 1985.

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW1638)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PHARMACOLOGY

UNIVERSITY LECTURER OR UNIVERSITY DEMONSTRATOR IN IMMUNOPHARMACOLOGY
New Blood

Applications are invited for a post of either a University Lecturer or a University Demonstrator (the equivalent of University Assistant Lecturer) in the Department of Pharmacology, appointment to commence on 1 October 1985 or as soon thereafter as possible.

The person appointed will be expected to develop a research programme in immunopharmacology and be involved in undergraduate teaching.

Applicants should have a PhD degree or equivalent and research experience in cellular or molecular immunology.

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee for the Faculty of Biology 'B', 19 Trumpington Street, Cambridge CB2 1QA, to whom applications (10 copies) including a curriculum vitae, list of publications and the names of not more than three referees, should be sent so as to reach him not later than 1 June 1985. (6262)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF GENETICS

Applications are invited for two posts:

UNIVERSITY LECTURESHIP OR DEMONSTRATORSHIP

(equivalent to UAL) in the field of Molecular Plant Genetics under the new Blood Scheme. The person appointed will be expected to establish a research programme and to undertake teaching.

UNIVERSITY DEMONSTRATORSHIP

preferably in the field of microbial genetics (either bacteria or yeast) though well qualified candidates from other areas of genetics will also be considered.

Relevant pensionable scales of stipends are as follows: UL £10,330 a year rising by eleven annual increments to £15,930. UD £7,980 a year rising by four annual increments to £9,860. Informal enquiries concerning either of these posts can be made to Professor J R S Fincham, Department of Genetics, Downing Street, Cambridge CB2 3EH.

Further particulars may be obtained from Secretary to the Appointments Committee, Faculty of Biology A, Zoology Department, Downing Street, Cambridge CB2 3EJ to whom application (3 copies) including curriculum vitae and names of three referees should be sent to reach him by 31st May 1985.

Applications for new blood post should be marked 'NBGEN'. (6276)A

UNIVERSITY OF DURHAM

POSTS IN ELECTRONICS/ ELECTRONIC ENGINEERING

Applications are invited for the following posts in a new School of Engineering and Applied Science being formed in Durham to consolidate expansion of existing electronics research and teaching in the University.

CHAIR OF ELECTRONICS

Applicants should have a wide range of interests in digital and analogue electronic circuits and systems. It is expected that the person appointed will have an established reputation for research combined with recent experience in industry or close collaboration with industry.

THREE LECTURESHIPS

The University has created three new Lectureships in Electronics/ Electronic Engineering. Candidates should preferably have experience in digital or analogue circuit or system design and their applications in communications, signal processing, or information engineering. Preference will be given to candidates who have recent experience in industry or have collaborated with industry in a research programme.

Salaries on the scales (under review) - Professors, basic, £18,172 -£21,156; Lecturers £7,520 - £14,925 with the usual pension arrangements.

Applications (chair - 12 copies; Lectureships - 3 copies) naming three referees by 31 May 1985 to the Registrar, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. (6301)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH BIOCHEMIST
(Scale 1A)

Applications are invited for the above position to pursue research into the mechanism of action of insulin. The post is funded under an MRC Programme Grant (to Dr R M Denton) and will be available from 1 July 1985 until 30 September 1988 in the first instance, with a starting salary in the range £7,520 — £9,860 (under review).

Studies will be largely concerned with exploring the mechanisms involved in the effects of insulin on the activity of pyruvate dehydrogenase and other enzymes in mammalian cells (especially fat cells).

Applicants with appropriate experience, for example in the study of reversible phosphorylation of proteins or mitochondrial metabolism should send a complete Curriculum Vitae, plus the names of two or three referees to Dr R M Denton, Department of Biochemistry, University of Bristol Medical School, University Walk, Bristol BS8 1TD (0272-24161 ext 798), from whom further information can be obtained.

Closing Date: 30 May 1985. (6289)A

THE ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen, AB2 9SB

DEPARTMENT OF MICROBIAL BIOCHEMISTRY

MOLECULAR BIOLOGIST

Applications are invited for a new 3-year post in the Department of Microbial Biochemistry to investigate gene manipultion in the exploitation and study of fungal cellulases.

The appointee would be expected to collaborate with other biochemists, molecular biologists and physical chemists at the Rowett Institute and elsewhere to obtain basic information on the structure/function relationships of cellulase components. The long term objective would be to obtain, by site specific mutagenesis, cellulases with new properties which would attract commercial interest.

Applicants should possess a first or second class honours degree in biochemistry or microbiology with a PhD and relevant experience in molecular biology.

Salary will be in the grades of SSO/HSO — £9,329 to £12,050 and £7,435 to £10,039 pa respectively (pay award pending). The Institute operates a non-contributory Superannuation Scheme although 1½% is contributed for dependent relatives. The Institute is an Equal Opportunities Employer.

Letters of application accompanied by a CV and the names and addresses of two referees should be sent to the Secretary at the above address by 23 May 1985 (6267)A

CRYSTALLOGRAPHY GROUP
UNIVERSITY CHEMICAL LABORATORY
Cambridge, UK

POST-DOCTORAL CRYSTALLOGRAPHER

Post-doctoral crystallographer wanted to join a group of chemists and crystallographers working on the structure of DNA fragments and DNA-drug complexes

The group have well developed techniques for synthesis and crystallisation, are currently working on four different structures with base pair mismatches and have data for many more. Experience in macromolecular crystallography would be an advantage but enthusiasm for tackling challenging problems and experimental/stucture-solving skills are more important. The project is a long term one supported by the MRC with salaries age-related on the University scale. One of the posts might be of interest to someone wishing to join in the work for a sabbatical year.

Please apply in writing to Dr Olga Kennard, Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK, or telephone 0223 66499 ext 331. (6258)A

UNIVERSITY OF EDINBURGH
Reproductive Endocrinology Laboratory
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY
CENTRE FOR REPRODUCTIVE BIOLOGY

SENIOR LECTURER (non-clinical)

Applications are invited for the post of Senior Lecturer (non-clinical) in the above Department.

As Director of the Reproductive Endocrinology Laboratory, the successful applicant will be responsible for providing a limited range of hormone assays for clinicians in reproductive medicine. The salaries of 9 technicians, 2 secretaries and running costs are reimbursed by the Lothian Health Board In addition to service activities, the successful applicant will be expected to take advantage of the excellent opportunities for research available within the Centre for Reproductive Biology which contains the Department of Obstetrics and Gynaecology (Professor David T Baird) and the MRC Unit of Reproductive Biology (Director: Dr D W Lincoln)

The salary will be within the range £14,135 - £17,705 per annum together with superannuation under USS.

Further enquiries to: Professor David T Baird, Department of Obstetrics and Gynaecology, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW. Tel No: 031-229 2575.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College South Bridge, Edinburgh EH8 9YL, with whom applications (12 copies) including curriculum vitae and the names and addresses of three referees, should be lodged not later than 18/5/85.

Please quote Reference No 538. (6142)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF FORESTRY

'NEW BLOOD' LECTURER IN TREE BIOTECHNOLOGY

A 'New Blood' post is available in the above department specifically to develop the roles of tissue culture, micropropagation and protoplast fusion in the breeding of forest trees. This will be part of an existing tree breeding programme concentrating particularly on broadleaved species.

Candidates should possess, or be about to obtain, a PhD and have a degree or expertise in Genetics, Biochemistry or Developmental Biology. Experience in manipulation of protoplasts would be an advantage.

Salary will be on the Lecturer scale £7,520 — £14,925 per annum (scale under review) with appropriate placing

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 5 July 1985. (6272)A

Natural Environment Research Council

Environmental Research

Directors of Science

Swindon based **£29,500**

The Natural Environment Research Council wishes to appoint three new Directors to make a contribution at the highest level in the formulation and implementation of scientific policy in the following areas

Earth Sciences:
Geology, solid earth geophysics, geochemistry, glaciology and aspects of physical geography applied to the understanding of the origin and structure of the earth and the processes instrumental in its formation and evolution

Marine Sciences:
Physical, chemical and biological oceanography, ocean/atmosphere interaction, including meteorology, climatology and marine biology

Terrestrial & Freshwater Sciences:
Hydrology, hydrochemistry and freshwater biology, terrestrial ecology and related sciences, including forestry and land/atmosphere interactions

Responsible to Council through the Secretary and Chairman, the Directors will advise on the allocation of funds for research programmes at NERC establishments and in universities, and have overall responsibility for the management of resources and implementation of research programmes within their own field

Candidates for these posts should have the highest international scientific reputations and an overview of their Science across Research Councils, Universities and Industries They should also have experience in the management of research in particular and of resource management in general The appointments will be made at Grade 3 level on a salary of £29,500. Period appointment or secondment preferred though permanent appointment will be considered The individuals appointed will have offices at Swindon but will need to travel extensively A contribution towards relocation expenses may be made

Further details and an application form, indicating which Director post you are applying for, are available from Jem Purkis on Swindon (0793) 40101, ext 423 Completed applications should be returned by 31 May 1985 to Dr E Buttle at the Natural Environment Research Council, Polaris House, North Star Avenue, Swindon, Wiltshire, SN2 1EU

(6329)A

UNIVERSITY OF ARIZONA

STRATIGRAPHY

The Department of Geosciences at the University of Arizona invites applications for a tenure-track academic position in stratigraphy. We are particularly interested in candidates whose research efforts are directed toward the application of stratigraphy to problems in paleobiology, biostratigraphy, sedimentology, or paleotectonics We seek an individual capable of creative and interdisciplinary approaches to conceptual issues as well as practical problems in the paleobiological and sedimentological aspects of stratigraphy Duties include teaching undergraduate stratigraphy, developing graduate course material to complement and extend existing programs, advising students, directing graduate research and developing a vigorous research program.

Rank and salary will be commensurate with experience and qualifications The position will be available August, 1986, or sooner The Application deadline is August 15, 1985 All qualified persons are urged to apply

Applicants should send curriculum vitae, bibliography, statement and research interests, and names and addresses of four references to Dr. George H Davis, Department Head, Department of Geosciences, The University of Arizona, Tucson, Arizona 85721. The University of Arizona is an Equal Opportunity/Affirmative Action Employer

(NW1721)A

POSTDOCTORAL POSITIONS

Lipids-Atherosclerosis-Nutrition Training Program funded by NHLBI Postdoctoral traineeships are available for research and training in areas of a) lipoprotein stucture, b) apoplipoprotein metabolism, c) interaction of apoproteins with lipoprotein lipase, d) effect of diet on plasma lipoprotein and apolipoprotein content and metabolism, f) development of cholesterol and bile acid homeostatic mechanism, g) pathogenesis of atherosclerosis including the role of prostaglandins, h) role of branched chain amino acids in lipid metabolism and nutrition, i) vascular pathology in juvenile diabetes, j) modulation of lymphocyte physiology by membrane bound lipoproteins and k) eipdemiological and behavioural aspects of hyperlipoproteinemia. Faculty includes W F Balistreri, M.D., J.A K. Harmony, Ph.D , J.E Heubi, M.D , J.E. Fischer, M.D., C.J. Glueck, M D., R L. Jackson, Ph.D., M.L. Kashyap, M.D. J.M Vander Bel-Kahn, M.D., E.A Stein, M.D , M.T.R. Subbiah, Ph D. and R.C. Tsang, M.D

Candidates with a Ph.D. degree in biomedical or behavioural fields and those with M D. degree, with appropriate research exprience, are eligible to apply. Applicants must be U.S. citizens or hold an immigrant visa.

Send curriculum vitae with 3 letters of recommendation indicating the special area of research interest to: Dr. M.T.R. Subbiah, Program Director, Lipids-Atherosclerosis Nutrition Training Grant, University of Cincinnati Medical Center, 234 Goodman Street, K-Pavilion, M.L. 540, Cincinnati, Ohio 45267. (NW1719)A

COLAISTE NA HOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK

LECTURESHIP IN ZOOLOGY

Applications are invited from qualified zoologists with practical experience in aquaculture for a contract lectureship in Zoology, contributing to an interdisciplinary M.SC. programme in Aquaculture. Salary will be within the range IR £12,718 — IR £15,042 p.a.

Application forms and further information may be obtained from Professor M.F. Mulcahy, Department of Zoology, University College, Cork. Closing date for receipt of completed application forms is Friday, 24 May, 1985. (6330)A

UNIVERSITY CHEMICAL LABORATORY
Cambridge, UK

POST-DOCTORAL CHEMIST

Post-doctoral chemist with interest in molecular biology wanted to join a group of chemists and crystallographers working on the synthetic DNA fragments and complexes. The group has just completed the determination of several structures with base pair mismatches

Future plans include the study of DNA and RNA fragments of biological interest, some with novel modified bases. Work is also in progress on DNA-drug complexes.

The projects are exciting and exacting with good opportunities for gaining new expertise and building up a strong publication list. Experience of oligonucleotide of peptide synthesis would be an advantage, but it is not essential. The project is supported by the long-term MRC grant with adequate funds at the Research Assistant or Associate level, depending on age and experience.

Please apply in writing to Dr Olga Kennard, Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK, or telephone 0223 66499 ext 331 (6257)A

UNIVERSITY COLLEGE CARDIFF

GEOLOGY DEPARTMENT

RESEARCH DEMONSTRATOR

Applications are invited for the post of Research Demonstrator in the Department of Geology, tenable for three years. Candidates should be pursuing a higher degree and preferably have had some relevant postgraduate experience. Interests may lie in any branch of the earth sciences though preference will be given to candidates with expertise in a major area of undergraduate teaching within the department.

Departmental commitments will amount to about 10 hours per week, plus occasional fieldwork in vacations. Salary will be within the range £6,600 — £7,980 per annum. Duties to commence 1st October or as soon as possible thereafter.

Applications, 2 copies, together with the names and addresses of two academic referees should be sent to the Establishment Officer, University College, PO Box 78, Cardiff CF1 1XL from whom further particulars are available. Closing date 24th May, 1985. Ref: 2942. (6251)A

ASSISTANT PROFESSOR

Yale University School of Medicine

Section of Neuroanatomy

NEUROBIOLOGIST

With experience in ultrastructural, pathway tracing, and immunohistochemical approaches to the analysis of organization and development of mammalian cerebral cortex. The position requires a small amount of teaching and organizing laboratory exercises for medical students.

Submit résumé to Chairman, Section of Neuroanatomy, Yale University, School of Medicine, New Haven, Ct. 06501. Yale is an Equal Opportunity/Affirmative Action Employer. (NW1708)A

NORTHWESTERN UNIVERSITY

Medical and Dental Schools

Department of Microbiology/Immunology and Cancer Center

POSTDOCTORAL POSITION

Applications are invited for a funded postdoctoral research position to study oncogene suppressors in human cells. Strong background in molecular biology preferred.

Applicants should send curriculum vitae and names, addresses and phone numbers of three references to: Dr Noel Bouck, Northwestern University, Department of Microbiolgy/Immunology, 303, E. Chicago Ave., Chicago, IL 60611. (NW1714)A

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited for a

LECTURESHIP IN ASTRONOMY

tenable in the Department of Physics from 1 October 1985. Applicants with experience in any branch of observational or theoretical astronomy will be considered.

Starting salary on the scale (under review) £7,520 - £14,925 plus USS.

Applications (3 copies) naming three referees should be sent by 15 June 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (6281)A

UNIVERSITY OF EDINBURGH

TEMPORARY LECTURER IN ZOOLOGY

Applications are invited for a temporary Lectureship in Zoology for one year only from 1 October 1985. This post is financed by the Nuffield Foundation and the maximum salary is point 4 of the Lecturer scale. The main teaching duties are in evolution and population biology. Further particulars can be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL with whom applications by letter (2 copies) including a curriculum vitae and the names of two referees should be lodged not later than 31 May 1985. Please quote reference number 1188. (6296)A

Southampton THE UNIVERSITY

INSTITUTE OF CRYOGENICS

Applications are invited for the post of Lecturer in the Institute of Cryogenics. Candidates should have a Ph.D. and research experience in applied low temperature physics or cryogenic engineering and a strong interest in teaching. The post will require development of links with industry via collaborative research and continuing education activities.

Salary within scale:— £7,520 — £14,925 per annum (under review). The appointment will be for an initial five year period.

Further particulars may be obtained from Mr. D.A.S. Copland, The University, Southampton SO9 5NH, to whom applications (7 copies from U.K. applicants) should be sent not later than 27 May 1985 quoting reference number 2155. (6282)A

UNIVERSITY OF GLASGOW

CHAIR OF COMPUTING SCIENCE

Applications are invited for a chair i computing science, in the area o computing systems design and imple mentation or in the practica application or computing languages databases, knowledge represen tation, special purpose compute architecture and graphics based man machine interfaces. The applican should have interests in one of thre topics or in a related area such a distributed processing, softwar engineering, or theoretical aspects o computing science.

The appointment will be tenabl from 1st October, 1985 or othe mutually convenient date.

Further particulars may b obtained from:— The Academi Personnel Office, University o Glasgow, Glasgow G12 8QQ, wher applications (3 copies, 1 copy in th case of overseas applicants), givin the names and addresses of thre referees, should be lodged on c before 10th June 1985.

In reply please quote Ref N 5472M. (6328)A

nature classified

NEW YORK OFFICE
Garth Walkes
65 Bleecker Street
New York, NW 10012
Tel (212) 477-9600

TORONTO OFFICE
Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1L1
Tel (416) 690 2423

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel 01 240 1101
Telex 262024

UNIVERSITY OF BIRMINGHAM

Faculty of Medicine and Dentistry

RESEARCH ASSOCIATE: PHYSIOLGY

Applications invited for the above post funded by the Muscular Dystrophy Group of Great Britain and Northern Ireland. Applicants should have a good honours degree in Physiology, Biochemistry or a related discipline. The appointment is temporary and begins on 1 October 1985.

Salary scale £6,600 — £8,920 with superannuation.

Further details can be obtained from Dr M E Smith (Tel 021 472 1301 ext 2446). Applications with curriculum vitae and the names and addresses of two referees should be sent to the Assistant Registrar, Medical School, Birmingham B15 2TJ by 21 May 1985. (6314)A

UNIVERSITY COLLEGE CARDIFF

BIOCHEMISTRY DEPARTMENT

RESEARCH ASSISTANT (3 YEARS)

Applications are invited for the above 3 year post on 'Comparative aspects of the effects of heavy metals on lipid metabolism and photosynthesis in marine algae'. Salary Grade 1B £6,600 — £7,520 per annum. Duties to commence 1st October, 1985.

Applications, 2 copies, together with the names and addresses of two referees should be forwarded to the Establishment Officer, University College, PO Box 78, Cardiff CF1 1XL. Further particulars are available from Professor J L Harwood, Tel: Cardiff 44211 ext 2268. . Closing date 23rd May, 1985. Ref: 2939 (6252)A

THE UNIVERSITY OF SYDNEY

School of Biological Sciences (Biophysics Laboratory)

POSTDOCTORAL FELLOW — Reference no 16/10

For research in plant electrophysiology. Experience in patch-clamp and/or perfusion techniques an advantage. A PhD in relevant area is required. Appointment is for two years.

Salary range: $A21,411 — $23,903 per annum (under review).

The University reserves the right not to proceed with any appointment for financial or other reasons.

Applications, quoting reference no, including curriculum vitae and the names and addresses of three referees, to the Registrar, University of Sydney, NSW 2006, Australia, by 31 May 1985. (W1834)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ZOOLOGY

RESEARCH ASSOCIATE

Required to work on the molecular genetics of the cell cycle in fission yeast. The position, supported by the Cancer Research Campaign for a period of three years, is available immediately.

The project is concerned with the cloning and analysis of cell cycle genes and their products in fission yeast. The techniques employed will mainly be those of recombinant DNA and nucleic acid manipulations, yeast transformation, and genetic analysis.

Candidates should have experience in these areas and an honours degree in a relevant subject. Salary is according to age and experience on the RA 1B scale (£6,600 — £8,920).

Applications (2 copies) including a curriculum vitae and names and addresses of two referees, should be submitted by 24th May to Dr P A Fantes, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, from whom further particulars may be obtained. Please quote reference number 5248. (6269)A

YOUNG RESEARCH worker required to participate in project on renal disease (hyperfiltration syndromes). The ability and dedication to carry out high precision radioimmunoassays (RIA) will be essential and familiarity with RIA and computer handling of data, an advantage. The project is suitable for work towards a higher degree. Salary up to £8,753, according to age and experience. The position is available for 3 years (first six months are a probationary period). Applications with full c.v. and names and addresses of 2 referees should be sent not later than (2 weeks after publication) to The Administration Officer, The Middlesex Hospital Medical School, Cleveland Street, London W1P 7PN. (6260)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF GEOLOGY

EXPERIMENTAL OFFICER (COMPUTING) IN GEOPHYSICS

This new post is to provide laboratory, field and computing support in geophysics teaching and research programmes to members of staff.

Applications are invited from Science graduates with physics or geophysics or computer science training. Familiarity with BASIC and FORTRAN 77 will be an advantage.

Salary on the IB scale for Other Related Staff: £6,600 to £10,330 a year (under review).

Further particulars from the Registrar, University of Leicester, University Road, Leicester LE1 7RH, to whom applications should be sent on the form provided by 17 May 1985. (6279)A

LECTURER IN MINERALOGY AND GEOLOGY

Applications are invited for the above post, vacant as from 1st January 1986 Appointment will be made, according to qualification and experience, within the salary range R17 267 — 28 889 p.a. with an annual bonus and attractive staff benefits.

The successful applicant will be required to teach undergraduate courses in mineralogy and igneous petrology and to supervise postgraudate research in these fields Applicants should have a sound knowledge of these sub-disciplines and should be actively engaged in research.

Applicants should submit a full curriculum vitae stating present salary and the names and addresses of three referees not later than 31 July 1985 to the Registrar, (Attention: Appointments Office) AC/5413, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa. Further information may be obtained from the Registrar or The Secretary, SA Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE.

The University's policy is not to discriminate on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable on request. (W1835)A

MOLECULAR BIOLOGIST

Applications for an assistant professorship are invited from individuals with major commitment toward research and whose interests are in the structure, regulation or expression of viral, or mammalian, genes. Expertise in genetic engineering of enzymology-protein chemistry, an asset. Knowledge of or readiness to learn French essential. Send applications to: Dr Pierre Bourgaux, Directeur, Département de Microbiologie, Faculté de Médecine, Centre Hospitalier Universitaire, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4. (NW1728)A

UNIVERSITY OF NEWCASTLE UPON TYNE

JUNIOR RESEARCH ASSOCIATE IN CELLULAR IMMUNOLOGY

Applications are invited from graduates in biological sciences for the post of Junior Research Associate to join a project studying the mechanism of antigen presentation to T lymphocytes, which is funded by the MRC.

The post is available for two years from 1st August 1985. Commencing salary up to £7,980 per annum on the National Range 1B salary scale £6,600 - £8,920.

Written applications including full curriculum vitae and the names and addresses of three referees to Dr John H Robinson, Department of Anatomy, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH. Tel 0632 328511 Ext 2946 not later than 23rd May 1985. (6303)A

THE BEATSON INSTITUTE FOR CANCER RESEARCH GLASGOW

POSTDOCTORAL RESEARCH SCIENTIST

A vacancy is immediately available for a postdoctoral research scientist to study the involvement of cellular genes in benign and malignant human tumours associated with papillomaviruses. The appointee will join a group studying gene organization and expression of papillomaviruses, their involvement in oncogenesis, and the cellular response to viral infection and transformation.

He/She will collaborate with other members of the group and will have ample opportunities for interaction with other scientists both within the Institute and in the University scientific departments, the Medical School and the Veterinary School. Excellent facilities and supporting background in molecular biology, virology and cell biology are available.

Applicants should have a PhD in molecular or cell biology, and experience with current techniques in manipulation of nucleic acids is essential.

The post is funded by the Cancer Research Campaign, and the appointment will be for three years, with possibility of extension to five, at an appropriate point on the University Research Grade IA Scale (£7,520—£12,150 per annum) according to qualifications and experience.

Applications, together with full CV and the names of two referees, should be sent to Dr M Saveria Campo, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD (6259)A

Please mention

nature

when replying to these advertisements

THE UNIVERSITY OF MANITOBA
Department of Microbiology

PROFESSOR AND HEAD OF DEPARTMENT

The department of microbiology is a constituent department of the Faculty of Science and has broad responsibility for the teaching of microbiology in honours and majors programs in biochemistry and genetics. The department has twelve full-time faculty members and offers M Sc. and Ph.D programs in a spectrum of prokaryotic and eukaryotic microbiology, molecular biology, and biochemistry

The university encourages both women and men to apply for positions. In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. **Deadline for applications is July 1, 1985.**

Applications should be directed to: **P.K. Isaac, Chair, Microbiology Headship Selection Committee, University of Manitoba, Faculty of Science, 250 Machray Hall, Winnipeg, Manitoba, Canada, R3T 2N2.** (NW1723)A

UNIVERSITY OF ULSTER
Faculty of Science

RESEARCH OFFICER, BIOMEDICAL SCIENCES

Ref: C85/82

(One year appointment at Coleraine)

To undertake an investigation, sponsored by the Department of Health and Social Services and the Ulster Cancer Foundation, into the influence of macrophage-derived products on the suppression of T-lymphocyte proliferation in cancer patients

Applicants should hold, or expect to obtain in the near future, a PhD in the area of Cell Biology/Cellular Immunology.

Salary for this appointment, tenable initially for one year from 1 August 1985, will be determined by age, qualifications and experience within the range £7,520 — £8,920 per annum of the Research and Analogous 1A Scale (under review)

Further details are available from the Staffing Office, University of Ulster at Coleraine, Cromore Road, Coleraine, Co Londonderry BT52 1SA (Telephone Coleraine 4141, Ext 225) to whom applications, including a full curriculum vitae and the names and addresses of three referees, should be sent not later than 17 May 1985. (6309)A

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place,
London W2 1PG

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post to work on a MRC funded project on the microdissection and microcloning of the mouse X-chromosome. The project involves the construction of detailed molecular maps of individual regions of the mouse X-chromosome for the isolation and characterisation of interesting genetic loci, in particular mdx (X-linked muscular dystrophy). The applicant should have experience of recombinant DNA technology.

The post is available immediately. Salary is on the 1A scale (starting point, £7,520 plus £1,233 pa London Allowance) for research staff.

Enquiries to Dr S D M Brown tel (01) 723-1252 ext 333. Applications including full cv and names and addresses of 2 referees to Assistant Secretary (Personnel) at above address by 13 May 1985. (6322)A

TRENT POLYTECHNIC
Department of Life Sciences

RESEARCH ASSISTANT/ DEMONSTRATOR

(£5,910 — £6,657)

Honours graduates with a first or second class Honours degree or equivalent in an appropriate Biological discipline are invited to apply for a research project which will lead to an M Phil or PHD Degree. The successful candidate will be appointed to one of the following projects:

i. The Effects of Mine Effluents on Fresh Water Invertebrate Animals (E/204)
ii. Protein Profiles in Physiological Fluids in Health and Disease (E/206)
iii Proteolytic Enzymes of Plants (E/203)

Please state the ref no of the Project for which you wish to apply.

Further details and application forms are available from the Staff Officer, Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Tel (0602) 418248. Closing date 17 May 1985. (6294)A

SOUTH MANCHESTER HEALTH AUTHORITY
CHRISTIE HOSPITAL & HOLT RADIUM INSTITUTE
Paterson Laboratories

SENIOR RESEARCH TECHNICIAN

Required in the Department of Molecular Biology at The Paterson Laboratories, a large multidisciplinary cancer research institute.

Applications are invited from enthusiastic, independent research-orientated technical personnel to join a team working on the molecular biology of Epstein-Barr Virus and its relationship to human cancer.

In addition to performing research activities, the successful candidate is expected to organize and supervise four other technical staff in the day-to-day running of the laboratory and to take charge of orders and accounts.

Applicants should have several years' relevant experience, preferably in molecular biology or related disciplines. Experience in handling tissue culture cells would be an advantage. Salary within the scale £7,521 - £9,935 pa, (under review), according to age, qualifications and experience.

Further information can be obtained from Dr J A Arrand, Tel 061-445 8123 ext 557.

Send sae for application form to the Laboratory Administrator, Paterson Laboratories, Christie Hospital, Wilmslow Road, Manchester M20 9BX, quoting reference PL/85/4. Closing date: 13 May 1985. (6316)A

PHARMACIST INDUSTRIAL

to supervise and coordinate research to develop parenteral emulsions for pharmaceutical use Applicant must have a Ph.D. in Pharmaceutical Sciences with two years of industrial experience in research and development of lipid emulsion products for parenteral, oral and topical use. Salary $40,800. per year Job in Menlo Park, CA.
Please send résumé to Job No. 2895, P.O. Box 865, Sacramento, CA 95804 not later than May 17, 1985. (NW1718)A

OXFORDSHIRE HEALTH AUTHORITY
Chest Unit Churchill Hospital

SENIOR PULMONARY FUNCTION TECHNICIAN

required to run small laboratory and to be responsible for the current research programme. Ideal for recently qualified staff or graduate with some experience as medical physics technician. The position is for 2 years duration.

Salary dependent on qualifications and experience commencing at £5,404/6,408.

For application form and job description apply to the Personnel Officer, Churchill Hospital, Headington, Oxford. Tel. Oxford 64841 Ext. 228. (6324)A

IMPERIAL CANCER RESEARCH FUND
RESEARCH FELLOW

required for post-doctoral appointment to work on murine immunogenetics, immunoregulation, and/or lymphocyte development and activation at the Fund's Tumour Immunology Unit, at University College Medical School, London WC1.

Appointment for three years. Salary range £10,220 to £12,505 entry depending on qualifications and experience.

Applications with CV and names and addresses of three referees should be sent to: Professor N A Mitchison, FRS, Tumour Immunology Unit, University College London, Gower Street, London, WC1E. (6226)A

POSTDOCTORAL POSITION

The Division of Oncology, Department of Medicine, University Hospital of Zurich, Switzerland. Ph.D. in biochemistry, biology or related field, to study surface antigens and transformation processes of human respiratory tumors. Duration two, possibly three years Experience in purification of membrane antigens, immunochemistry or recombinant DNA techniques desirable.

Please send CV and names of three referees to: Dr Rolf A. Stahel, Box Number NW1713, c/o Nature, 65 Bleecker Street, New York, NY 10012. (NW1713)A

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

Scientific Appointment in the Laboratory of Gene Structure and Expression

Applications are invited for a scientific position in the Laboratory of Gene Structure and Expression, (Head: Dr F.G. Grosveld) in the field of studies on the regulation of globin gene expression. The candidate will be expected to carry out independent research and should preferably have postdoctoral experience in the globin-DNA field. Extensive experience with recombinant DNA technology and tissue culture techniques is definitely required

The position might be filled either by a career or a short-term appointment, according to the age and experience of the candidate.

The salary will be at an appropriate point on the Council's range equivalent to University non-clinical academic staff scales, plus £1,233 London allowance and superannuation provision.

Applications, giving the names of three persons to whom reference may be made, should be made not later than 31st May 1985 to the Director, National Institute for Medical Research, Mill Hill NW7 1AA. Please quote ref: GSE/10/C. (6323)A

MEDICAL RESEARCH COUNCIL
National Institute for Medical Research
Computing Laboratory

COMPUTING TECHNIQUES IN SEQUENCING

Applications are invited for a short term non-clinical scientific appointment, tenable for 3 years, from candidates with experience of the use of computing techniques in DNA and protein sequencing. The successful applicant will be expected to set up facilities in the Institute for the handling of international sequence data banks and for the manipulation of experimental data.

Renumeration will be at an appropriate point on the scales for university non-clinical academic staff plus £1,233 London allowance and Superannuation provision.

Applications, together with a full curriculum vitae and the names of two professional referees should be sent to the Assistant Director, National Institute for Medical Research, Mill Hill, London NW7 1AA by 31 May 1985 quoting reference C/1/G.

The successful candidate will be expected to start not later than 1st August 1985. (6313)A

POSTDOCTORAL RESEARCH ASSOCIATE; (In a research setting). Chemist to conduct research work in Organic Chemistry on the synthesis of derivative of Adamantane and Diamantane and their possible polymerization. PhD in Organic Chemistry. Must have manipulative and maintenance knowledge of NMR, IR, MS, and GC. Job Site: Los Angeles. Salary $1605 per month. Send this ad and your résumé to: Job 7573, PO Box 865, Sacramento, California 95804, no later than May 3, 1985. (NW1673)A

THE DANA FARBER CANCER INSTITUTE

Is seeking to fill one research position at the Assistant Professor level. Joint appointment will be held in an appropriate department at Harvard Medical School. Candidates should be qualified to develop an interactive research program emphasizing the molecular biology of lymphocyte cell surface antigens, should hold a doctoral degree (M.D. and/or Ph.D) and, preferably, should have postdoctoral research experience in a related area

Applications should submit a résumé, list of publications, statement of research interests and the names and addresses of 3 references to Dr. David Kiszkiss, Director for Research, DANA-FARBER CANCER INSTITUTE, 44 Binney St., Boston, MA 02115. An Affirmative Action/Equal Opportunity Employer.
(NW1709)A

ROYAL HOLLOWAY AND BEDFORD COLLEGES

RESEARCH ASSISTANT

Applications are invited from Physicists, Engineers or Applied Mathematicians, to join a team engaged in a range of projects on fundamental aspects of Electromagnetic Antennas for Millimetric Wavelenghts. This project involves both theoretical work (using analytical and computational techniques) and supporting experimental studies in one or more of the following areas:—

(1) Electromagnetic Theory

(2) Numerical Control Engineering

(3) Applied Optics

Candidates should have an aptitude for research and a good degree or a postgraduate qualification in a relevant subject. Salary scale £9,863 – £11,093 (under review).

Applications (2 copies) with curriculum vitae and names and addresses of 2 referees should be sent immediately to the Personnel Officer, Royal Holloway and Bedford Colleges, Egham Hill, Egham, Surrey TW20 0EX.
(6318)A

Central Haematology Laboratory Inselspital, Berne, Switzerland

Applications are invited for the post of

RESEARCH BIOCHEMIST
(POSTDOCTORAL RESEARCH FELLOW)

for a period of two years which may be renewed upon mutual agreement. The post is available immediately. The project is concerned with the relationship between structure and function of factor VIII/von Willebrand factor Candidates should have experience in biochemistry of biological macromolecules and interest in clinically oriented research. Salary will be approximately SFr. 4500.-- per month before taxes

Applications including a curriculum vitae and the names of two referees, should be sent to:- Prof. M. Furlan, Central Haematology Laboratory, Inselspital, CH-3010 Bern.
(W1833)A

RESEARCH SCIENTIST

Research in protein chemistry, enzyme chemistry, yeast cell biology and yeast molecular biology including cloning and expression of genes in yeast. PhD in Biochemistry and 2 years experience in research of yeast cell and molecular biology required. Job site. La Jolla Salary. $42,800 per year. **Send this ad and your résumé to Job 2796, P.O. Box 865, Sacramento, CA 95804 not later than 5/20/85.**
(NW1722)A

Please mention
nature
when replying to these advertisements

ROYAL HOLLOWAY AND BEDFORD COLLEGES

NEW BLOOD LECTURESHIP IN GEOPHYSICS

For research in the fields of seismic reflection processing, modelling and interpretation and to assist in teaching a new MSc course in Basin Evolution and Dynamics. The new Geology Department will be based at Egham and is being created by the amalgamation of the Departments from Bedford, Chelsea and King's Colleges.

Salary scale £8,753 — £16,158 including London Allowance. Candidates must be under 35 years of age.

Please send a self addressed envelope for further details to: Mrs D J Odds, Personnel Officer, Royal Holloway and Bedford College, Egham Hill, Egham, Surrey TW20 0EX, to whom applications (two copies) with cv and the names and addresses of two referees should be sent. Closing date 15th June 1985.
(6319)A

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF ANCIENT HISTORY & ARCHAEOLOGY

SOIL SCIENTIST

Applications are invited for a Research Associateship to work on soils and sediments associated with archaeological horizons in deposits in a Wiltshire river valley. Applicants should have a degree which includes a substantial soil science component. The appointment is funded by the SERC and is for up to three years from 1 July 1985 or as soon as possible thereafter.

Salary on the scale: £6,600 — £8,920.

Applications (six copies), naming three referees, should be sent by 28 May 1985 to the Assistant Registrar (Arts), University of Birmingham, PO Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. (6315)A

POSTDOCTORAL POSITION

available to determine DNA sequences essential for the transcriptional expression of T-DNA and higher plant genes. The research involves cloning, *Agrobacterium* vector construction, *in vitro* mutagenesis, transcript mapping and characterization of chimeric plant promoters. Experience in recombinant DNA technology, DNA sequencing and plant molecular biology desirable.

Send curriculum vitae and the names of three referees to: William B. Gurley, Department of Microbiology and Cell Science, 1059 McCarty Hall, University of Florida, Gainesville, Florida 32611. *An equal opportunity/affirmative action employer.* (NW1725)A

Research Award in Immunoregulation

Centocor, Inc., will award a $10,000 unrestricted research grant for the most outstanding human Interferon-Gamma scientific paper published (or accepted for publication) between 1/1/85 and 6/30/86.

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Fax (215) 644-7558
Telex 834823
(NW1726)H

EQUIPMENT WANTED

Wanted to purchase: a second hand general purpose E.P.R machine in working order.
Box No. 6325
c/o Nature,
4 Little Essex St.,
London WC2R 3LF
(6325)L

STUDENTSHIPS

UNIVERSITY OF OXFORD
Dyson Perrins Laboratory
(Organic Chemistry)

CASE STUDENTSHIPS

Applications are invited from suitably qualified graduates, or from those expected to graduate shortly, for two SERC financed CASE studentships in synthetic organic chemistry. One is concerned with thiotetronic acid synthesis, the other is involved with the development of a total synthesis of some macrocyclic diterpenes.

The studentships are tenable from 1st October 1985. Applicants should write, giving the names of two referees, as soon as possible to Dr E J Thomas, The Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY. (6253)F

UNIVERSITY OF OXFORD
Dyson Perrins Laboratory

RESEARCH STUDENTSHIPS

Applications are invited for research studentships in the following areas (i) Organic Synthesis:- asymmetric carbon-carbon bond formation using chiral organoiron auxiliaries (ii) Organotransition Metal Chemistry:- mechanistic studies related to the reactions of metal carbonyl ligands.

The studentships leading to the degree of D Phil are for three years from October 1st 1985. Applications with curriculum vitae and names of two referees, as soon as possible to Dr Stephen G Davies, Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY (6254)F

UNIVERSITY OF OXFORD
Department of Human Anatomy

The Department of Human Anatomy invites applications from students interested in post-graduate study. Developmental biology, neurobiology, endocrinology, or membrane physiology are currently active areas. Radioimmunoassay, autoradiography, tissue culture, electron and scanning electron microscopy and quantitative image analysis are among the facilities offered by the Department. Details of research in the Department can be obtained by applicants.

Applicants, who should have or expect to obtain a good honours degree, should send a copy of their curriculum vitae, a statement of their research interests and the names of three referees to Professor R W Guillery FRS, Department of Human Anatomy, South Parks Road, Oxford, OX13QX, by 14 May 1985. (6271)A

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BIOCHEMISTRY

The department has

5 STUDENTSHIPS

available for research leading to the degree of PhD. These include 1 MRC, 2 SERC and 2 SERC-CASE studentships for work in the fields of cell biology, enzymology, microbial biochemistry and bioenergetics.

Applications with curriculum vitae and the names, addresses and telephone numbers of 2 referees should be sent to Dr A G Lee, Department of Biochemistry, University of Southampton, School of Biochemical and Physiological Sciences, Bassett Crescent East, Southampton, SO9 3TU. (6300)F

UNIVERSITY OF SOUTHAMPTON
Clinical Pharmacology/Medicine 1

SERC (CASE) Studentships

Applications are invited from good honours graduates in pharmacology, biochemistry or physiology for the following SERC (CASE) studentships to study:

1. The biochemical pharmacology of dispersed human skin mast cells (in collaboration with Roussel Laboratories Limited).

2. The development of bronchial hyper-reactivity in the guineapig (in collaboration with Roussel Laboratories Limited).

3. The oxidative metabolism of arachidonic acid in human and primate mast cells (in collaboration with Frisons Pharmaceuticals PLC).

The studentships are available for 3 years from October 1st 1985 and the successful applicants expected to register for a higher degree. Further information and applications, including curriculum vitae and the names of two referees to: Dr M K Church, Clinical Pharmacology, Southampton General Hospital, Southampton S09 4XY. Telephone 0703-777222 Ext 4312. Applications close June 3rd 1985. Please quote ref no AJS/85/26. (6273)F

UNIVERSITY OF LEICESTER
SCHOOL OF BIOLOGICAL SCIENCES
(BOTANY DEPARTMENT)

SERC — CASE STUDENTSHIP
(ICI Corporate Biosciences Group)

An SERC-CASE Studentship in collaboration with Dr W Schuch of the ICI Corporate Biosciences Group is available to work on 'Photocontrol of phenylalanine ammonia-lyase mRNA levels in oil-seed rape seedlings under radiation conditions simulating crop stands'. This project is concerned with the molecular biology of phytochrome action in light-grown plants, with particular respect to the control of growth and lignification in crop plants experiencing mutual shading. The work will involve the use of cDNA probes to estimate PAL mRNA levels and to measure specific transcription rates. An academic background in molecular biochemistry would be an advantage; prior experience with plants is useful but not essential and training will be given in plant photomorphogenesis.

Applicants having, or expecting to achieve, at least an Upper Second Class Honours Degree in an appropriate subject should submit a curriculum vitae, with the names of two referees, as soon as possible to Professor H Smith, Department of Botany, University of Leicester, University Road, Leicester. (6290)F

Medical Research Council Research Studentship (Partnership Award)

University Laboratory of Physiology, Oxford and the National Institute for Medical Research, Mill Hill, London

A 3-year M.R.C. Studentship leading to a D.Phil. is available from 1st October 1985, for a project investigating contractile mechanisms in 'skinned' muscle fibres employing 'caged' ATP and the fluorescently labelled derivative of the calcium sensitive regulatory protein, troponin (TnC-DANZ). The training will involve the combination of physiology and biochemical methods, particularly the use of novel optical and laser techniques in muscle research, introduced by Dr C.C. Ashley and Dr. D.R. Trentham, F.R.S., the co-supervisors of the project.

The overall aim is to correlate mechanical and biochemical experiments on skinned muscle fibres with those on intact fibres in order to provide a clearer insight into the basic contractile mechanism and its regulation.

Applicants should have or expect to obtain a 1st or upper 2nd class honours degree, and have a background in physiology, biochemistry or physical sciences.

Applications including a CV and the names and addresses of 2 referees should be sent to Dr C.C. Ashley, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, as soon as possible. (6312)F

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

RESEARCH STUDENTSHIPS

The department occupies two sites — at the Bute Medical Buildings and the Gatty Marine Laboratory. Excellent facilities are available for research in the following areas: cell physiology, neurobiology and neuropharmacology, muscle physiology, human exercise and respiratory physiology and environmental biology. The following Research Council studentships are available:

1 "Effects of temperature on muscle contraction in fish" or "Hypoxia and activity metabolism in fish" supported by NERC under the supervision of Dr Ian A Johnston.

2 SERC-CASE studentship on "Physiological, neuroanatomical and biochemical studies on the function of dopamine in the insect nervous system" under the joint supervision of Dr Robert M Pitman and staff at The Wellcome Research Laboratories, Berkhamstead.

3 MRC studentship in muscle and cell physiology.

Further particulars of the department and research projects may be obtained from Mrs M Noble, Secretary, Department of Physiology and Pharmacology, Bute Medical Buildings, University of St Andrews, St Andrews, Fife KY16 9TS, Scotland, to whom applications (which should include a full curriculum vitae and the names of two referees) should be sent to arrive not later than 20 May 1985. (6297)F

SERC STUDENTSHIP IN SEA-USE

The London School of Economics is offering two Advanced Course studentships, funded by the Science and Engineering Research Council, on its MSc Sea-Use Programme for the academic year 1 October 1985 — 30 September 1986. Preference will be given to applicants possessing a good first degree in economics who wish to specialise in the economics option; but applications from other specialities such as law, engineering, political science, history, geography or environmental studies will be considered. The programme consists of a basic course in Marine Science, Biology and Technology, with a choice of two options chosen from: International Law of the Sea, Financial Reporting and Management, Economics and Problems of Sea-Use Policy-Making, together with a research essay of 10,000 words on an agreed topic.

Candidates should write at once to the Secretary of the Graduate School, The London School of Economics and Political Science, Houghton Street, London WC2A 2AE, for application material. (6270)F

KINGSTON POLYTECHNIC
School of Industrial, Organic and Polymer Chemistry

SERC "CASE" STUDENTSHIPS

Applications are invited from good honours graduates in chemistry, materials technology or related subjects for three SERC "CASE" Studentships concerned with organic polymer composites. All projects involve industrial collaboration (ICI, Westland Helicopters, National Physical Laboratory) in the study of the properties of fibre reinforced polymers using instrumental methods.

Application forms and further details from Staffing Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel 01-549 1366 ext 205. (6278)F

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MEDICINE
DEPARTMENT OF CLINICAL PHARMACOLOGY

RESEARCH STUDENTSHIP

Applications are invited for a research studentship within the Clinical Pharmacology Group to study the pharmacokinetics of therapeutically used amino acids such as L-dopa and tryptophan. Applicants should have a first or upper second-class honours degree in Pharmacology, Biotechnology or a related subject. The studentship will be for three years but the successful applicant will be expected to register for a PhD. The grant will be at the appropriate MRC rate.

Applications (2 copies) giving a full curriculum vitae and the names, addresses and telephone numbers of two referees should be sent to Dr A G Renwick, Department of Clinical Pharmacology, University of Southampton, Basset Crescent East, Boldrewood, Southampton, Hampshire from whom further details may be obtained. Please quote Ref: AJS/85/25. (6265)F

UNIVERSITY OF READING
DEPARTMENT OF PURE & APPLIED ZOOLOGY

STUDENTSHIPS

for postgraduate study are available in the following areas:

1. Animal behaviour in wild and domesticated mammals.
2. Genetics aspects of development or competition between insects.
3. Effects of parasite infections.
4. Evolution of life cycle strategies.
5. Mechanical properties of biological materials.
6. Metal or pesticide accumulation and detoxification.

Applicants should send a curriculum vitae with the names of two referees to Professor K Simkiss, Department of Pure & Applied Zoology, University of Reading, PO Box 228, Reading, RG6 2JN. (5727)F

ROYAL POSTGRADUATE MEDICAL SCHOOL

MRC RESEARCH STUDENTSHIP

Applications are invited for a three year MRC Studentship from graduates holding, or expecting to obtain, an upper second second or first class honours degree. The successful candidate will work on the measurement and pathophysiology of novel peptide neurotransmitters.

Applications in the form of cv with the names of two referees together with a letter of application should be sent to Professor S R Bloom, Department of Medicine, Royal Postgraduate Medical School, 150 Ducane Rd, W12 OHS from whom further particulars may be obtained.

Closing Date: 23 May 1985.
(6320)F

UNIVERSITY OF OXFORD

NUFFIELD DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

Applications are invited for an MRC research studentship leading to a D Phil, tenable for 3 years from October 1985, under the direction of Dr Phyllis Starkey. Applicants should have (or be about to obtain) a good Honours degree in a biological science.

The successful applicant would investigate the cellular and enzymic basis of ripening of the cervix in labour, as part of a Department studying various aspects of human pregnancy and labour including the immunological interaction between mother and fetus, the causes of pre-eclampsia and recurrent abortions, the factors controlling the initiation of labour, and the cell biology of the human decidua, placenta and fetal membranes.

Further details from, and applications to: Dr Phyllis Starkey, Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Headington, Oxford. Closing date 31st May 1985.
(6311)F

KING'S COLLEGE LONDON (KQC)

DEPARTMENT OF PHYSIOLOGY

SERC - CASE STUDENTSHIP

Applications are invited for a collaborative studentship under the SERC - CASE scheme between the above department and the National Institute of Biological Standards, Hampstead. The project will involve studies of the biosynthesis, secretion and characterisation of the 'major proglucagon fragment' which is produced together with glucagon by the pancreatic A cell.

Applicants should have, or expect to obtain, a first or upper second class honours degree in Biochemistry, Physiology or a related subject. Applications, including a curriculum vitae and the names of two referees, should be sent to Dr S L Howell, Physiology Department, Queen Elizabeth College, University of London, Campden Hill Road, Kensington, London W8 7AH by 17th May 1985.
(6284)F

MEDICAL RESEARCH COUNCIL

Mechanisms in Tumour Immunity Unit

RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship, tenable for a three year period, from Autumn 1985 to work on one of two projects:

1 Investigation of the inhibitory profile of C1-inhibitor — a plasma protease inhibitor active against both complement and coagulation proteases.

2 Growth factors involved in the establishment of a human B lymphoblastoid (EBV) lines.

The successful candidate should possess by Autumn 1985 a good honours degree in either biochemistry or a biological science and will be expected to register for a PhD with the University of Cambridge.

Applications, including a full curriculum vitae and the names and addresses of two referees, should be sent to: Professor P J Lachmann, MITI Unit, MRC Centre, Cambridge CB2 2QH by 16th May 1985.
(6299)

UNIVERSITY OF LEICESTER

DEPARTMENT OF MICROBIOLOGY

SERC CASE STUDENTSHIP

Applications are invited for a CASE studentship to study the expression of eukaryotic genes in Pseudomonas putida and Methylophilus methylotrophus, in collaboration with Dr G S Sharpe, Corporate Biosciences Laboratory, ICI.

Applicants should possess, or expect, a first or an upper second class degree in biochemistry, genetics or a related field.

Applications, in the form of a curriculum vitae with the names of two academic referees should be sent to Dr G J Boulnois, Dept of Microbiology, Medical Sciences Building, University Road, Leicester LE1 7RH (Tel. 0533-551234, ext 262).
(6292)F

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GENETICS

NERC CASE RESEARCH STUDENTSHIP

Applications are invited for the above studentship to work on an investigation of marine polychaetes as indicator organisms for genotoxic effects of pollutants. The project will be jointly supervised by Dr D P Fox of Aberdeen University, Genetics Department and Dr J Davies, DAFS Marine Laboratory, Aberdeen.

Candidates should hold, or expect to obtain, first or upper second class honours in Genetics or another appropriate biological subject.

Applicants should send a curriculum vitae together with the names of two referees to Dr D P Fox, Department of Genetics, University of Aberdeen, 2 Tillydrone Avenue, Aberdeen, AB2 2TN not later than Friday 17th May. Informal enquiries may be made to Dr Fox, tel: 0224-40241 ext 6455. (6255)F

QUEEN MARY COLLEGE (University of London)

DEPARTMENT OF CHEMISTRY

SERC-CASE RESEARCH STUDENTSHIPS

Applications are invited for the following Research Studentships awarded under the SERC Cooperative Awards in Science and Engineering schemes for October 1985 The Chemistry Department has recently expanded considerably with the restructuring of experimental science in the University of London.

Dr P A Hamilton — 'Mechanism of Atmospheric Sulphur Reactions' (with Central Electricity Research Laboratory)

Dr. P. O'Brien — 'Novel Precursors for the Deposition of Cadmium Sulphide for MOCVD' (with Polaron Equipment Ltd.)

Professor J. Pritchard — 'Hydrogen Chemisorption on Copper Catalysts' (with ICI Agricultural Division)

Dr N S Simpkins — 'Asymmetric Synthesis of Potential Inhibitors of 5-Lipoxygenase' (with May and Baker Ltd.)

Professor J.H.P. Utley — 'Electrosynthesis of Liquid Crystals and Related Intermediates' (with BDH Chemicals Ltd.)

Dr. D.S. Urch — 'High Resolution XRF-XPS: Electronic Structure and Analysis' (with Health and Safety Executive)

Further information on any of these projects can be obtained from the appropriate supervisor. Applicants must have, or expect to obtain this year, a degree in chemistry with at least upper second class honours. Application forms from Dr. J.R.C. Light, Chemistry Department, Queen Mary College, Mile End Road, London E1 4NS to be returned as soon as possible.
(6321)F

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

(University of London)
Keppel Street,
London, WC1E 7HT

SERC CASE STUDENTSHIP IN IMMUNOLOGY

Applications for a Research Studentship leading to a PhD degree are invited from candidates who hold or expect to obtain this summer a first or upper second class honours degree in a biological science. Experience in Immunology is desirable but not essential. The studentship is tenable at the School, in conjunction with Imperial Chemical Industries, from October 1st 1985. The project will be supervised by Professor M W Steward of the Immunology Unit, Department of Medical Microbiology and Dr A F Wright of ICI and will be concerned with the production and control of high affinity antibody responses.

Applications, including a full cv and the names of two referees, should be sent to Professor Steward at the School. (6283)F

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

SERC CASE STUDENTSHIP

Applications are invited for a SERC CASE Studentship to work with Dr A W Robards in collaboration with ICI Agricultural Division on "Separation of subcellular granules from bacterial cells". It is expected that the appointed student would have a background in micro-biology and/or cell biology and should have a strong interest in applications of cell biology to biotechnology.

Applications should be made to Dr Robards, Department of Biology, University of York, Heslington, York, YO1 5DD from whom further details can be obtained. Candidates must be ordinarily resident in Great Britain and should have (or expect to obtain) a First or Upper Second Class Honours Degree from a British University or Polytechnic.
(6275)F

UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

CASE STUDENTSHIP

Applications are invited for a CASE studentship, in collaboration with the Agricultural Scientific Service of the Department of Agriculture and Fisheries for Scotland at East Craigs (Edinburgh) and the Scottish Crop Research Institute (Invergowrie), to study the role of components of the outer membrane in the antigenic properties of soft rot Erwinia species that are pathogenic to potatoes.

Candidates should have, or expect to attain this summer, a good honours degree in biochemistry or a related discipline.

Further information can be obtained from Dr I D Hamilton, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, to whom application, including a curriculum vitae and nominating 2 academic referees, should be made by 31st May, 1985. (6310)F

UNIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

Applications are invited for a

RESEARCH STUDENTSHIP

funded by the SERC CASE award scheme to work on the Structure and Evolution of cereal grain proteins. The research will be with Dr M Richardson at the University of Durham and Dr P Shewry (Biochemistry Department, Rothamsted Experimental Station) and involves protein chemistry (amino acid sequencing), molecular biology and recombinant DNA techniques. It is envisaged that the student's time will be split equally between the two Institutes.

Applicants should have, or expect to obtain, a first or upper second class honours degree, preferably in biochemistry, botany or a biological science.

Applications (cv and the name of two referees) should be sent to Dr M Richardson, Department of Botany, University of Durham, Science Laboratories, South Road, Durham DH1 3LE no later than 6 June 1985.
(6282)F

STUDENTSHIPS Continued

ASTON UNIVERSITY
Department of Pharmaceutical Sciences
RESEARCH STUDENTSHIPS
in the Pharmaceutical Sciences Institute

The Pharmaceutical Sciences Institute has been established to coordinate the research work of the Department of Pharmaceutical Sciences. The Institute is concentrating its resources within well-funded research groups and the following research areas will be supported in work commencing 1 October 1985.

Targeting of Drugs by Phagocytic Cells: SERC CASE Studentship (Ciba-Geiby)	(Dr D A Lewis)
Hypoxia, Ischaemia and Peripheral Vasodilator Mechanisms: SERC CASE Studentship (ICI Pharmaceuticals)	(Dr O A Downing)
Drug-delivery Systems Design	(Dr W J Irwin)
Computer-graphics Aided Design and Synthesis of Chemotherapeutic Agents	(Prof M F G Stevens)
Cardiovascular Pharmacology	(Prof C B Ferry)
Microbiology	(Prof M R W Brown)
Cancer Chemotherapy	(Dr J A Hickman)
Toxicology	(Dr E S Harpur

A number of research studentships will be available for highly qualified graduates, or those expecting to graduate in 1985, to work on a variety of topics leading to the award of PhD degrees. The named individuals should be contacted for further information on projects in identified areas and a copy of the Departmental research and postdoctoral prospectus.

Department of Pharmaceutical Sciences
Aston University
Aston Triangle
Birmingham B4 7ET (6287)F

UNIVERSITY OF SURREY
DEPARTMENT OF CHEMISTRY
STUDENTSHIPS

Applications are invited from Chemistry graduates, or those about to graduate, for the following research studentships which will become available as from 1st October, 1985

1. Two University of Surrey sponsored research studentships to work with Professor J M Pratt in the general area of Bio-inorganic Chemistry.

2. An internal departmental studentship to work on the Structure Determination of High Valent Porphyrin Complexes Related to the Peroxidases and Cytochrome P-450. (Drs K B Nolan and D C Povey).

3. A Ministry of Agriculture, Fisheries and Food sponsored studentship to study the Effects of Phytate on Mineral Bioavailability and Heavy Metal Contaminants. (Dr K B Nolan).

4. A CASE studentship in conjunction with ICI Pharmaceuticals to study A Thermodynamically-Based Scale of Hydrogen Bond Basicities. (Dr M H Abraham).

5. A CASE studentship in conjunction with the Imperial Cancer Research Fund to study Aromatic Hydroxylations. (Dr J R Jones).

6. A Ministry of Defence sponsored studentship to study Aromatic Amine Cure of Epoxy Resins. (Drs G J Buist and J R Jones).

Interested persons should send their curriculum vitae and names and addresses of two referees to Dr J R Jones, Chemistry Department, University of Surrey, Guildford, GU2 5HX as soon as possible. (6261)F

UNIVERSITY OF LIVERPOOL
DEPARMENT OF BOTANY
RESEARCH STUDENTSHIPS

The following research studentships are available within the Department of Botany tenable from October 1st, 1985.

NERC Nitrogen economy of wood-decay Basidiomycetes (Professor D H Jennings).

The role of mutation in the origin of metal tolerance (Professor A D Bradshaw, Dr T McNeilly and Dr H A Collin).

Dynamics of annual plant communities (Dr A M Mortimer and Dr P D Putwain).

SERC The theoretical and practical significance of plasmid transfer between streptomycetes. (Dr S T Williams, Professor D A Ritchie and Dr A M Mortimer).

SERC/CASE Ion transport in the fungus Dendryohiella salina (Professor D H Jennings).

Further details of these studentships are available from Professor A D Bradshaw, Department of Botany.

Applicants should have or be expecting at least a 2:1 Honours Degree in an appropriate biological subject. The Department of Botany has a very active research school with excellent facilities, concerned with pure and applied aspects of botany.

Applications should be made as soon as possible and at least by June 3rd to The Registrar, The University, PO Box 147, Liverpool L69 3BX. They should include details of academic experience and the names of two referees. Quote Ref: RV/255/N. (6288)F

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY
SERC CASE RESEARCH STUDENTSHIPS

Applications are invited for the above from undergraduate students who expect to obtain at least an upper second class honours degree in biophysics, biochemistry, microbiology, genetics, botany or related subjects. Biotechnology research projects, involving collaboration between an academic staff member and an individual partner, in:

(1) Cloning of the nitrate reductase structural gene of barley in collaboration with Long Ashton Research Station (Dr J L Wray)

(2) Biochemical and genetic studies of the mushroom Pleurotus in collaboration with Mitchell Mushrooms (Dr W M Ledingham)

(3) An investigation into the interrelationships of the cell wall properties of Thiobacillus ferrooxidans and bioleaching processes of ferrous iron and sulphide oxidation in pyritic ores in collaboration with Shell (UK) PLC (Dr W J Ingledew).

For further information, contact the staff member concerned.

Applications, naming at least two referees and stating the project(s) of interest to The Secretary, Department of Biochemistry, University of St Andrews, St Andrews, Fife KY16 9AL. Telephone enquiries to 0334-76161 ext 295. (6298)F

QUEEN MARY COLLEGE
(University of London)
School of Biological Sciences
SERC-CASE STUDENTSHIP

An SERC-CASE Studentship in collaboration with the Biological Evaluation Division, Shell Research Ltd., is available to investigate host plant selection and feeding behaviour in the rice brown plant hopper, Nilaparvata lugens. The project will include the development of new techniques for monitoring food uptake and for assessing the role of different classes of sensory receptor in the regulation of settling and feeding in this and in other small leaf and plant hopper pests of rice. An important part of the investigation will be to examine the way in which motility and feeding behaviour may be affected by a novel class of insecticide both in individual hoppers and in populations of N lugens exposed to sublethal doses.

Applicants should have or expect to obtain a first or upper second class honours degree and will be expected to register for a PhD degree. Applications, together with a cv and the names of two academic referees, should be sent to Dr L J Goodman, School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS as soon as possible. Further details can be obtained from the above address. (6295)F

INSTITUTE OF AQUACULTURE
UNIVERSITY OF STIRLIN
Applications are invited for a
NERC-FUNDED STUDENTSHIP

in the following areas: The toxicity aluminium to Atlantic Salmon Acidic Waters or Quantitative a Qualitative Aspects of Filter-Feedi on cyanobacteria by Fish.

Further details on these proje and application forms may obtained from the Academ Administrator, the Institute Aquaculture, University of Stirlir Stirling FK9 4LA, Scotland. (6305)F

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place, London W21 1PG
SERC CASE STUDENTSHIP

Applications are invited for a SER Case Studentship in collaborati with Shell Research Ltd (Dr D H Hi son) for research (leading to a Ph degree) into the effect of environme tal conditions on the metabolism chemicals by fish.

Applicants should have, or expe to obtain, a first or second cla honours degree in biochemistry chemistry, and have an interest chemical and environment toxicology.

Applications, including the nam of two referees should be sent as so as possible to Dr P Millburn, Depa ment of Biochemistry, at above a dress, from whom further particula may be obtained (tel 01-723-12 ext 118). (6277)F

FELLOWSHIPS

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF BIOCHEMISTR
POST-DOCTORAL FELLOWSHIP

A Post-doctoral Fellowship fund by the Cystic Fibrosis Research Tru is available to work on alginate bi synthesis in mucoid strains Pseudomonas aeruginosa, under t direction of Drs P Gacesa and N Russell. Candidates having a PhD the field of biochemistry/micr biology, particularly those wi experience in enzymology or NM are invited to apply.

The appointment is for 3 yea subject to annual renewal, and t salary will be £7,520 in the first ye (RA 1A scale, under review). Duti to commence September, 1985.

Applications (2 copies) including curriculum vitae, together with t names and addresses of two referee should be forwarded to t Establishment Officer, Universi College, PO Box 78, Cardiff C 1XL. Closing date 23rd May, 198 Ref: 2952. (6307)E

FELLOWSHIPS continued

MAX-PLANCK-INSTITUTE FOR BIOPHYSICS
Postdoctoral Research Fellowship in

BIOPHYSICS

Characterization of polydisperse lipid systems by photon correlation spectroscopy.

Applications are invited from candidates interested in joining a multi-disciplinary research group studying transport processes in membranes. The fellowship is offered for a one to two year period starting May 1985 or later. Salary will be from DM 26.000 to DM 32.000 p.a., net, depending on experience.

Send application (c.v., list of publications, names of two referees) to Dr. E. Grell, Max-Planck-Institute for Biophysics, Heinrich-Hoffmann-Str. 7,6000 Frankfurt 71, (West Germany). (W1826)E

UNIVERSITY OF LEICESTER
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
MERCK SHARP & DOHME NEUROSCIENCE RESEARCH FELLOWSHIP

Applications are invited for a MSD Neuroscience Research Fellowship to study cholinergic receptor-effector mechanisms in brain. The project involves receptor labelling and an assessment of receptor-effector coupling by examination of cyclic nucleotide and inositol phospholipid metabolism in brain. Applicants should have, or shortly expect to have, a PhD and possess suitable experience in pharmacology/biochemistry/neurobiology. Some experience of discrete cerebral lesioning or tissue culture would be an advantage. Although this position is with Professor S R Nahorski's research group in Leicester, it is possible that a short period may be spent at the MSD Neuroscience Research Centre, Harlow, with Dr L L Iversen. The Fellowship is from 1st October 1985, or nearest conveient date, and is for a period of one year in the first instance and for which funding may be extended for a further two years.

Salary is on the scale £7,520 -£12,150 (Research 1A).

Additional information can be obtained from Professor S R Naborski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester, LE1 7RH, UK, (0533-551234, Ext 361) to whom applications, including CV and names of two referees, should be sent. (6306)E

UNIVERSITY OF BRISTOL
DEPARTMENT OF ZOOLOGY
POSTDOCTORAL POSITION IN MOLECULAR BIOLOGY)
(Neurobiology)

Applications are invited for a three-year fellowship to investigate the regulation of expression of multi-functional genes in the mammalian nervous system implicated in the regulation of cellular differentiation and neuromuscular synapse formation. The project will be funded by the Wellcome Trust.

The successful applicant will hold a PhD in Biochemistry/Molecular Genetics or a related field, and have experience in DNA manipulation and hybridisation techniques with a record of research into any aspect of the control of transcription in eukaryotic cells.

Laboratory facilities will be provided in the newly-established Molecular Genetics Unit at Bristol.

An appointment will be made on the University Academic Staff Range IA at a starting salary of £7520-£9390 per annum.

Initial enquiries may be made to Dr L W Haynes, Department of Zoology, The University of Bristol, Woodlands Road, Bristol BS8 1UG (0272 24161 ext 57), to whom applications, accompanied by cv and the names of three referees should be addressed before 24 May. (6116)E

POSTDOCTORAL FELLOWSHIP
in the area of
Immunological control of Membrane Transport
Primary responsibility:

production and study of monoclonal antibodies to membrane transport proteins. Position available July 1, 1985, one year renewable, salary competitive.
Send curriculum vitae and names of three referees to Dr. Philip Dunham, Department of Biology, Syracuse University, Syracuse, New York 13210. Equal opportunity employer. (NW1724)E

1985 — 1986
L.S.B. Leakey Foundation
RESEARCH FELLOWSHIP
FIELD STUDIES OF THE GREAT APES

The fellowship will be awarded to an individual to facilitate the establishment of a long-term research project on a wild population of great apes. Preference will be given to studies with significance for understanding human origins and to those which explore the causes of variation in ape behaviour, particularly in previously unstudied habitats. The fellowship, worth up to $20,000, is for field expenses only

Applicants should submit curriculum vitae and a one-page statement of research goals by 1 May 1985 to: The L.S.B. Leakey Foundation, Attention: Kathleen F. Galvin, Grants Officer, Foundation Center, Pasadena, Calif. 91125. Eligible candidates will be asked to provide further details by 15 June 1985. The recipient will be announced in October 1985. (NW1717)E

CONTINENTAL EUROPEAN FELLOWSHIP AWARDS 1986–87 FOR ACADEMIC STAFF

Applications are invited for awards by the CIBA-GEIGY Fellowship Trust for the academic year 1986/87

Senior CIBA-GEIGY Fellowships

Senior CIBA-GEIGY Fellowships will be awarded to start from an agreed date during the academic year 1986/87 These will be available to lecturers, senior lecturers or readers who hold, and will return to, permanent teaching positions at United Kingdom or Republic of Ireland universities, polytechnics or comparable teaching institutions, who wish to undertake research at an agreed continental European university or technological university These posts should have been held for a period of at least five years in the field of chemistry, biochemistry, chemical technology, chemical engineering and biology, particularly in its relation to chemistry.

Awards are made for a period of six to twelve months

Value of the awards £10,000 per annum plus travelling expenses

CIBA-GEIGY Fellowship awards are intended to supplement Fellows' salaries for the period of tenure

Further details and application forms, are obtainable from: The Secretary, The CIBA-GEIGY Fellowship Trust, 30 Buckingham Gate, London SW1E 6LH (Telephone 01-828 5676).

The closing date for applications is 1 November 1985

CIBA–GEIGY

(6248)E

UNIVERSITY OF WESTERN ONTARIO
Department of Obstetrics and Gynaecology

POSTDOCTORAL FELLOWSHIP

Funds are immediately available for two postdoctoral scientists to work within the newly established Gynaecology Cancer Research Laboratory at Victoria Hospital. The principal interests with this laboratory focus on reproductive endocrinology and the identification of tumour markers. One of the projects for which funds have been obtained will initially involve sequencing of cloned cDNAs for two human plasma steroid binding proteins and studies of the hormonal regulation of their synthesis by cells in culture. A second project will largely involve the isolation of mRNA from human ovarian tissue and the construction and analysis of cDNA libraries with the ultimate objective of identifying one or more specific ovarian tumour marker proteins.

Candidates should hold a Ph.D. in Biochemistry or Molecular Biology and be familiar with the principles and practical aspects of recombinant DNA technology. Applications, including a curriculum vitae, should be addressed to:

Dr. Geoffrey L. Hammond
The University of Western Ontario
Gynaecology & Cancer Research Laboratory
375 South Street London, Ontario. N6A 4G5.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents.

(NW1711)E

INDIANA UNIVERSITY

NEW PERSPECTIVES ON NUCLEIC ACIDS

A SYMPOSIUM

September 29 — October 2, 1985
Presented by the
Institute of Molecular and Cellular Biology
at Indiana University
Bloomington, Indiana

This Symposium will address recent developments in the structure and function of polynucleotides. Session speakers, leaders in their fields, will provide broad coverage of topics of interest to workers in both academic and biotechnology settings.

Program topics will include: physical techniques in determination of polynucleotide structure, role of higher order structures in RNA function, RNA-protein interactions, RNA processing, RNA roles in regulation of translation, transport and replication.

Session speakers include:

John Abelson	Judith McClarin	Brian Reid
Struther Arnott	Donald Mills	Phil Sharp
Andrea Branch	Peter Moore	Joan Steitz
Tom Cech	Harry Noller	Olke Uhlenbeck
Larry Gold	Norman Pace	Peter Walter
Christine Guthrie	Dinshaw Patel	Carl Woese
Michael Mathews	Barry Polisky	Michael Yarus

Sponsored in part by the Indiana Corporation for Science & Technology, Abbott Laboratories, Boehringer Mannheim Biochemicals, Boehringer Mannheim Diagnostics, Inc., Eli Lilly & Company, International Minerals and Chemical Corporation, and Monsanto Company.

For information and application write to: Director, Institute for Molecular and Cellular Biology, Jordan Hall 322A, Indiana University, Bloomington, Indiana 47405. (NW1707)M

LEUKAEMIA RESEARCH FUND

International Research Symposium on
"GROWTH FACTORS, HAEMOPOIESIS AND MALIGNANCY"
at Royal College of Physicians, London NW1
on WEDNESDAY 29TH MAY 1985
Registration Fee (including lunch) £15.
For programme details and registration form apply to:
LEUKAEMIA RESEARCH FUND,43 Great Ormond Street, London WC1N 3JJ, (tel: 01-405 0101) (6100)M

CIBA FOUNDATION SYMPOSIUM BURSARY SCHEME

AIM: To enable young scientists to attend Ciba Foundation symposia and subsequently spend 4 weeks in the laboratory of one of the symposium participants.

The bursary will cover —
a) travel expenses
b) board and lodging for the duration of the bursary

Qualifications: Applicants must be aged between 25-35 years on the closing date for application, and actively engaged in research on the topic covered by the symposium of their choice.

Topics:
1 FUNCTIONS OF THE PROTEOGLYCANS
2 THE JUNCTIONAL COMPLEXES OF EPITHELIAL CELLS
3 MECHANISM OF DEATH OF NERVE CELLS
4 AUTOIMMUNITY AND AUTOIMMUNE DISEASE

Applications: In writing, addressed to:
Mrs S Venables, The Ciba Foundation, 41 Portland Place, London W1N 4BN including the following information.
a) Full name, address and date of birth
b) Symposium of choice (one from the above list)
c) Qualifications and short résumé of further education
d) Career history, including full list of publications
e) Details of current research and aims of future career
f) Names and addresses of two referees

Closing Date for Applications: 30 September 1985. (6293)M

CONFERENCES and COURSES

UNIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

MSc BIOTECHNOLOGY

This new one-year course (starting 27 September 1985) results from collaboration between the Department of Botany and Imperial Chemical Industries PLC. The course, which combines lectures and practicals with a research project, will provide an overview of modern concepts and techniques in biotechnology Topics include: techniques in molecular biology and cell biology; genetic engineering and eukaryotes; tissue culture and monoclonal antibodies; industrial microbiology; fermentation and chemical engineering; microbial leaching and metal stripping; enhanced oil recovery and degradation air and microorganisms; biomass conversion; novel plant products; legal and regulatory aspects.

Instruction will include a significant contribution from ICI staff, especially from Biological Products Business at Billingham. The course is especially suitable for biological chemists, microbiologists, botanists and applicants from related disciplines, and will also offer a rapid and efficient retraining programme for applicants seconded from industry.

Application forms and further particulars may be obtained from Dr B A Whitton, MSc Biotechnology, University of Durham, Durham DH1 3LE, England. (6266)C

UNIVERSITY OF LEICESTE

SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF BOTANY

BIOTECHNOLOGY SHORT COURSE CO-ORDINATOR

Applications are invited for the po of Biotechnology Short Course C ordinator from those with curre research interests in the field of pla biotechnology. This post has lectur status. The person appointed w assess the demand for pos experience courses in biotechnolo and subsequently in other areas biotechnology. He or she will expected to pursue a researc programme within the Departme of Botany.

This appointment will be for fi years, supported by a grant from t DES under its PICKUP programm

Initial salary will depend qualifications and experience on t scale £7,520 to £14,925 (und review).

Further particulars ar application forms can be obtain from the Registrar, University Leicester University Road, Leiceste LE1 7RH, to whom applicatio should be returned by May 1985. (6280)C

IMPERIAL COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY

POST-EXPERIENCE COURSE

A post-experience course on "Par cle Sizing and Characterisation Light Scattering" will be given 4th-5th July 1985.

For further information contact A R Jones, Department of Chemic Engineering and Chemical Tec nology, Imperial College, Lond SW7 2BY. (6246)

KING'S COLLEGE LONDO (KQC)

University of London

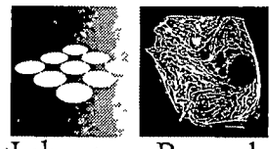
MSc DEGREE IN GENERAL BIOCHEMISTRY

Both part-time and full-time cours will commence in October 1985 a will be held at the Campden Hill Sit Kensington. The part-time cour extends over two academic years a involves attendance for one aft noon and one evening per week. T full-time course extends over o academic year and involves research project.

Qualifications. Candidates shou normally hold a first or second cl honours degree (or an approv equivalent qualification) in a scien subject. Graduates in Medicin Dentistry or Veterinary Medicine also admitted. The course is appropriate to recent graduates fro honours Biochemistry courses.

For further information and app cation forms write, in the fi instance to the Secretary, Bi chemistry Department, Chels College, Manresa Road, Lond SW3 6LX. (6304)C

Continued on page



Labsystems Research Symposium

Synthetic Peptides in Biology and Medicine June 6–8, 1985 Hotel Aulanko, Hämeenlinna, Finland

Labsystems has initiated a series of biannual research symposia to molecular biology. The theme of the first meeting will be "Synthetic Peptides in Biology and Medicine".

Topics include:

Protein structure and the basis for peptide synthesis
Immunology of synthetic peptides
Synthetic peptides in microbiology
Synthetic peptides in cell biology
Peptide hormones

The proceedings will be published.

Invited speakers will include F. Audibert (France), E. Beachey (USA), J. De Larco (USA), R. Eisenman (USA), G. Evan (UK), T. Hopp (USA), H. Jornvall (Sweden), S. Kent (USA), V. Marchesi (USA), R. Neurath (USA), S. Oroszlan (USA), M. van Regenmortel (France), E. Ruoslahti (USA), J. Schlessinger (Israel), G. Schild (UK), G. Terenghi (UK), L. Terenius (Sweden), G. Walter (USA) and E. Wimmer (USA).

The registration fee is 750 FIM, which will cover accommodation and meals. Participants are expected to pay their own travel expenses.

Applications should be sent to: Organizing Committee, Labsystems Research Symposium, P.O. Box 8, 00881 Helsinki, Finland, Telex 121949 Labsy sf, not later than April 30, 1985. They should contain a curriculum vitae, including the applicants scientific background and research interests.

The Organizing Committee

Kari Alitalo, Jorma Keski-Oja, Veli-Pekka Lehto, Ale Närvänen, Paul Partanen, Ralf Pettersson, Jukka Suni, Antti Vaheri.

(W1831)M

CONFERENCES and COURSES continued

1985 COLD SPRING HARBOR CONFERENCE ON CANCER CELLS

DNA Tumor Viruses: Control of Gene Expression & Replication
Wednesday, September 4 — Sunday, September 8, 1985

Organized By

TERRI GRODZICKER, Cold Spring Harbor Laboratory
PHILLIP SHARP, Massachusetts Institute of Technology
MICHAEL BOTCHAN, University of California, Berkeley

The 1985 Cold Spring Harbor Conference on Cancer Cells entitled, "DNA Tumor Viruses: Control of Gene Expression and Replication" will be held from Wednesday, September 4, through Sunday, September 8, 1985. We anticipate a schedule with approximately fifty major speakers who will present talks of 20-25 minutes with ample time for discussion.

The meeting will be devoted to a broad discussion of aspects of genetic regulation using SV40, polyoma virus, adenoviruses, papilloma viruses and herpes viruses as model systems. Planned session include:

Control of transcription: enhancers and promoters
Control of transcription: regulatory proteins
In vitro approaches to transcription
Regulation of RNA processing and translation
DNA replication and recombination
Transforming proteins
Stages of Transformation

Requests for registration material should be directed to the Meetings Coordinator, **Cold Spring Harbor Laboratory,** P.O. Box 100, Cold Spring Harbor, NY 11724.

(NW1712)C

ETTORE MAJORANA CENTRE FOR SCIENTIFIC CULTURE

International School of Biophysics

14th Course: GENERATION OF DIVERSITY AND SPECIFICITY IN THE NERVOUS SYSTEM

Erice-Trapani-Sicily: 14-24 July 1985

Invited speakers include: C. Barnstable (USA), G. Berlucchi (Italy), P. Calissano (Italy), A. Cattaneo (Italy), J.P. Changeux (France), U. Di Porzio (Italy), A. Gorio (Italy), N.M. Le Douarin (France), G. Levi (Italy), R. Levi-Montalcini (Italy), J. Mallet (France), R. Miledi (USA), R.J. Milner (USA), J.G. Nicholls (Switzerland), P. Rakic (USA), U. Rutishauser (USA), J. Szentagothai (Hungary), D. Trisler (USA), B. Zipser (USA).

Topics include: Monoclonal antibodies and neuronal specificity; recombinant DNA approaches to nerve cell function; epigenetic factors in neuronal development; molecular system for cell recognition; neurotransmitter receptors; nerve cell lines and primary cultures; neurite generation and regeneration; synaptic plasticity; neuronal organization and brain function.

Application: Closing date for application is **15th June, 1985.** No special application form is required. Applicants should specify: i) date and place of birth, together with their present nationality; ii) degree and other academic qualifications; iii) list of publications; iv) present position and place of work. Young persons with limited experience should enclose a letter of recommendation from the head of their research group or from a senior person active in the field. The total fee including full board and lodging (arranged by the School), is US $600. Thanks to the generosity of the sponsoring Institutions, partial support may be given to deserving students who need financial help. This must be specified and justified in the application letter.

Application to: Dr. G. Boera, Institute of Cell Biology CNR, Via Romagnosi 18A, 00196 Rome, Italy. Tel. 6/3611407. Telex 622195 INGENR I, Telefax 3609402.

'M.G.F. Fuortes' Scholarships: Two 'M.G.F. Fuortes' Scholarships will be awarded to deserving neurobiologists one by the 'M.G.F. Fuortes Memorial Fund' (reserved for a USA applicant), and the other by the 'Ettore Majorana Centre'. The scholarships will cover both travel expenses and school fees. The letter of application for these scholarships should be sent, together with a duplcate of the application for these scholarships should be sent, together with a duplicate of the application for the attendance to the Course to: **MGF Fuortes Memorial Fund, Laboratory of Neurophysiology, NIH Bldg, 36 Room 2C-02, Bethesda, Md.20205, USA.**

R. Levi-Montalcini, P. Calissano Direcots of the Course. A. Borsellino Director of the School. A. Zichichi Director of the Centre. (W1830)C

AWARDS

DEPARTMENT OF HUMAN ANATOMY, OXFORD
MRC BRAIN METABOLISM UNIT, EDINBURGH

MEDICAL RESEARCH COUNCIL PARTNERSHIP TRAINING AWARD

Applications are invited from final year students expecting to graduate with a first or upper second class honours degree in appropriate branches of science, medicine or veterinary medicine for a 'partnership Training Award'. The successful applicant will study the metabolism of and the development of neural connections and neurotransmitter receptors in hypothalamic grafts in hypogonadal mice, a mutant strain totally deficient in hypothalamic luteinizing hormone releasing hormone It is expected that this research will lead towards the degree of either D.Phil. (University of Oxford) or PhD (University of Edinburgh)

Applications including a full curriculum vitae together with the names of 2 professional referees should be addressed to either Dr. H. M. Charlton, Department of Human Anatomy, South Parks Road, Oxford OX1 3QX. or Professor G. Fink, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ not later than May 20th, 1985. (6204)N

THIRD MEADOW BROOK CONFERENCE ON MOLECULAR MECHANISM OF STEROID HORMONE ACTION SEPTEMBER 15-18, 1985

The Conference will address recent developments in steroid hormone action and will be held at the Meadow Brook Mansion of Oakland University. Invited speakers include:

W. Bardin, J. Baxter, M. Beato, J. Clark, J. Gorski, G. Greene, J. Gustafsson, M. Haussler, K. Horwitz, O. Janne, S. Liao, E. Milgrom, G. Mueller, B. O'Malley, J. Oppenheimer, G. Ringold, A. Roy, W. Schrader, T. Spelsberg and J. Tata.

For additional information concerning registration and poster presentation contact:

Dr. F.H. Sarkar, Conference Coordinator
Department of Biological Sciences
Oakland University
Rochester, MI 48063
(313-370-3585) (NW1705)C

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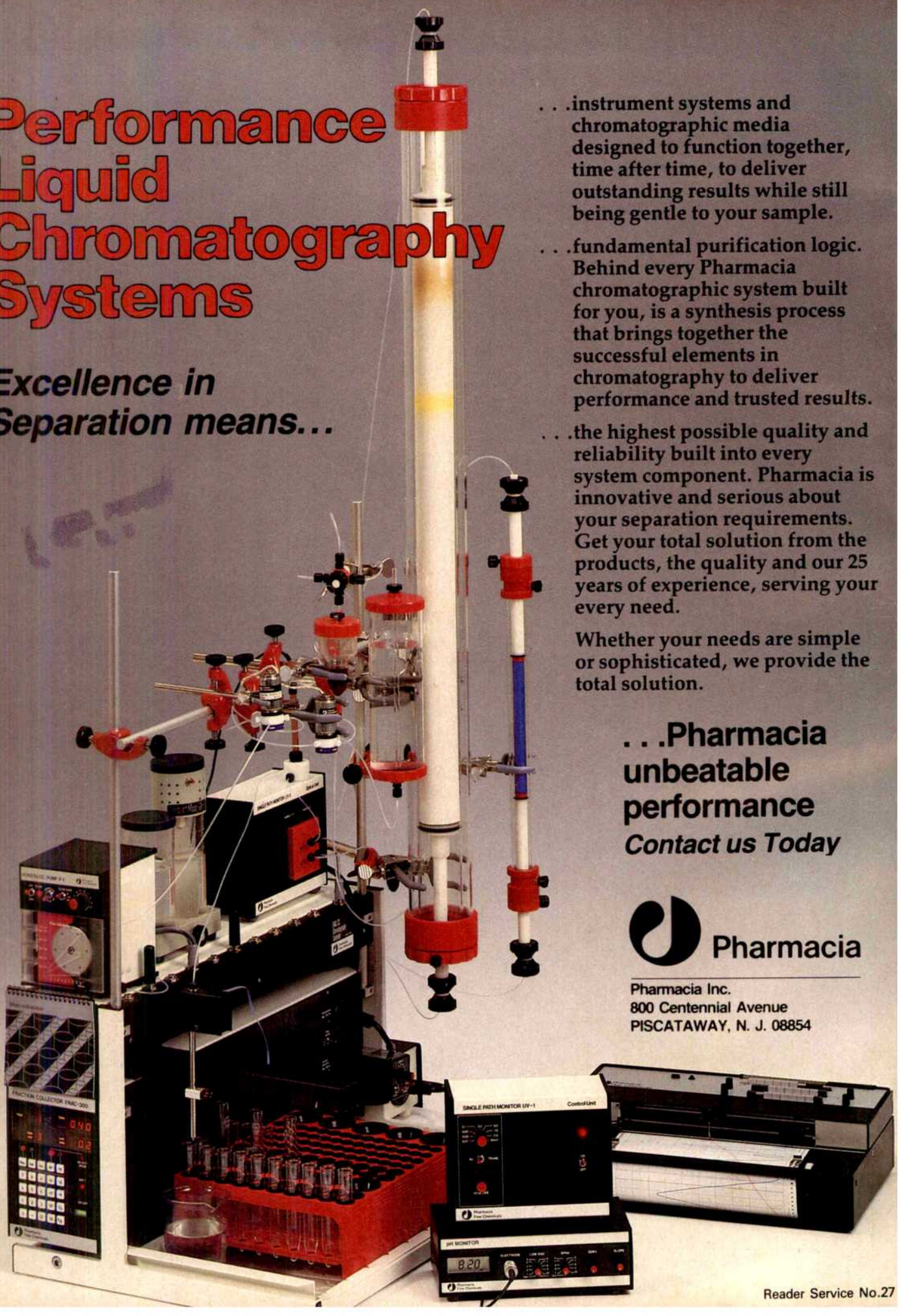
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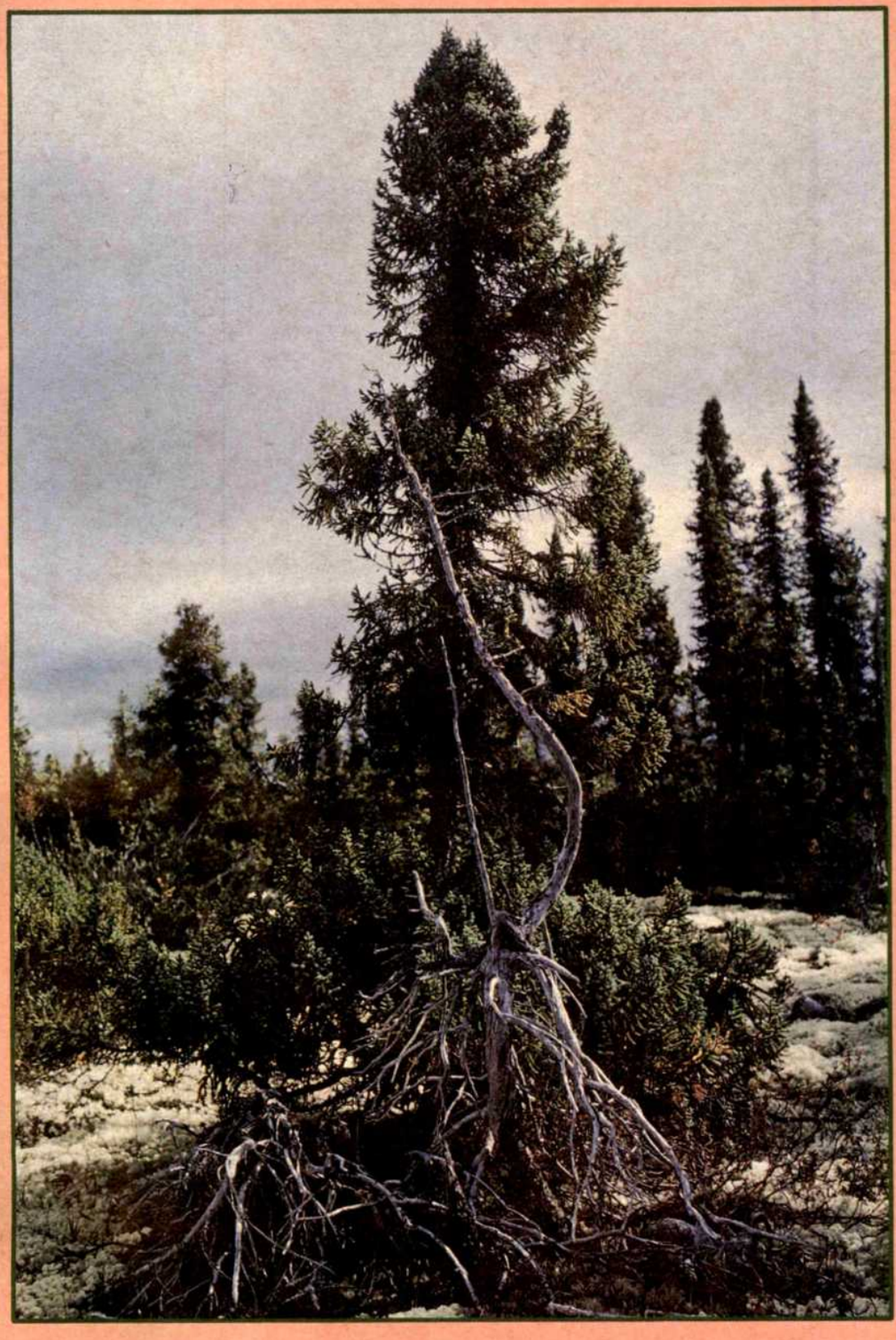


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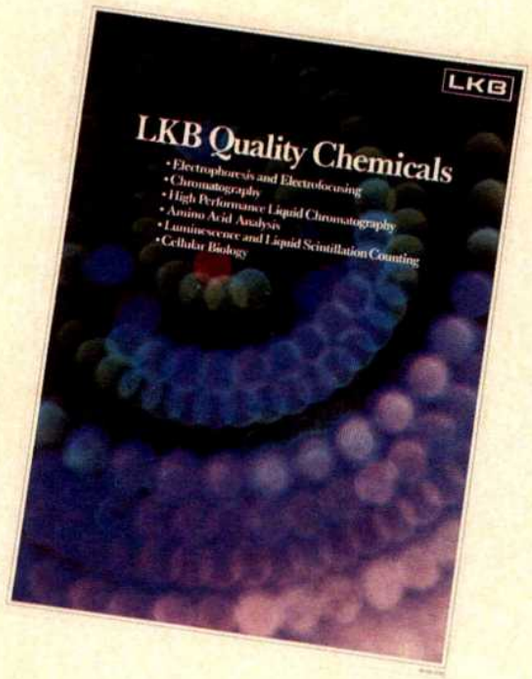
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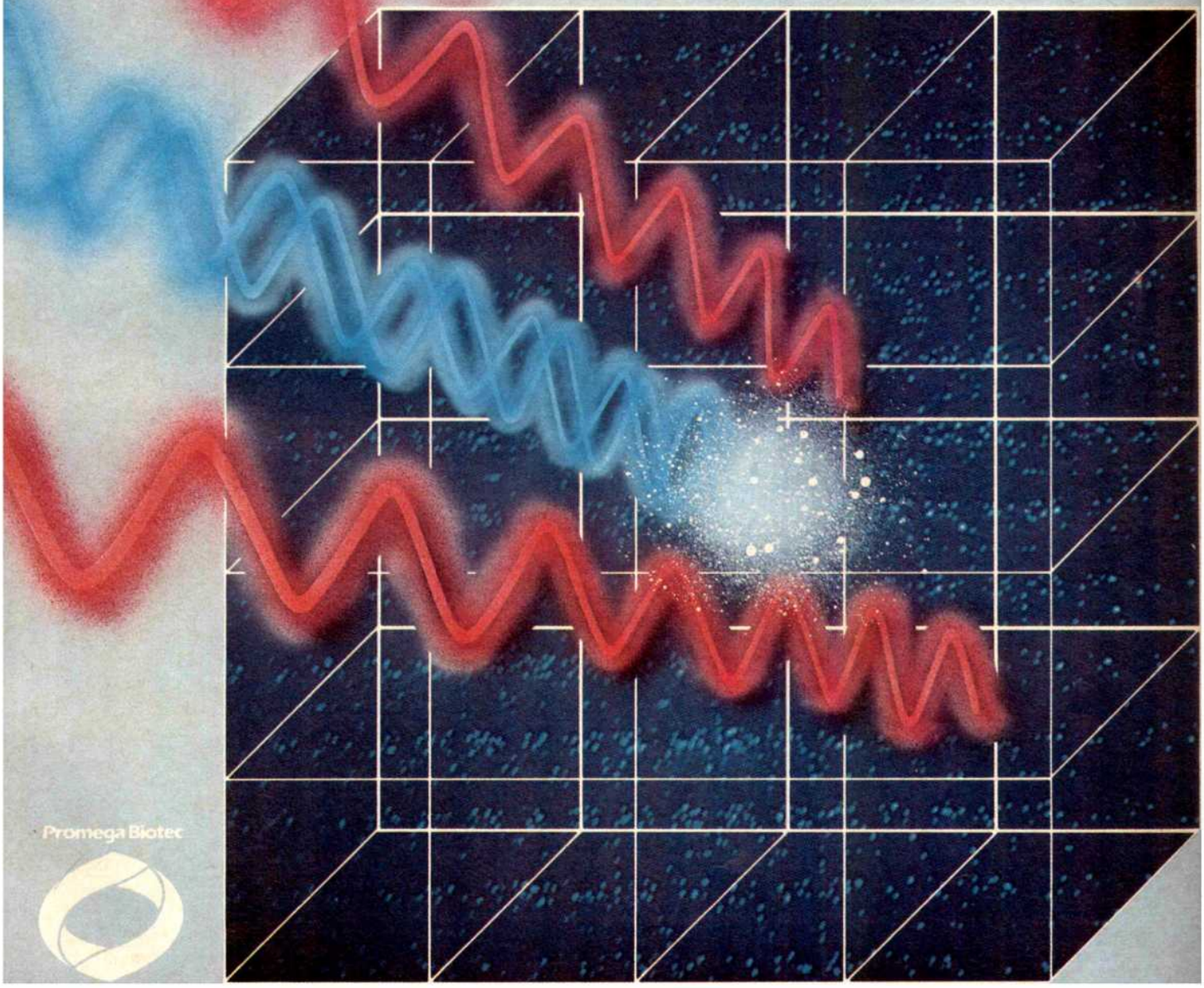
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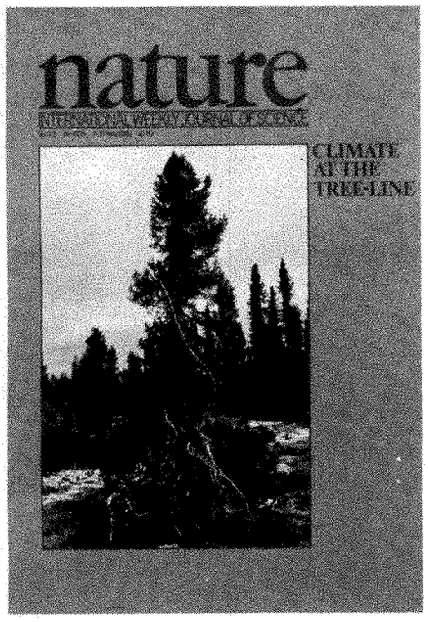
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COVER



An area of lichen/black spruce woodland near Bush Lake in Quebec, Canada. A tree-ring chronology has been constructed for this woodland for the years 1398 to 1982, giving some pointers to conditions during the Little Ice Age and providing the longest tree-ring chronology yet available for eastern North America. (See page 135)

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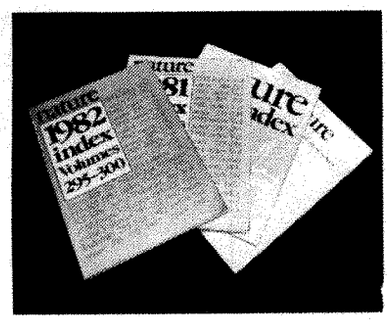
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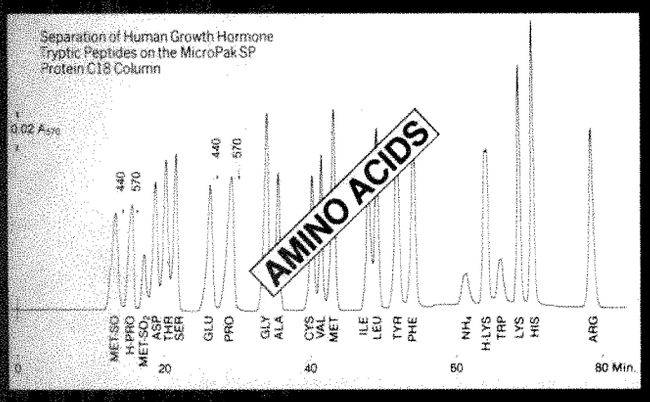
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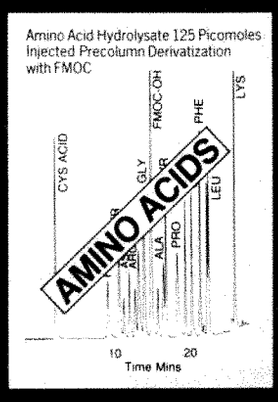
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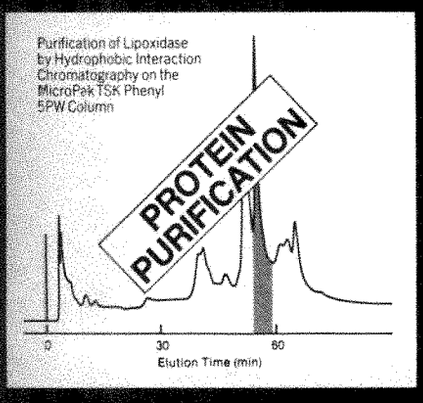
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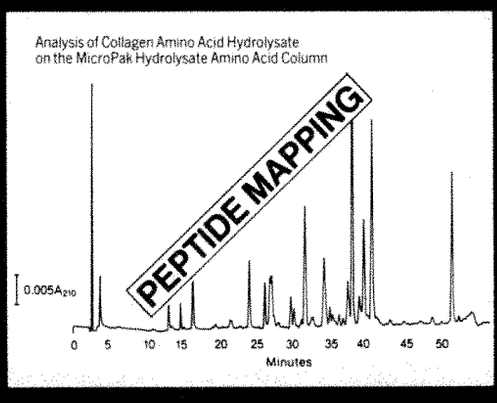
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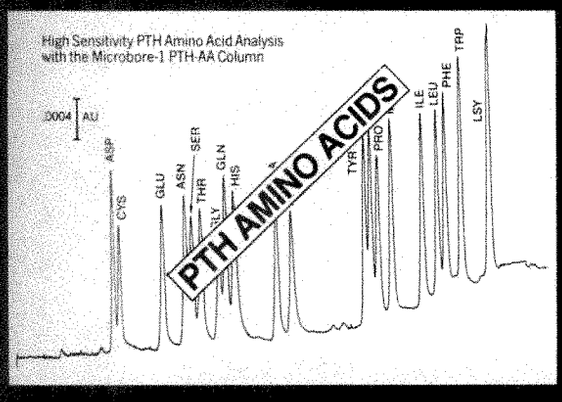
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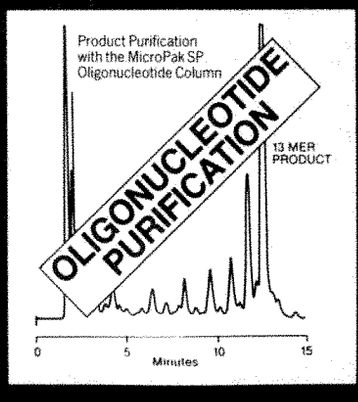












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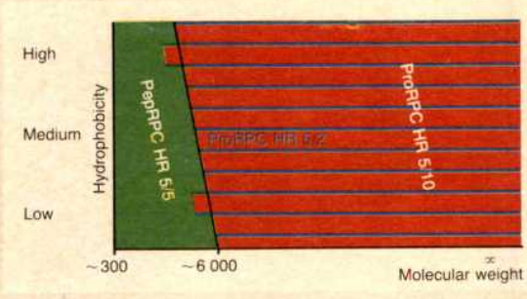
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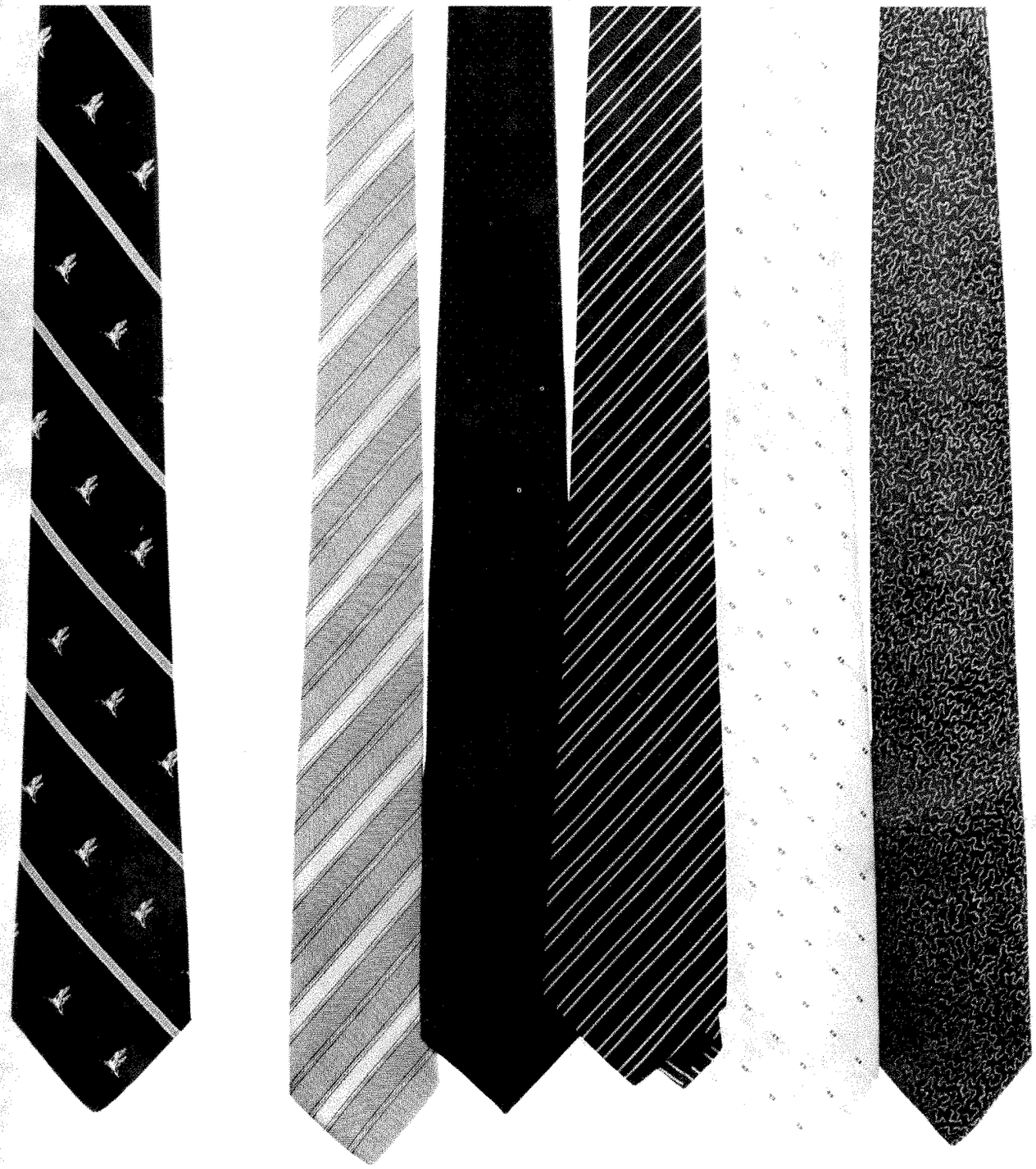
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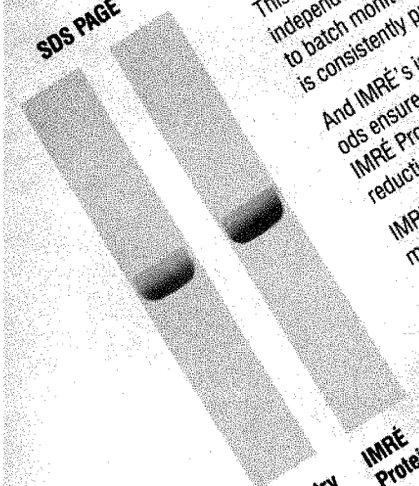
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NEW TECHNOLOGY in BIOTECHNOLOGY

In the past five years, even the enthusiasts of the new biotechnology industry have often been surprised at the speed with which new opportunities have emerged. The initial focus was on the use of *Escherichia coli* for manufacturing polypeptides and proteins.

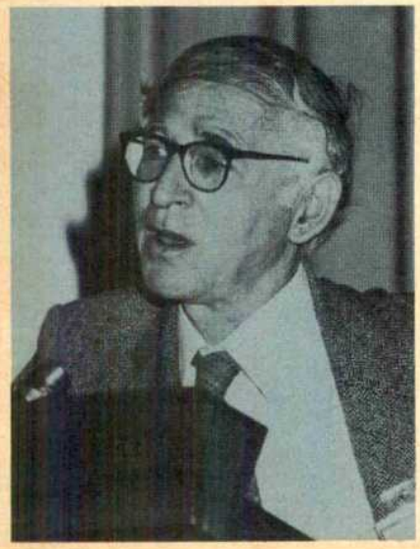
NOW...

- other bacteria as well as yeasts are also being used and attention is turning to mammalian and plant cell systems.
- improvements to the polypeptides and proteins produced are being achieved by deliberate mutagenesis of the genes introduced into the cells.
- new strains of plants and animals are being established from engineered embryonic cells.
- the manipulation and production of monoclonal antibodies and enzymes are becoming ever more refined and the goal of representing a large protein by a much simpler peptide is within reach.
- progress is being made in coupling biotechnology to electronics, so producing sophisticated measuring devices.

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THE OBJECTIVE of this *Nature* conference (the second to be held in Britain) is to provide a survey of the recent research that will influence the evolution of biotechnology in the next few years.

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Dr. A. Klug, MRC Laboratory of Molecular Biology, Cambridge.

AFTERNOON

Antibodies
Dr. J. Birch, Celltech Ltd., Slough.
Dr. M. Neuberger, MRC Laboratory of Molecular Biology, Cambridge.
ROUND TABLE DISCUSSION

Day 2 Wednesday, June 26th

Biosensors
Dr. M. Flanagan, University College, London
Professor I.J. Higgins/Dr. A. Turner, Cranfield/Leicester.

Micro-organisms
Dr. S. Emtage, Celltech Ltd., Slough.

Micro-organisms continued
Professor D. Hopwood, John Innes Institute, Norwich.
Dr. B. Carter, Searle R&D, High Wycombe.
ROUND TABLE DISCUSSION

Day 3 Thursday, June 27th

Mammalian systems
Professor P. Tiollais, Institut Pasteur, Paris.
Dr. A. Levinson, Genentech, San Francisco.
Dr. R. L. Brinster, University of Pennsylvania

Plants
Professor M. Fowler, Wolfson Institute, Sheffield.
Professor J. Schell, Max Planck Institut, Köln.
ROUND TABLE DISCUSSION or FINAL TALK

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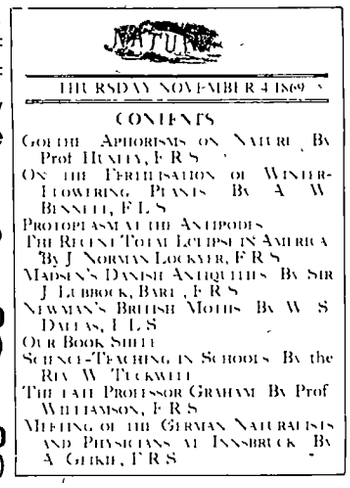
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Too much fall-out from summit

Last week's summit meeting in Bonn was no more of a failure than its predecessors. These meetings do not settle issues, but have the virtue of putting them on the international agenda.

TAKE seven heads of government, each preoccupied with urgent domestic business, and throw them together for a couple of days with instructions to consider the world's urgent problems from the other fellow's point of view. It is a daunting assignment. Usually, after the event, the public relations people are hard pressed to conceal the disturbing truth that even world statesmen, as they are called, do not rise to the grand occasions. Their simplest trick is to make public the quarrels there will certainly have been on relatively minor issues, but at the same time to suggest that none of this can presage permanent disunity (which is usually correct). So what can the statesmen claim they have accomplished when they return to face their own electors? Prudently, they arrange that each among them has some cheerful souvenir to take home. On this occasion, there was lukewarm support for President François Mitterrand's plan that high technology should be used to help Africa feed itself, general approval almost indistinguishable from indifference for the British government's economic policies and muted acquiescence in US plans for star wars research. But the statesmen at Bonn failed to agree on the US demand that there should be a further round of talks next year on the liberalization of international trade and on the French demand that international monetary reform should be a precondition of such talks.

The temptation to blame the French for the failure at Bonn should be suppressed, if only because the French, and especially President Mitterrand, have consistently done their best to tackle other than immediate problems. Four years ago, at Fontainebleau, the cry was that technology should be made the instrument of renewed economic growth. This year, at Bonn, the plan was to rid Africa of famine by an engineered green revolution coupled, wherever appropriate, with the use of Earth resources satellites and the like. President Mitterrand is entirely right to believe that technology can work wonders, and would now be within his rights to be irritated that his fellow-statesmen do not share his vision. Where he goes wrong is to suggest that technology, admittedly a necessary part of the solution of these problems, is for that reason also sufficient. That is the illusion. While the climate and the soil of much of developing Africa are inimical to agriculture, the reasons why food production is inadequate have more to do with the willingness of African governments to sacrifice the prosperity of their peasant and farming populations to the interests of their urban voters than with the physical conditions under which crops must be grown. A summit of the seven will not put that to rights.

The French demand for monetary reform is similarly stronger on good intentions than on realism. Rapid currency fluctuations are unsettling, and bad for world trade (which may be true), so let us legislate against them. That seems to be the argument. It is a classic case of therapy by the removal of symptoms. But President Mitterrand is well placed to know that before fixed currency rates were finally abandoned in 1972, international financial crises happened just as often, but took a different form. The immediate cause of the troubles of the past few months is the large budget deficit of the federal government of the United States, and the way in which this is being financed (by borrowing from abroad rather than by printing domestically inflationary dollar bills). Since there is no way in which sovereign governments can be prevented by their trading partners from running budget deficits or even, if they choose, from letting domestic inflation run riot, the only present basis for monetary reform is an agreement between the summit states to limit the effects of bad budgeting on the international money markets. If Mr. Mitterrand had been pressing for true reform along such lines, rather than for a return of the bad old days, the United States would probably still have disagreed with him, but then he would have had reason on his side.

The US demand for a further round of talks on international trade beneath the umbrella of the General Agreement on Tariffs and Trade (GATT) is a more complicated issue. That GATT should exist is a sign of grace. That the United States should have taken the initiative in pressing for an extension of the free-trade regime is decidedly another. Politically, it might have been easier for the US administration to give in to the recent demands in Congress for a greater measure of protection from imports from overseas. But the United States was asking last weekend for an extension of the rules governing international trade that would strike at the roots of many shaky institutions elsewhere. The proposal that the rules on free trade should be extended to agricultural produce might yet make a market for Texas beef in Japan, but would certainly spell trouble for the practice of the European Community of subsidizing farmers by means of artificially high prices for food in domestic markets, while retaining the right to dump the surplus on the international markets. (One of last weekend's little ironies is that, while Chancellor Helmut Kohl was seeking emollient ways of making the Bonn communique seem good, his agriculture minister had dug in his heels, on behalf of West German farmers, against a proposal that the price of European cereals should be reduced.) The truth is that, on free trade, the United States has reason on its side and should be helped to have its way.

Star wars, the other agenda item, is more of an embarrassment for all. Two years ago, when the Strategic Defense Initiative, as it was later called, took much of the US administration by surprise, most other governments were either sceptical (as in Western Europe) or hostile (the Soviet Union). With the passage of time, the administration seems to have calculated that doubters might be turned into supporters if they were offered participation, but the terms on which this will be possible are not clear. But most of the summit governments, and certainly their electors, would be happier if the whole scheme had never been thought of. Some of them will talk about participation in the hope that helping will give them influence, but are alarmed that star wars may jeopardize the missile talks under way at Geneva.

Agreement on that issue, like most of the others raised in Bonn, lies in the future. To next year's summit, perhaps? Many of the issues raised in Bonn cannot wait that long. The US deficit will either have been made to go away, or there will be a different set of economic problems with which to grapple. But President Mitterrand may find, by next year, that he has been luckier than he now thinks likely on monetary reform, given the way in which bankers have taken fright over recent developments. If star wars have confounded the Geneva negotiations, or if these have broken down for other reasons, there will be more urgent things to talk about, which only goes to show that these summits, whatever they fail to accomplish, have become a valuable agenda of our anxieties. □

Running telescopes

Managing British astronomy is up for talks. Here is a recipe for the committee responsible.

THE first tangible product of the review of the pattern of British academically related research to which the Science and Engineering Research Council committed itself six months ago is hardly the revolution some had feared (or, sometimes, hoped for), but is none the less interesting on that account. The nub of the decision about research in astronomy announced last week is for the time being unremarkable, even hackneyed: there is to be another committee, the composition of which will be decided only next week. But in the course of inviting comments, the council says its objective is to decide what arrangements there should be for managing the two modern observatories in which the British have a majority share, at La Palma and in Hawaii. (The Netherlands, Spain, Denmark and Sweden are differently involved in one or other or even both of the projects.) By putting the question in those terms, the council has side-stepped what in the past has seemed the central question in British astronomy, that of whether the two observatories (at Herstmonceux and Edinburgh) should be merged and, if so, on which site. To ask instead the question how the new observatories should ideally be managed should provide a more constructive agenda for discussion than the many earlier enquiries on related subjects.

As things are, the Royal Greenwich Observatory (at Herstmonceux) is responsible for the La Palma observatory, to which it has donated its largest telescope, the 92-inch Isaac Newton reflector. Edinburgh, on the other hand, has had operational responsibility for the Anglo-Australian telescope, roughly 18,000 kilometres distant. (Nothing is said in last week's announcement of the importance of that instrument in the future scheme of things, confirming earlier guesses that the research council plans to trade its share of the capital equipment for a measure of observing time.) But yet another laboratory, the Rutherford Appleton Laboratory in the middle of southern England, has an important place in the management of British astronomy, both in the operations of the computer network by which data are exchanged between university laboratories and in supervision of the millimetre-wave telescope being built on Mauna Kea. On the face of things, British astronomers are overwell catered for in these respects. What the new committee should be asking is whether they should now be given a chance to look after themselves.

There is now plenty of recent experience to suggest how this should be arranged. Like other kinds of laboratories, observatories need directors, preferably persons of distinction in the field concerned, astronomy in this case. Even when the chief users are from universities, observatories also need to have a resident scientific and technical staff. Given the geography of the new British ventures, these will presumably be chosen to be as small as possible. For the rest, there need to be oversight committees, essentially trustees, and committees for the allocation of observing time, which again need not always meet in the shadow of the instruments they service. To give this pattern of management conviction with the users, some way should be found of incorporating users or their representatives in these managerial functions. These are the essential ingredients of the management structure, which happen not to involve the existing observatories at Herstmonceux and Edinburgh.

But this, of course, is not the whole of the fabric of support for a lively astronomy enterprise. The development of instruments has been shown in recent years to have been crucial to the improvement of techniques, at both telescopes, while the management and storage of data is likely to become just as important in the years ahead. But the people best placed to know what instruments will yield worthwhile results are potential users and their frequent attendants, the people with a zeal (and flair) for making things a little more sensitive or accurate, from which it follows that the research council should make grants to university astronomers for the development of instruments (which already happens), keeping up its sleeve some means for building what the enthusiasts design (best done, alongside the development of instruments for satellites, at the Rutherford Appleton Laboratory). Data management needs to be done by astronomers, not toilers in some huge laboratory, which argues for transferring this responsibility to one of the two observatories in the United Kingdom. With its recent success in designing data handling techniques, Edinburgh seems a better bet than Herstmonceux.

This is the kind of pattern to which logic leads — a single smaller observatory based in Britain, more responsibility (as for instrument design) given to the university users of the new equipment and central support in engineering from a large laboratory. So why bother with a committee? Because committees are about politics as well as reason. The choice of which observatory to close is bound to be invidious, and so is most easily made by people with an appearance of detachment. That is how the argument goes. So the research council ultimately in charge should simplify the committee's work by making clear in advance how it would deal with the people, possibly counted in hundreds, whose jobs would vanish under a brisk plan for reorganization. And the council should do its level best to practise what it has been preaching in the past few years, that astronomy is not only an end in itself but also a way of acquiring skills of general applicability, by shouldering the task of finding acceptable and useful jobs for those who may be displaced. At a time when Britain, against the recent trends, is about to be better blessed with facilities for observation in astronomy, it would be absurd that the dole queues should be lengthened by an influx of professionals in the field.

Beware junk bonds

Business in the United States, always inventive, seems bent on turning fiction into truth.

THE board-game "Monopoly" was designed in Atlantic City to mimic the real world of property speculation. Now, the financial wizards of Wall Street and similar places seem bent on adapting "Monopoly" to real life. Shadowy figures with colourful names such as T. Boone Pickens have hit on a novel way of buying up desirable companies in the stock market. The stratagem is ingenious and simple. A publicly-quoted company is singled out as a target, often on the strength of its diversity and the likelihood that its business assets are undervalued. Then a second corporation is created which offers to buy a proportion of the stock in the target in return for securities of its own, usually in the form of interest-bearing certificates. To make the deal attractive to the shareholders, the rate of interest is fixed at a level great enough to promise a return greater than the dividends earned from the target company. To lend reality to the offer, the company usually has to spend cash buying a proportion of the outstanding stock in the markets. Under United States law, it will suffice for the raider's purpose to acquire just that proportion of stock to gain control of the target company.

That is phase one. The problem now is that the shell company that has won control of its target is saddled with a load of debt, which can only be discharged by selling off the assets of what was originally the target company, exchanging the cash proceeds for the outstanding debt. In principle, these schemes will work so long as the assets can be sold for more than the nominal cost of buying half the target company. In practice it is often more profitable to offer some cash with the original purchase, for then the purchase price may be much lower. One way and another, the junk bonds outstanding from the recent frenzy of takeovers in the United States continue to be serviced. So far as anybody can tell, none of the schemes financed by junk bonds has yet come unstuck. But the amounts of money involved are so large ($9,000 million for Gulf Oil) and the basis of these deals so shadowy, that some of them are bound at some stage to run into trouble, perhaps because the assets are no longer as saleable as they seemed. Then a lot of people who thought themselves rich will find themselves poor, the common experience after the Great Crash of the 1930s from which "Monopoly" sprang. □

US agricultural research

Mission-agency seeks relief from Congress

Washington

THE Agricultural Research Service (ARS), the US Department of Agriculture's sprawling intramural research agency, is emerging from three years of austerity (some self-imposed) and reorganization with a mixed success in its efforts to polish its tarnished image with the scientific community.

ARS's administrator, Dr Terry Kinney, has scored one major success by reducing administrative overheads by $12 million out of the agency's $487 million annual budget. Less successful have been Kinney's attempts to challenge Congress's insatiable appetite for building new laboratories that for the most part serve only parochial interests.

And the most long-standing criticism of ARS — that it is not keeping pace with new developments in basic research, especially molecular genetics — persists, in spite of one notable ARS initiative, the creation last year of a plant gene-expression centre in conjunction with the University of California.

That criticism was reiterated most recently by a National Academy of Sciences panel which scrutinized ARS's research programmes at the request of ARS itself. The panel's report again emphasized the need to concentrate on developing basic understanding of plant physiology and development, insect neurobiology, immune responses in animals and other fundamental molecular processes in domestic plants and animals as well as pests.

Standing in contrast to the panel's recommendations is ARS's own 1984 annual report of its research achievements, which highlights applications almost exclusively — a new rotary disk for harvesting soya beans, a pelletized guayule seed that improves germination, a pilot plant for automatic tanning of cattle hides, a new kind of spaghetti made with 10 per cent bran.

The academy panel, chaired by Ralph Hardy, recently head of life sciences at DuPont and now president of BioTechnica, also recommended some drastic steps for ARS if it is to create the proper "climate" for good research. For example, the panel said ARS needs to be a lot tougher about offering permanent positions to scientists.

At present, the panel said, virtually all ARS scientists who are reviewed at the end of a one-year probationary period are promoted to permanent staff positions; the probationary period should be extended to five years and candidates should be evaluated by an outside group of experts.

The panel also suggested a substantial increase in ARS's postdoctoral programme; instead of 25 postdocs (actually, ARS officials say, the true figure is closer to 275), there should be at least 750. And instead of devoting 90 per cent of research budgets to salaries — as at some of the ARS's 147 laboratories and research centres — salaries should be at most 75 per cent, and in some cases as low as 60 per cent, the remainder going towards properly equipping the laboratories.

The 147 laboratories are a problem in themselves. The panel noted, as have many others before, that that is simply too many. Research groups are fragmented, preventing the formation of a "critical mass" of scientists needed to make progress.

ARS officials say that they have already dealt with many of these complaints, but that their hands are tied on the others. Federal personnel rules, for example, prohibit use of outside consultants in hiring and promotion decisions. But ARS is now negotiating with the federal Office of Personnel Management to lengthen the current one-year probationary period to three years.

Congress has been the greatest obstacle to ARS's attempts to shut down centres and laboratories that have outlived their usefulness, or which fragment the research effort. ARS did succeed in transferring the staff of the fire-ant research laboratory in Gulf Port, Mississippi, to its Gainesville, Florida, insect research centre. It hopes to consolidate several other laboratories this year.

But Congress has effectively turned down a request to rescind funds appropriated in fiscal years 1984 and 1985 for construction at eight facilities (including a Forage Seed Production and Research Center and the National Soil Tilth Center). Congress has also continued to create new facilities, such as a national centre for leptospirosis in Ames, Iowa, and a Children's Nutrition Research Center in Houston, Texas.

One way that ARS hopes to avoid being saddled with still more laboratories in the future is by writing new language into the farm bill, which is up for renewal this year. As things stand, the only way for a state to obtain federal money for a research facility is by having ARS build and operate it. The new language would open an escape valve, allowing Congress to appropriate matching funds for states to build their own facilities when they consider this necessary.

Dr Mary Carter, ARS's associate director, says that the agency has already taken steps to meet the panel's criticism of high personnel costs, and that many laboratories are already down to 65 or 70 per cent. Some laboratories where the figure is deemed too high have been ordered not to fill vacancies to correct the problem.

Carter also said that ARS is committed to expanding its programmes of postdoctoral research. Besides the 50 positions that are being created directly by the administrator's office, the research laboratories themselves hire some 250 or so postdocs.

But ARS, always under pressure from Congress — particularly Representative Jamie Whitten (Democrat, Mississippi), chairman of the Appropriations Committee — to "do something for the farmer", is drawing the line at any major tilt in favour of basic research. "We're a mission-oriented agency," Carter said, emphasizing the new ARS party line that biotechnology is merely a group of "methodologies" that can be put to use at all of its laboratories. ARS has explicitly rejected the notion of establishing biotechnology or basic research centres.

Stephen Budiansky

Land bows out from Polaroid

EDWIN Land, the 76-year-old founder of the Polaroid Corporation, has broken his last tie with the company. Last week, Land announced he would sell his remaining shares, which account for 8.3 per cent of Polaroid stock, and turn over a sizeable portion of the proceeds to the non-profit Rowland Institute for Science, a vaguely utopian haven for basic research that Land set up in 1981 by selling off $38 million worth of stock.

Land's remaining stock has an estimated market value of $71 million, $28 million of which is to go to the Rowland Institute.

Land resigned as chairman of Polaroid in 1982 (see *Nature* 298, 701; 1982) during a rough period for the company. The company's Polavision instant movie film, which hit the market in 1977, just as home video equipment was becoming available, proved a colossal flop, costing the company a reported $68 million. Although Polaroid's earnings began to recover from that setback in 1983, the company hit rough times again in the third quarter of last year.

The Rowland Institute, along with the Rowland Foundation that Land established in 1960 to support research and education, has increasingly been his consuming interest in recent years.

The institute, with a staff of about 80 and a new building on the Charles River in Cambridge, Massachusetts, was described by Land at the time of its founding as a place where scientists could return to "an older way of doing science", without large research teams or big machines.

Stephen Budiansky

France

New scourge for universities

IN a rather extraordinary and for the universities somewhat disturbing development in French higher education, a principal critic of the French university system has been appointed president of a new universities "evaluation committee".

The man in question, M. Laurent Schwartz, is a highly-respected mathematician at the Ecole Polytechnique, one of the "ivy-league" *grandes écoles* of France. But he is also the author of two influential diatribes attacking the French universities and prescribing certain medicines, such as a greater dose of selection and élitism, which do not appeal to the majority of university staff. These were appointed in the egalitarian boom years after the 1968 student rebellion when the universities both shifted well to the political left and quadrupled in size. To them, a Schwartzian new broom is a fearsome thing.

But how much power will M. Schwartz have? This will not be clear until his new committee, the *Comité National d'Evaluation des Universités* (CNEU), has been in place for a year and delivered its first report. But that this may come around the time of next year's general elections in France, when, according to present opinion polls, the right will return to government, will cause further alarm.

In principle CNEU is being presented as equivalent to Britain's University Grants Committee (UGC), which distributes British universities' annual "recurrent grant" from the Department of Education and Science to cover salaries and basic university expenditure such as heating and building costs. But there are important differences. On the whole, UGC is composed of university heads of department representing a cross-section of academic disciplines. In the face of university cuts, therefore, it has tended to act as a defensive body, the only notable exception being when UGC cut grants to certain smaller, mostly technological universities on the debatable basis that these universities attracted students with poorer A-level grades than others such as Oxford and Cambridge. This, in a sense, was passing the buck.

But the French CNEU promises to be different. Apart from M. Schwartz, the nine members of the "academic" side of the committee will include physicist M. Pierre Aigrain, former science minister under the previous (and conservative) French President, Giscard d'Estaing, alongside other representatives of the *grandes écoles*, and few from the university system itself. The committee is completed by five members of what might be described as an "industrial" side of the committee. Altogether, then, CNEU cannot be described as representative of the universities, as can UGC. Rather, CNEU represents a new structure on top of the universities: a new level of control.

The appointments to CNEU were announced only last week. But its terms of reference were defined in a higher education bill last year. According to these, CNEU will regularly review the work funding of the universities, considering both their research and teaching. CNEU will also measure each university against its multi-annual plan — under which a university agrees development plans with the ministry of education in exchange for a secure 3-5 year budget.

The committee will also review the new PhD system, which began only this year, and will not report to the minister of education but to the Elysée itself: to the President of the Republic, presently M. François Mitterrand.

It seems possible that M. Mitterrand, new scourge of the universities, while entering from the left, will end up by leaving from well to the right. **Robert Walgate**

NIH

More competition, more disquiet

Washington

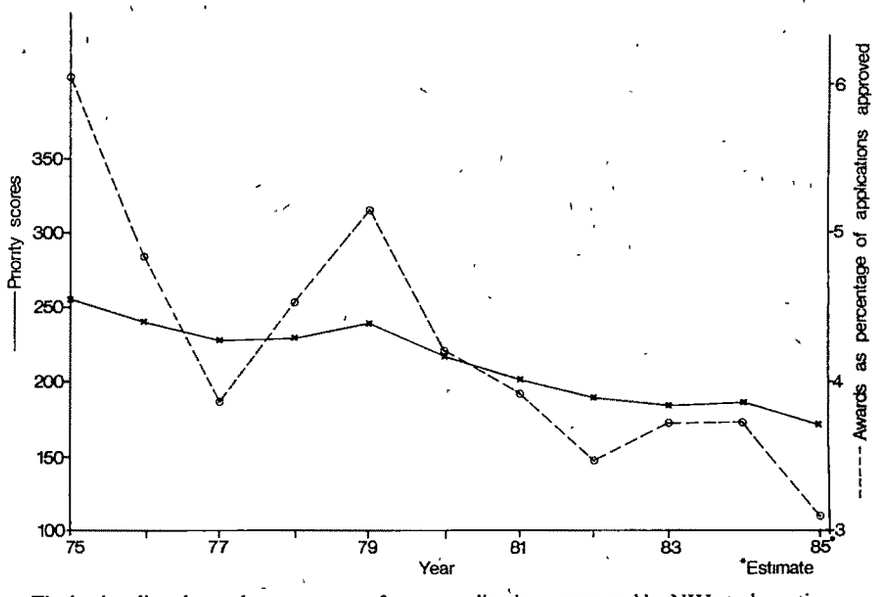
THE Reagan administration's plan to restrict to 5,000 the number of extramural research project grants awarded by the National Institutes of Health (NIH) is having a marked effect on the priority score required of successful applicants. The estimated aggregate cut-off point for 1985 is 170, although because there have been more high-quality applications than expected, the figure may turn out to be as low as 160. The payline, as the cut-off is called, varies between institutes from less than 150 to over 200.

The Congress had requested 6,500 grants for 1985; if it prevails in the argument with the administration, the payline would be around 190. According to the latest rumours, a compromise of around 5,800 grants is likely.

Meanwhile, intramural research at NIH is also facing problems. The administration's proposed budget for NIH intramural research in 1986 is $561 million, down $16 million from 1985. NIH officials estimate that $619 million would be needed in 1986 to maintain research at its present level.

In addition, the institutes as a whole have to lose 450 man-years from their staff by 30 September, with a reduction of a further 150 man-years during the following year. The number of staff that will have to be lost by September will probably be over 600, because the instruction to reduce staff (from the Office of Management and Budget) was received only in January, one-third of the way into the fiscal year.

The problem is further exacerbated in those institutes already employing more staff than the Office of Management and Budget had assumed. Institutes in this category have initiated a "semi-freeze" policy, whereby two jobs must be lost before a single replacement can be hired. NIH officials are concerned that young untenured researchers are being frozen out and that administrative and domestic staff, who are usually tenured, will as a consequence be a growing proportion of the whole. **Tim Beardsley**



The broken line shows the percentage of grant applications approved by NIH study sections that were actually funded; the solid line shows the so-called "payline" — the priority score that 90 per cent of the funded applications had to better in order to receive funding. Priority scores range from 100 (excellent) to 500 (poor). The 1985 estimates assume 5000 grants this year; the actual number is still under negotiation.

Creation science

Rot sets in in Queensland

St Lucia, Queensland

IN Australia's sunshine state of Queensland, worried academics and teachers are preparing for a re-enactment of the "creation science" debate of the past few years in the United States. Their concern is that Queensland's Minister for Education, Mr Lin Powell, has ordered the teaching of creationism in state high schools. Whenever teachers raise the subject of evolution, they are also now obliged to present the conflicting arguments for creation and catastrophism.

Hitherto, many of Queensland's teachers have exploited a convenient loophole in Mr Powell's edict: they were not instructed how to teach creation science. But the minister now intends to close that loophole by spelling out to teachers exactly how they should present creationism in the classroom. His plans are being formulated with advice from the Creation Science Foundation (CSF), an organization that maintains a constant barrage of creationist propaganda from its headquarters in the Brisbane suburb of Sunnybank. At present, Queensland is the only Australian state where creationism is officially included in the school science curriculum, although some academics and teachers believe that it is being taught unofficially elsewhere.

CSF, which describes itself as a "faith-funded organization", publishes the glossy magazine *Ex Nihilo* at an annual cost of more than $40,000. The magazine is delivered to all state high schools in Queensland (with approval from the Minister for Education), and also finds its way into school libraries in other states.

Late last year, the librarian at a high school in the Northern Territory judged *Ex Nihilo* to be unsuitable for children and requested CSF to send no further issues. CSF gave a prompt response in its newspaper *Prayer News* — it published the name and address of the offending school and urged its readers to write expressing disapproval. The editor of *Prayer News* commented that it was a "dangerous situation" when a library refused to accept a gift of *Ex Nihilo*. Eventually, the hapless school librarian capitulated under mounting pressure from CSF and its supporters.

While *Ex Nihilo* brings creationism to a general audience (including children), CSF has also launched a second periodical destined for only the most erudite creationists. The *Ex Nihilo Technical Journal* is laid out in the format of many reputable scientific journals and purports to give an in-depth treatment of the latest development in creation research.

CSF, not content merely to make inroads into the educational system, now also offers its own correspondence courses in creation science, which may be attractive to teachers seeking additional "qualifications". The gratifyingly large number of enrolments (more than 90) has prompted CSF to start selling its courses in the United States, where it already has representatives to promote sales of its literature. Most recently, CSF has revealed plans for a "world class museum as a teaching centre on creation".

So far, the creationist campaign has met no organized opposition from the scientific community. Not a single scientific society has spoken out against the bogus science of creationism. The Australian and New Zealand Association for the Advancement of Science has remained silent, as has the Australian Academy of Sciences. Representatives from all major scientific organizations, gathered in Canberra on 16 April for the inaugural meeting of the National Committee for the Promotion of Science and Technology, might well have been expected to voice some opinion on the issue, but did not.

Nevertheless, many individuals — scientists and teachers, students and parents — are beginning to express concern about the unchecked growth of creationism. On 22 March, an audience of nearly 400 gathered at the University of Queensland to hear a series of lectures exposing the fraudulent basis of creation science, as a result of which an action group has been formed in resistance to the creationist campaign.

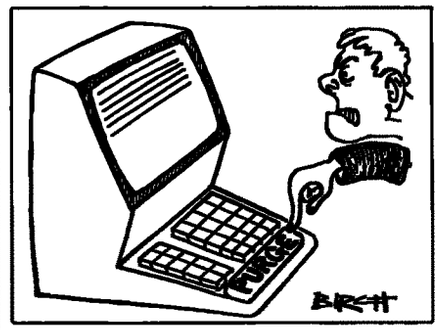
The action group has adopted the name Australian APE (Australian Association for the Protection of Evolution), and aims publicly to refute the claims made by creationists and to press for the removal of creationism from the school science syllabus. It is assembling "survival kits" for schoolteachers who find themselves unwillingly obliged to teach creationism.

Tony Thulborn

Soviet computers

Keeping up with the students

THE Soviet Union has launched a major programme for computer literacy in schools, apparently in response to complaints by Dr Vyacheslav Elyutin, Minister of Higher and Specialized Secondary Education, that the poor performance of certain sectors of the Soviet economy is attributable to the lack of computer-mindedness among middle managers. During the coming academic year, a course in "Fundamentals of information science and computer technology" is to be introduced in all secondary schools, initially in the ninth and tenth forms (for 15 to 16 year olds), and later for younger students. Experiments are also planned in the use of computers in teaching other school subjects, as for example in processing the data obtained in physics or chemistry lessons.



Experiments in the use of computers in schools seem to have been going on for some time. According to Professor Vadim Monakhov, director of the Institute of Teaching Content and Methods of the Soviet Academy of Pedagogic Sciences, one such course was introduced in a Moscow school (specializing in mathematics) as early as 1960. Shortly before the recent Politburo decision, a Soviet television feature showed children from another Moscow secondary school using computers and visual display units under supervision and even making microcalculators and assembling printed circuit boards for local industry as part of their "work training experience".

The logistical problems of the new courses are considerable. Soviet school textbooks are usually published in editions of 4 million and software packages will have to be produced on the same scale. Dr Monakhov last month visited the United Kingdom for an international study group on the introduction of computers in schools but, on his return, told a Moscow radio reporter that although he had had considerable opportunity to get acquainted with teaching software of British companies, including Acorn and Sinclair, he found them for the most part unsuitable for Soviet use. The Soviet educationalists were therefore, he said, faced with the "tremendous" task of developing their own software.

Hardware is also liable to cause problems. It is not simply a matter of choosing the computer that is "most suitable" to the teaching profession, Monakhov explained, but also to ensure that Soviet industry is capable of producing such a computer by September. The plans for training of teachers for the new courses also seem unrealistic. Crash courses for teachers of mathematics and physics will be organized (presumably during the summer vacation). Since the Politburo decision also supposes a vast expansion of extra-curricular computer facilities for young people, organized through creativity clubs and the Pioneer youth movement, many teachers may well be faced with pupils more familiar with computers than they are themselves.

Vera Rich

Soldiers' sickness

Compensation for US veterans

Washington

ACTING under instructions from Congress, the US Veterans Administration (VA) has now spelled out its proposals for regulating compensation claims based on exposure to dioxin-contaminated herbicides and radiation from atomic tests. But VA's proposals, which were required to be based on "sound scientific and medical evidence", are unlikely to end the long-running debate over when cause and effect should be inferred in dealing with such claims.

Nineteen million gallons of dioxin-contaminated herbicides were used during the Vietnam war, but veterans have been largely unsuccessful in claiming compensation from the government for disabilities allegedly resulting from their exposure. Only 20 claims have so far been recognized.

VA's proposed regulations continue its policy of assuming some dioxin exposure for all Vietnam veterans, but are unlikely greatly to increase the number of successful claims. Chloracne is the only disease recognized by VA as likely to result from exposure to the most potent dioxin isomer, 2,3,7,8-TCDD, and then only if symptoms appear within 3 months of military service.

Generally, compensation is paid for all medical conditions which appeared during service, and for some chronic diseases appearing within a year of discharge. In borderline cases, compensation is awarded if there is reasonable doubt. Most claims fall outside these categories, however, as they are based on diseases appearing years later.

VA was requested by Congress to consider specifically how it would deal with claims linking dioxin exposure with soft tissue sarcoma and porphyria cutanea tarda, a liver disorder, but has concluded that neither disease has been convincingly shown to result from dioxin.

Most of the evidence on the human health effects of dioxin comes from studies of industrial exposure and from the Air Force's Ranch Hand study, which found some suggestive but equivocal evidence linking relatively high levels of dioxin exposure in spraying crews to increased incidence of skin cancer, birth defects and neonatal deaths.

Firmer answers should emerge from a $75 million epidemiological study by the Centers for Disease Control (CDC) of health effects of Vietnam service and dioxin exposure, recently mounted. Some 30,000 veterans will be interviewed by telephone, of whom 10,000 will be given thorough physical examinations at special clinical facilities constructed at Albuquerque, New Mexico. CDC are also carrying out a case control study of those particular cancers suspected of having being caused by dioxin. The results should be available before the end of the decade.

Veterans who participated in above-ground nuclear weapons tests in Nevada and in the Pacific have been just as unsuccessful in prosecuting compensation claims as the Vietnam veterans. Only 20 cancer cases have been recognized as due to service-related exposure to ionizing radiation. VA's proposed regulations allow possible claims based on leukaemias and bone cancer appearing more than two but less than 30 years after exposure (excluding chronic lymphatic leukaemia), and those based on thyroid, breast, lung, liver and skin cancers occurring more than 10 years after exposure. But, because many of these conditions are relatively common in the general population, few cases are likely to be recognized.

The central argument in radiation-linked claims has always been over the size of the estimated dose. Estimates based on film-badge records, for example, take no account of ingested and inhaled radioactivity, which were probably not negligible in tests where soldiers had to run towards a bomb crater throwing hand grenades. The Defense Nuclear Agency has attempted to fill the gap by making estimates of dose through these pathways, and these are used in a major study of mortality among atomic test veterans that is to be released later this month by the National Research Council's Medical Follow-up Agency. This study, directed by Seymour Jablon, traces mortality among 50,000 test veterans, roughly one-fifth of the total.

But even this will not be enough to satisfy veterans' organizations and the Congress. Acting again under instructions from Capitol Hill, VA is considering the feasibility of an epidemiological survey of test veterans to determine the incidence of morbidity. VA's deliberations are being overseen by Congress's Office of Technology Assessment (OTA), which will shortly publish a feasibility study on the survey. According to Michael Gough of OTA, the Defense Nuclear Agency's dose estimates are likely to be challenged by veterans' representatives, so the question boils down to by how much the dose estimates must be wrong for detectable excess cancers to occur. Whatever OTA recommends, however, the final decision on the epidemiological survey will rest with VA, which may well decide to proceed with the study in order to silence criticism.

Tim Beardsley

Environment

Japanese move to save forests

Tokyo

A GROUP consisting largely of academics has formed in Japan, the world's first society for the promotion of "Green Civilization", which it is hoped will grow into an international organization. Founder members include Kenichi Fukui, Nobel prize-winner in chemistry, and Mostafa Tolba, secretary-general of the United Nations Environment Program.

The organization has been set up largely because of concern over increasing deforestation and desertification of the Earth through poor agricultural practices and industrial pollution. The move is significant in that Japanese companies have been heavily involved in the destruction of tropical rain forest in South-East Asia. Indeed, the nation was dubbed an "ecological menace" in a report adopted last month by the environment commission of the European Communities for "pillaging natural resources in the most irresponsible way possible, and ravaging tropical rain forests for hard woods".

During Japan's "high-growth" phase, huge tracts of land were stripped of all vegetation in many parts of South-East Asia to provide building materials and pulp for Japan; although direct involvement by Japanese companies in the worst of these practices has now lessened, the amount of tropical forest lost each year has increased and Japan remains the major Asian market. The Green Civilization Society says it intends to try to put the ecological point of view over to industrial companies involved in logging but it is clearly not about to become a grass roots political pressure group.

Unlike Friends of the Earth, much of whose ideology it appears to share, the Green Civilization Society is to be a more academic body. It is believed that by building an interdisciplinary academic movement — for example of ecologists, biotechnologists and forestry researchers — more rational and less destructive means of forest management could be found that could be incorporated into recommendations to the government. Within Japan itself, forestry is regulated by laws devised in the nineteenth century which were meant to provide a balance between exploitation and regeneration of the forest. These laws are no longer effective but will only be changed, Green Civilization members say, by a joint effort between ecologists, forestry researchers and lawyers.

The society has now around a hundred members, most of them drawn from the universities. At the inaugural meeting last week, Seiji Kaya, former president of the Tokyo University, was elected chairman and Michio Tsutsui, professor of forestry at Tokyo University, vice-chairman. So far, funds are being raised mainly from individuals, with some contributions from industry. One hope is that enough money can be raised to provide a "Green Nobel Prize". The establishment of the new group coincides with the designation of this year as International Forestation Year by the United Nations.

Alun Anderson

Japanese tokamak

Plasma now in production

Tokyo

PLASMA was successfully produced in Japan's giant JT-60 tokamak fusion machine for the first time last week. Although this milestone has long since been passed by both of JT-60's competitors — the Tokamak Fusion Test Reactor (TFTR) at Princeton in the United States and the Joint European Torus (JET) at Culham in England — any of the three might be first sometime around 1987 to reach the immediate goal of break-even.

JT-60 scientists were clearly very pleased at how smoothly the tokamak had run. Operation began in April and produced plasma exactly as expected almost immediately. The machine was then run in a discharge cleaning mode to remove impurities from the inner sides of the vacuum chamber and shut down for the installation of diagnostic apparatus which will be used to monitor plasma parameters in the next phase of development.

In the first test run, ohmic heating alone was used and temperatures in the 100-200,000°C range achieved for around 80 milliseconds — a long way from the 100 million degrees and 1-second confinement needed to achieve the break-even condition when the reactor produces as much energy as it consumes. But to achieve that goal, JT-60 will not be following quite the same route as either TFTR or JET.

Unlike either of its competitors, JT-60 will use radio-frequency waves to drive current in the plasma. In the other designs, currents are induced to flow in the plasma by a set of coils around the tokamak that act as the primary winding of a d.c. transformer — the plasma itself acts as the secondary winding. But such a system allows only pulses of current to be produced, for in a d.c. transformer the current in the primary would have to increase indefinitely to continue to drive current in the secondary, a clearly impossible condition. Driving the current by radio-frequency waves, however, allows operation to continue as long as needed as electrons are pushed along the magnetic field by transfer of the waves' momentum.

The JT-60 team is also proud of the neutral beam injector equipment that will be used to heat the plasma in the future. All three tokamaks are using neutral beams but, according to Masaji Yoshikawa, director of Large Tokamak Research at JT-60, the Japanese designs are at least a year ahead of the others in their technical development.

JT-60 also incorporates a unique method of dealing with impurities — metal atoms or gas molecules that may be knocked out of the tokamak inner wall by the plasma — in its magnetic limiter or divertor, and if it works as predicted, will provide a crucial advance towards practical fusion. The idea is to give the plasma in the tokamak a figure of eight, rather than circular, cross-section, and to keep impurities away from the main body of the plasma in the upper "circle" of the figure of eight.

JT-60 is now expected to be run until the end of June with ohmic heating and then shut down for six months while both neutral beam and radio-frequency heating systems are installed. Next year, power will be put into the plasma from all the systems and, if all goes well, break-even will be reached in the winter of 1987.

All these experiments are to be run with a hydrogen-deuterium plasma; there is no intention to switch to the deuterium-tritium plasma that would be used in a commercial reactor and will be the subject of second-stage experiments for both TFTR and JET. Neutrons produced in the latter reaction are very energetic and make the whole tokamak radioactive; expensive and difficult remote handling techniques thus become essential.

The Japanese are leaving this stage to their next reactor, the fusion experimental reactor, which will follow JT-60. That is, rather, if some form of massive international effort does not follow on from the three projects now running. Increased costs are making the advantages of international cooperation more and more obvious and recent talks have produced hopes that it might eventually take place.

Alun Anderson

Hungarian education

New law spawns discontent

HUNGARY's new education law, passed last month, lays special stress on the unity of education and research, and the need to provide more flexible forms of post-university training, according to the Minister of Education and Culture, Mr Bela Koepeczi. Presenting the bill to the National Assembly (Parliament), Koepeczi said that although it is not the task of the law to lay down specific conditions for higher and postgraduate education, the new law makes it clear that the provision of proper material conditions in the education sector is the duty of the government. And the government, he assured the assembly, has undertaken to continue its investment in the reconstruction of the universities and to improve the supply of equipment and other necessities for university-based research.

The new law, which replaces the existing legislation of 1961, covers the whole educational process from kindergarten to postgraduate and is intended not as a radical reform but as a gradual process of development, Koepeczi explained. The prospect of new legislation, however, triggered a debate in the universities on academic autonomy, and, in particular, the allocation of senior appointments. A number of academics, Koepeczi said, have gone so far as to press for "decentralization". This term is at present used in Hungarian political circles to mean the reform of 1982, by which industrial enterprises elect their own managers (choosing from at least two candidates). In other words, these scholars advocate that the universities should be allowed to elect their own rectors and senior administrators.

This, Koepeczi says, is not a true "materialization of democracy" since the appointments in question are the concern not only of the institutions individually, but also of the wider scientific and professional community. Although, therefore, the new law gives the universities considerably more control over syllabuses, teaching methods and day-to-day administration, senior appointments will still be the concern of the government or of the relevant minister. "Democratization" will continue to be ensured by the government or minister consulting with the relevant committees of the Hungarian Academy of Sciences, which thus serve as a kind of watch-dog for scientific public opinion.

Since, in Hungary, members and researchers from the Academy of Sciences frequently simultaneously hold university posts, the system has so far worked reasonably well. The "decentralization" lobby, therefore, seems to have based its stand rather on a matter of principle than on the need to reform any current shortcomings.

This inevitably evokes the question whether the "decentralizers" have not, to some extent, been influenced by the present endeavours of the Polish universities to retain the autonomy won in 1980-1981, in which the right of the universities to elect their own rectors and deans is one of the most burning issues.

Naturally, Mr Koepeczi made no reference to the Polish situation in his address to the National Assembly. The new law was presented as a purely Hungarian concern. Nevertheless, on one point at least, those responsible for drafting the law seem to have had an eye on developments in Poland. In drawing up the bill, the government, Koepeczi said, considered it extremely important to provide a legal background to the operation of student "self-government bodies"; an issue that has so far aroused no great controversy in Hungary, but which in Poland is at present a major point of confrontation between the vast majority of the student body (over 90 per cent in some universities) and the governmental Committee for Social and Political Questions which seeks to reduce the self-government committees to a mere adjunct of the Party youth movement.

Vera Rich

The embryo's right to protection

SIR — The opponents of all human embryo research seem to have shifted ground, from the meaningless proposition "life begins at fertilization" to the argument that a human embryo is inherently entitled to full human rights by dint of genetic uniqueness and potential. R. Watson (*Nature* 7 March, p. 10) goes so far as to assert that there can be "no objections" to the idea that a unique potential individual is created "at fertilization".

Leaving aside the difficult questions of when fertilization can be said to have occurred (at sperm penetration, at pronuclear formation, or at activation of the paternal genome some hours or days later?), and of clear differences in potential between embryos, the assertion does not bear close scrutiny. If anything, the beginnings of genetic uniqueness occur in the forming gametes, during the second meiotic division. Here genetic information is exchanged between pairs of homologous chromosomes (the chromosomes of the embryo's *grandparents*, in fact): in the egg this will have occurred several hours, and in the spermatozoon, several weeks before fertilization. If the embryo is to be granted "rights" on the grounds of genetic uniqueness and potential, how can we logically withhold similar "rights" from the gametes? What of the "rights" of the polar bodies, so wantonly cast aside during oogenesis, and yet clearly of human origin and containing genetic information capable, if suitably nurtured and combined, of potential humanity? One could push the argument for "rights" back still further, to parental embryos, when the germ cell lineage separates from that of the somatic tissues: who can deny that to destroy a primordial germ cell prevents it from fulfilling its life-transmitting potential?

My purpose is not to argue that embryo research should not be constrained, nor that the embryo is worthless. Rather, it is to point out, yet again, that the transmission of life and of human identity forms a continuum, and that there is no point at which human potential suddenly jumps into existence. Setting such arbitrary points may give us the warm glow of moral certainty, but the exercise is not supported by the real complexity of biological systems.

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SIR — The legal and moral status of embryos and the way in which they are handled are the subjects of a complicated debate. R. Watson (*Nature* 7 March, p. 10) seeks to simplify the topic by referring to a clear topological event, fertilization, "at which a unique member of our species is created". One problem with such an oversimplification is that it may be applied to give silly conclusions.

I contend that each member of a pair of identical twins is a unique member of our species, even though both result from one fertilization. I believe that it is (and should be) illegal to murder one, or to use him or her destructively in medical research. I reach the decision that it is morally wrong to kill a member of a pair of identical twins by deciding that each is a separate, thinking, feeling person, and not by some simple topological argument about the moment when a genome is created.

Of course one might argue that any mitosis (like that which produces identical twins) is an event "at which a unique potential member of our species is created", since it is probably possible to freeze any human diploid cell, await the development of practical human cloning by nucleus transfer, and make an individual of that cell. The killing of single human cells is neither legally nor normally the equivalent of homicide nor, I contend, should it be. This line of argument, too, is an oversimplification.

Whether the benefits of research on embryos (or of abortions) outweigh the disadvantages of destroying embryos is a complex and difficult question. We should address that complex question without looking for quick and easy definitions of what is a person.

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Genes and intelligence

SIR — Summarized briefly, John Hartung's hypothesis (*Nature* **311**, 515; 1984) is that ill health caused by genetic factors may indirectly affect the intelligence. The principle point of my letter (*Nature* **313**, 425; 1985) was to question that *a priori* assumptions that intelligence and school attendance are linked and that hay fever impairs intellectual performance. Both these assumptions may be true, but they are unproven. Since Dr Hartung's hypothesis depends directly on the validity of these assumptions and since they may be tested empirically, it is essential to his argument that they should be examined experimentally.

His reply (*Nature* **314**, 398; 1985) deals largely with the viscosity of gases. Of course he is right that the viscosity of gases increases with temperature; he was wrong in his original letter to suggest that humidity also increases viscosity. The problem is, however, far more complex. For instance, the air flow in the nose is turbulent and resistance will therefore depend on gas density, being in general terms less in humid air than in dry air. The shape of the nose is obviously not wholly dependent on climate. This is illustrated by the observation that the inhabitants of the dry desert regions of North Africa and the Middle East, where natural conditions provide the most viscous air to be found on Earth, are not noted for short noses with wide nostrils.

I agree with Dr Hartung that the search for genetic differences that may also affect intelligence is a proper subject for scientific enquiry. My familial credentials are even more extensive than his, since I can claim to be the son and nephew of both men and women who served in the fight against Hitler's Germany. I think, though, that Dr Hartung would agree with me that neither moral nor political rectitude are genetically determined characteristics. The assertion of familial virtue is no guarantee of the value of his opinions or of mine.

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Goldfarb's letter

SIR — In your latest article on the Goldfarb case (*Nature* 28 March, p.307), my name is mentioned as follows: "During his recent conversation with KGB officer Gusev, Dr Goldfarb was confronted with a copy of a letter he had written to Professor Elie Wollman of the Institut Pasteur in Paris, supporting the idea of the moratorium." Gusev said that the letter might constitute a case of 'anti-Soviet propaganda' and made Dr Goldfarb sign a document to that effect".

I wish to make a few points clear.

- I have known David Goldfarb for many years, because he was a pioneer in introducing bacteriophage and bacterial genetics research in the Soviet Union. I consider myself a close friend of this exceptional man, both professionally and personally.
- Our correspondence is brief, containing only personal news of the usual kind, and neither him nor I ever approached in writing any subject that could be interpreted as being controversial in the broad sense of the word.
- I have received no letter from Goldfarb for about a year and was astonished at not even getting a Christmas card, although I wrote to him at the New Year.
- I never received the letter mentioned in your article. There are therefore three possibilities:

(a) such a letter does not exist and has never existed;
(b) the letter exists and has been diverted by the Soviet secret police;
(c) a fake letter has been manufactured.

Hypothesis (b) seems to me to be excluded for the reasons given above.

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Black hole at the galactic centre

The latest accurate measurements of the size of the galactic core at centimetre radio-wavelengths leave no reasonable escape from the conclusion that there is a black hole at the centre.

WE HAD best learn to live with the idea that there is a black hole at the centre of our Galaxy. That is the implication of the article by K.Y. Lo *et al.* on p.124 of this issue. Perceptive readers will think the paper, which is the culmination of eight years of observation of the galactic centre with coordinated radiotelescopes separated by great distances, is over-modest. Others may think it over-cautious. The phrase "black hole" appears only once, and then as a quotation from an article by Lynden-Bell and Rees, who first floated the idea.

The new development is essentially nothing but a more accurate measurement of the diameter of the radio source known, for the past decade and more, to be located at the galactic centre. The result is startling. At the shorter of the two radio-wavelengths (1.35 cm) at which measurements have been made, the angular diameter of the source is merely 0.002 second of arc (2.1 milli-arc seconds), which at the distance of the galactic centre corresponds to a physical diameter of about 20 astronomical units, the size of the Solar System within the orbit of Saturn. At the longer wavelength (3.6 cm), the source at the centre of the Galaxy appears to be shaped like an ellipsoid with a long axis lying only 8 degrees away from the rotational axis of the Galaxy as a whole.

The case for supposing that there is a black hole at the centre rests almost entirely on the difficulty of accounting for such a prolific source of radio emission in any other way. Lo *et al.* argue that the supposition that the galactic centre might be the site of a rapid burst of star formation, tenable when the limits on the size of the central source were more than a hundred times greater than they have now become, plainly cannot be accommodated within a region no bigger than the orbit of Saturn. A stellar system which is a powerful source of radio emission might fill the bill, but only artificially; such systems are short-lived compared with the lifetime of the Galaxy. Black holes, on the other hand, endure (unless they are very small compared with the masses of typical stars) and even grow more massive with the passage of time. By default, the presence of a black hole at the galactic centre emerges as the simplest explanation of the new observation.

But is it not disconcerting that such an important and interesting conclusion should rest on such a negative argument? That is a natural but not a particularly pointed question. Black holes as such are by their nature invisible, although that does not apply to whatever matter may be dragged by gravitation into such an entity. Captured material, whatever its chemical composition, will be unspecifically ionized and will yield synchrotron radiation if there are magnetic fields to play with. Visible radiation will however be absorbed (or converted into low-frequency radiation) by the molecular and dust clouds lying around the galactic centre. Infrared observations are also limited in this way, as well as by the mismatch between the resolution of the telescopes for the time being available and the angular diameter of the source. This is why long-baseline radio-interferometry is, for the time being, the best hope for an unambiguous description of the object at the galactic centre.

In the circumstances, the temperature of the source estimated by Lo *et al.* means very little, for the radiation distribution is clearly very different from that of a black body. But the temperature (100 million K) is an accurate measure of the intensity of the radio-emission from the central object and its surroundings. The variation of the apparent angular size of the object with the wavelength of observation is surprisingly tidy — a simple proportionality to the square of the wavelength. The interaction of radiation with electrons is implicated in some way, but just what form this takes will depend, Lo *et al.* say, on the construction of a brightness map for the source.

There is little that can at present be said about the mass of the black hole at the centre of the Galaxy, and indeed a mere measurement of the geometrical size of the region from which radiation is emitted can be neither here nor there. But measurements such as have been attempted in the past few years of the motion of objects, stars and clouds in the neighbourhood of the centre should eventually help to define the mass of the object. As things are, systematic studies of the velocity distribution of nearby objects are inconclusive and open to conflicting interpretations. But presumably it is a matter of time spent on observations before something can be said about the variation with distance of angular velocities of nearby objects about the centre of the Galaxy, from which it should be possible to infer what mass there is at the centre.

Meanwhile, studies of this kind are for some time likely to be more profitable when applied to other galaxies than this. So much should be clear from the account two weeks ago by C.M. Gaskell (*Nature* **314**, 672; 1985) of the recent demonstration by J.L. Tonry that there is a black hole at the centre of the dwarf galaxy M32, a satellite of M31 (Andromeda). The technique is to determine the non-radial velocity of central objects as a function of radial distance from the centre, which should be a measure of the mass lying within that distance. Gaskell explained some of the pitfalls in this process, and in particular the failure by these means to demonstrate the presence of a black hole in M87.

The implications of these developments for the present understanding of galactic evolution are clearly important. Any galaxy, if left alone, should eventually collapse under its own weight after several repetitions of the cycle of stellar nuclear synthesis. So there should be no surprise that the end point of galactic evolution should often be a black hole, one whose mass is not very different from that of the original galaxy. That black holes begin forming (at least in M32 and in this Galaxy) is not surprising either, but it is helpful that at least two cases of this phenomenon are now known. To generalize from two cases, one an elliptical galaxy (M32) and one a spiral (this), may seem rash, but it would be no great upset for the applecart if most galaxies turn out to have a black hole at the centre.

As is now generally understood, this seems to be the case for active galaxies, quasars spectacularly but other active galaxies such as Seyfert galaxies as well. But it remains to be told whether the difference between such a galaxy and this, where the central black hole is only a modest source of radiation, lies simply in the amount of material accessible to the central attracting source or whether there is something extra special about quasars and similar galaxies. The importance of answering that question is that it will decide whether quasars are exceptional galaxies rather than ordinary galaxies in a particular (and brief) stage in their evolution.

Meanwhile, this development has no immediate bearing on the grander cosmological question of whether there is enough hidden mass to close the Universe, or eventually bring the present expansion to a halt. The mass of the black hole in M32 is estimated at 5 million solar masses, a tiny fraction of the whole. The black hole at the centre of this Galaxy, whatever its mass, is unlikely to be much bigger. So the missing mass that matters must still be sought elsewhere.

John Maddox

Neurobiology

Why flying locusts do not crash

from Jonathan Bacon

LOCUSTS manage to remain aloft for long periods while avoiding crashing into obstacles, their companions in the swarm, or the ground. Studying how they achieve this has kept some biologists busy for almost 40 years but only comparatively recently has the technology been available to examine the neuronal interactions that control these manoeuvres. Intracellular recordings can now be obtained from restrained locusts that still produce the motor rhythm of flight. A paper on page 142 of this issue[1] describes how sensory interneurones that convey information about flight-course deviation interact with the neurones of the flight motor to mediate steering responses.

When a locust is attached to a support so that the legs do not contact the ground, it will 'fly' for as long as a windstream is blown on its head[2]. The early observations were made in the Sherringtonian climate of the time, when it was thought that sensory input was needed to stimulate each reflex burst of motor activity. In terms of the locust flight motor, this implies that each beat of the wing would produce the necessary feedback to stimulate the next wing beat. In 1961, D.M. Wilson performed one of the classical experiments that was to change this way of thinking[3]. By systematically removing the wings, the top half of the thorax, the abdomen and even the head, he showed that what remained of the nervous system (the thoracic nerve centres, or ganglia that contain the flight motor neurones) was still able to produce a burst of motor activity in response to a puff of wind or an electric shock, although no rhythmic sensory feedback remained. This demonstrated irrefutably that the animal can produce a patterned motor output in the absence of patterned sensory input and established the locust flight motor as a classical example of a central pattern generator (CPG).

Although sensory inputs are patently not required for the locust flight CPG to function in the laboratory, they are necessary in proper flight where turbulence is encountered and the ground and other obstacles are to be avoided. Two sensory cues that help the animal to fly a straight, level course are wind direction and the position of the horizon. Wind direction is monitored by banks of tiny hairs on the head, which the animal uses to determine the attitude of its flying body with respect to the air mass. By constantly making tiny adjustments to its direction of flight through changes in wing stroke, the animal strives to maintain a symmetrical air flow over its head[2,4].

Apparent changes in the slope of the horizon are detected by the large compound eyes and the ocelli, simple light-detecting organs on the head. If the horizon suddenly moves upwards in the animal's field of view, the wing beats are modified to correct the dive. Similarly, L.J. Goodman reported that when the horizon appears to slope to the right, the wing beats are adjusted until the horizon again appears horizontal[5]. The horizon stimulus is very powerful — a locust flying in a device that allows it to rotate freely about its longitudinal axis can be made to fly upside down by inverting the artificial horizon[5].

Clearly, if the neurones of the thoracic ganglia responsible for producing the correctional changes in the motor output to the wings are to react swiftly to deviations in the flight path, they must be constantly appraised of, among other things, wind direction and horizon position. Several descending sensory interneurones that run from the head ganglion to the thoracic ganglia are candidates for the fast sensory pathways[6,7]. Early attempts to investigate the cellular basis of steering behaviour sought an explanation in terms of direct connections between these descending interneurones and flight motor neurones, but the synaptic potentials recorded in the motor neurones are very small (and variable) in relation to the massive membrane potential oscillations seen in flight motor neurones in real flight[8]. Although direct synaptic connections between descending interneurones and flight motor neurones certainly exist, they are probably too puny to mediate the rapid steering responses.

Progress in analysing the steering reactions was hampered because the flight motor neurones were the only components of the neural network in the thoracic ganglia that could be identified with certainty. Because sensory neurones are easy to stimulate and muscles easy to record, most workers concentrated on the sensory influences on the flight motor rather than on the working of the motor itself. This contrasts with work on locomotor patterns in soft-bodied invertebrates, such as the leech and the mollusc, where the sensory apparatus is difficult to stimulate precisely and muscles are more difficult to record but the central neurones of the CPG are easy to see and impale.

Further progress could not be made unless the rhythmically active elements of the flight CPG, the cells that must have been active in Wilson's original deafferented preparation[3], could be monitored directly. The breakthrough came when R.M. Robertson and K.G. Pearson developed a physiological preparation in which intracellular recordings from thoracic interneurones could be obtained during a burst of activity in the flight CPG[9]. With much painstaking probing of the neuropile, they have identified sets of interneurones that show rhythmic activity at different phases of the flight cycle, of which some are premotor and some receive sensory inputs[10]. A third type belongs to the flight CPG — current injected into such a cell will reset the whole flight rhythm[10]. Though much work remains to be done, it seems that a description of the neuronal interactions producing the locust flight CPG is likely to emerge from studying the synaptic interactions between some of these thoracic interneurones[11].

Reichert *et al.* have now modified this preparation for investigating the neuronal control of flight steering[1]. They provide the locust with a combined horizon and wind stimulus that can be moved to simulate the pitches, rolls and yaws that the animal would experience in flight. The authors reliably identify three pairs of descending interneurones that convey sensory information from the head to the flight circuits of the thoracic ganglia. Each neurone responds to different combinations of wind and visual stimuli with an output that has specific directional characteristics, termed by the authors the "emergent modality" of the neurone.

Even more interesting is the interaction of the output from these deviation detector neurones with thoracic flight neurones. Essentially, Reichert and his colleagues have repeated Goodman's experiment but with intracellular monitoring of interneuronal activity. If the animal could break free of the pins holding it stable for intracellular recording, the changes in flight motor output would enable it to correct for the movement of the horizon stimulus.

The descending neurones make only direct connections with flight interneurones but these have strong inputs to a rhythmically active set of thoracic interneurones. This set also receives a large input from the flight CPG and is presynaptic to flight motorneurones. They therefore effectively act to gate the tonic output of the descending sensory interneurones, so that it is transformed into a phasic input to the flight motor.

Although this newly described mechanism is important because it ensures that the signals from the deviation detectors are delivered to the relevant motor neurones only in their active phase, it is not certain that the information conveyed by the deviation detectors is "intrinsically phase in-

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dependent" in real flight. At least one pair of wind-sensitive interneurones in the locust is known to be phasically active in flight because the animal's own wing movements inevitably produce phasic wind turbulences around the head[12]. Since the three deviation detectors are also wind sensitive, they too may well be modulated at the flight frequency and so the information they carry about horizon movement would already be phasically modulated. However, this modulation would be lost in a fixed preparation.

While work on this kind of preparation is the only way to make progress in understanding flight stabilization behaviour, it does suffer the limitations inherent in immobilizing an animal to study locomotor behaviour. Inevitably, the feedback from the environment is very reduced or completely missing. Any study in neuroethology is bound to be an exercise in compromise because intracellular recordings cannot be made in freely behaving animals and intracellular prepartions cannot freely behave. Nevertheless, this work on restrained flying preparations is an important step in understanding the neural circuitry that converts sensory signals to motor output. □

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Superconductors

Induction of superconductivity by applied magnetic fields

from M. Brian Maple

PHYSICISTS have been fascinated with the interplay between superconductivity and magnetism for nearly three decades, during which period many new and interesting phenomena have been reported (see ref. 1). One of the latest, reported by H.W. Meul and colleagues[2], is the induction by a high magnetic field of superconductivity in europium-containing metallic compounds of the type $Eu_xSn_{1-x}Mo_6S_8$, where x ranges from 0.7 to 0.8. This is a remarkable observation in view of the fact that applied magnetic fields generally destroy superconductivity. It is perhaps equally remarkable that the mechanism apparently inducing the superconductivity, and known as the exchange field compensation effect, was originally suggested more than twenty years ago by V. Jaccarino and M. Peter[3].

Despite previous indications of S-N-S-N (S, superconducting; N, normal) behaviour in related compounds in high magnetic fields[4,5], it has not been possible to establish the occurrence of magnetic field-induced superconductivity (MFIS) because of the rather broad transitions encountered. Meul *et al.* overcame this problem when they found that the addition of small amounts of Br or Se to the compounds sharpens the transitions. At the same time, they adjusted the superconducting transition temperature, T_c, to within the narrow range of values required to observe MFIS, which was measured in terms of the electrical resistance, R, which has a finite value in the normal state and vanishes in the superconducting state. The inset in Fig. 1 shows normalized electrical resistance (R/R_N) versus applied magnetic field (H) at several temperatures for one of these compounds — $Eu_{0.75}Sn_{0.25}Mo_6S_{7.2}Se_{0.8}$. The data at the lowest temperature (0.37 K) clearly reveal a succession of transitions with increasing magnetic field — from superconducting to normal, to superconducting again, and finally, back to normal (S-N-S-N). From a series of measurements of R/R_N versus H at various temperatures, a phase diagram in the H-T plane was constructed (Fig. 1); two separate domains of superconductivity are found, one at low fields and another at high fields, whereas in a conventional superconductor only one domain of superconductivity at low fields is present.

In order to understand the sequence of superconducting to normal transitions observed, the nature of the superconducting material must be considered. The $Eu_xSn_{1-x}Mo_6S_8$ compounds are type II superconductors and hence an applied magnetic field can penetrate into their interior provided it is between the lower critical field value H_{c1} and the upper critical field value H_{c2}, above which superconductivity is destroyed. Under normal circumstances, the value of H_{c2} would be comparable with the paramagnetic limiting field value H_p. However, the $Eu_xSn_{1-x}Mo_6S_8$ compounds have magnetic moments associated with the divalent europium ions. Coupling between these magnetic moments and the conduction electron spins generates an 'effective' magnetic field, called the exchange field H_J, that acts on the conduction electron spins in the same manner as an applied magnetic field.

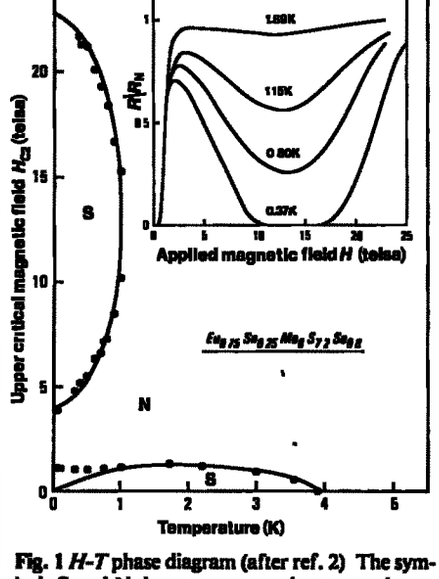


Fig. 1 H-T phase diagram (after ref. 2) The symbols S and N denote superconducting and normal regions, respectively. The solid circles represent the H_{c2} measurements of Meul *et al.*[2], the solid lines are the boundaries predicted by theory. Normalized electrical resistance, R/R_N, versus applied magnetic field data at several temperatures are shown in the inset.

If the sign of the coupling between the Eu magnetic moments and the conduction electron spins is negative, the direction of H will be opposite to that of H_J, that is, the effect of H_J will be 'compensated' by H (see inset to Fig. 2). The net magnetic field H_T is then given by $H_T = H - [H_J]$. The sequence of S-N-S-N transitions shown in the inset to Fig. 1 can be explained in terms of the relationship between H_T and H_p as illustrated schematically in Fig. 2. The upper and lower curves show the applied field H and the exchange field H_J respectively. Summation of these yields the net magnetic field H_T, and it is the variation in this with respect to the value of H_p that determines the superconducting behaviour of the material.

In zero applied field, the compound is superconducting. As the applied magnetic field is increased, the Eu magnetic moments become aligned in the direction of the field at a relatively low H-value and $|H_J|$ attains its maximum value $|H_J^{max}|$ of 30-50 tesla. Consequently, provided $|H_J|$ is greater than H_p, the magnitude of H_T (which is now negative) can become larger than H_p and drive the material into the normal state. As the applied magnetic field is increased further, the net magnetic field starts to decrease in magnitude, until its magnitude falls below H_p when the compound once more becomes superconducting. Thereafter, H_T increases linearly with H, vanishing and then changing sign at $H = |H_J^{max}|$ where H_J is completely compensated, until H_T (now positive) exceeds H_p, when the system once again becomes normal. Because the value of the exchange field can be rather large, the compensation effect can take place at very high magnetic fields.

In actuality, the second transition from superconductivity to normal state will also occur if H surpasses the orbital critical field H^*_{c2} of the compound. Thus, in order to observe MFIS, it is important that H^*_{c2}, which can also limit the value of the upper critical field H_{c2}, is large enough to allow superconductivity to occur in this magnetic field range (that is, $H^*_{c2} > |H_J^{max}| - H_p$), since the orbital effects are not compensated by the Jaccarino-Peter mechanism. The compensation of the exchange field by the applied magnetic field in several

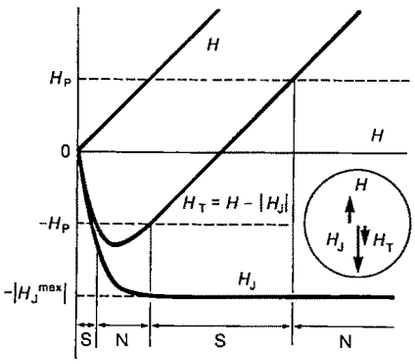


Fig. 2 Schematic diagram of the S-N-S-N transition sequence in an applied magnetic field H. H_T is the net magnetic field acting on the conduction electron spins, H_J is the exchange field produced by the Eu magnetic moments, and H_p is the paramagnetic limiting field. The material is superconducting (S) when $|H_T| < H_p$ and normal (N) when $|H_T > H_p$.

$Eu_xM_{1-x}Mo_6S_8$ systems (where M is a metal such as tin, lead, lanthanum[7] or ytterbium[8]) and in $EuMo_6S_8$ under pressure[9], had been inferred from an enhancement and anomalous temperature dependence of H_{c2} in these materials. Independent evidence for a negative coupling between the Eu magnetic moments and the conduction electron spins has emerged from the nuclear magnetic resonance and Mössbauer studies of Fradin *et al.*[10], electron paramagnetic resonance measurements by Odermatt[11] and the band structure calculations of Freeman and Jarlborg[12]. The phenomenon of MFIS was thus anticipated, but samples of the necessary high quality, in which the candidates for MFIS were satisfied, have not been available until the present study by Meul *et al*[2].

Following the initial suggestion by Jaccarino and Peter that MFIS could occur in a ferromagnet due to exchange field compensation, several theoretical investigations of MFIS in paramagnetic systems were carried out[13-15]. One model, based on the theory of type II superconductivity including the effect of the exchange field[14], was used by Meul *et al.* to analyse their $H_{c2}(T)$ measurements on the $Eu_xSn_{1-x}Mo_6S_8$ system. The calculated boundaries of the two superconducting domains observed for the compound $Eu_{0.75}Sn_{0.75}Mo_6S_{7.2}Se_{0.8}$ are indicated by the solid lines in Fig. 1 where the parameters of the theory (see ref. 2) have been adjusted to give the best fit to the data. The excellent description of the H-T superconducting phase boundaries shown in Fig. 1 within the context of the Jaccarino-Peter compensation mechanism can be regarded as singularly impressive. However, several important issues remain to be resolved before a complete understanding of this phenomenon can be attained. The behaviour of the superconducting energy gap and the flux-line lattice should be characterized, and the thermodynamic order of the magnetic field-induced transition needs to be estalished. It would, of course, be interesting to observe MFIS in other materials; preliminary evidence indicates that it may occur in the $Eu_xHo_{1-x}Mo_6S_8$ system[16].

Twenty-two years after its prediction by Jaccarino and Peter, MFIS due to the exchange field compensation effect seems to have been verified by Meul *et al.* on a paramagnetic system. But Jaccarino and Peter originally suggested MFIS in a weakly ferromagnetic material, assuming that it would be superconducting in the absence of ferromagnetic ordering. MFIS in a ferromagnet remains to be discovered. □

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Hybrid oncogenes

A multi-purpose tool for studying multi-step processes

from Peter W. J. Rigby

INTRODUCTION of exogenous DNA into the mouse germ line is a well-established technique that has been applied to many biological problems. Most previous work has concentrated on the analysis of DNA sequences that mediate tissue- and cell-type-specific gene expression[1-7], but more complex biological phenomena can also be studied using this method[8,9]. Insertion of oncogenes, of either viral or cellular origin, into the germ line provides an *in vivo* system in which the mechanism of tumorigenesis can be analysed and is also likely to facilitate the study of development, differentation and cellular interaction. The paper by D. Hanahan on page 115 of this issue[10] describes the most interesting application of this approach so far reported.

Hanahan has constructed hybrid oncogenes in which the coding sequences of simian virus 40 (SV40) large-T antigen — a nuclear DNA-binding protein that can transform various cell types, including primary cells[11] — is placed under the control of DNA from the upstream region of the rat insulin gene[10]. Transfection experiments using cultured cells have shown that this region contains a cell-type-specific transcriptional control element that functions in insulinoma cells but not in fibroblasts[12]. Hanahan has used two types of construct: in one, the insulin upstream sequences are oriented so that the insulin promoter drives transcription of the T-antigen gene; in the other, the upstream sequences have the reverse orientation and so transcription of the viral oncogene cannot be driven by the conventional insulin promoter.

Lines of mice carrying each of these constructs in their germ line have been established. The mice develop normally but die prematurely at around nine to twelve weeks of age; before death their behaviour is normal. Immunological studies show that expression of large-T antigen is confined to the β-cells of the endocrine pancreas, indicating that the upstream sequences of the insulin gene are sufficient to confer cell-type-specific expression *in vivo* as well as *in vitro*. Proper expression occurs in mice carrying either construct; in the case of the inverted control region it is not known where the messenger RNA initiates.

Histopathological and immunocytochemical analyses show that in young mice the islets of Langerhans are of normal size but that all of the β-cells express large-T antigen whereas the α- and δ-cells, which synthesize glucagon and somatostatin, respectively, are reduced in number and are disordered. With increasing age most of the islets become hyperplastic and are comprised almost entirely of β-cells expressing large-T antigen. Finally, a small pecentage of the hyperplastic islets give rise to well-vascularized β-cell tumours which do not metastasize.

Two other groups have recently developed lines of transgenic mice carrying oncogenes. In one study, the oncogene was again that encoding SV40 large-T antigen, but in this case expression was controlled by the virus's own promoter/enhancer region[13]. The mice develop normally but again premature death occurs, mainly as the result of tumours in a particular region of the brain, the choroid plexus. The other group constructed a hybrid oncogene in which murine c-*myc* encoding sequences were placed under the control of the promoter/enhancer in the long terminal repeat of the hormone-responsive mouse mammary tumour virus[14]. They observed expression of the hybrid oncogene in various tissues including the mammary gland and, most commonly, the salivary gland. In two

lines of mice, adenocarcinomas of mammary origin appeared in multiparous females at the time of pregnancy. The hormonal induction of expression that occurs in the lactating mammary gland is presumably prerequisite for the accumulation of sufficient levels of the c-*myc* oncogene product to induce the first step of tumorigenesis.

Although the *in vitro* experiments that suggest the existence of at least two classes of oncogene required for the transformation of primary cells[11-20] have aroused much interest, it is important to extend these findings to *in vivo* studies. Hanahan's results show that SV40 large-T antigen induces proliferation of the β-cells and the subsequent hyperplasia but suggest strongly that this is not sufficient to induce a tumour, otherwise all the hyperplastic islets would progress to tumours[10]. Similarly, tumours arise in only a few of the mammary glands of the mice carrying mammary tumour virus/c-*myc* oncogene. In these situations some second event must be required for tumorigenesis. An *in vivo* system in which the multi-step nature of tumorigenesis can be analysed has thus been established.

In vitro experiments are severely limited by the availability of suitable lines of cultured cells and by transfection efficiencies, whereas *in vivo* experiments allow expression of the oncogene to be targeted to any cell type for which an appropriate transcriptional control sequence is available. Large-T antigen could thus be expressed in many different cell types to ask whether the second event always affects the same gene or whether there is cell-type specifity for the subsequent events(s). Conversely, the insulin upstream region could be used to target the expression of other oncogenes to the β-cells to ask, first, whether hyperplasia and subsequent tumorigenesis are induced and, second, whether the subsequent event(s) is general, or specific to the initiating oncogene. Finally, two or more oncogenes could be targeted simultaneously to the same cell type. Such studies will greatly advance our understanding of the multi-step nature of oncogenesis. Equally important, they will enable molecular studies on tumour types for which there is no good *in vitro* model. The system should also make possible the study of processes, such as angiogenesis, metastasis and immune surveillance, that are hard to study *in vitro*.

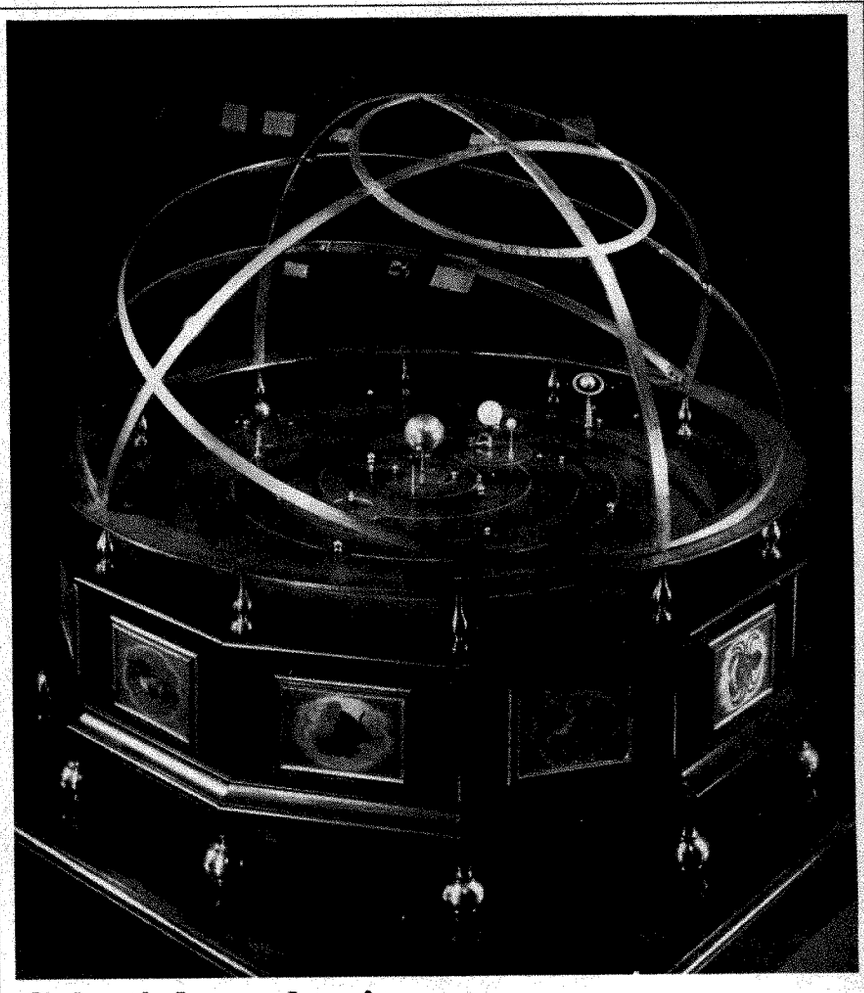
It is curious that the β-cell tumours seem not to induce an immune response. SV40 itself is an extremely weak carcinogen in mice; tumours arise very rarely and with a long latent period[21,22], presumably because the small proportion of the large-T antigen that is displayed on the surface elicits a powerful immune response.

The severe disorganization of the other cell types in the endocrine pancreas induced by β-cell proliferation and hyperplasia is also of interest. The ability to perturb the growth of one cell type in complex interacting system will allow studies of the mechanisms that normally control the orderly growth and behaviour of an organ.

One further use of this type of study derives from the ability of many oncogenes, including those discussed here, to 'immortalize' cells. Thus, it may be possible to establish cell lines derived from all tissues of mice carrying such genes in their germ line. Indeed, it has already been shown that cells can be readily grown from a number of the tissues of the SV40 transgenic mice (A.J. Levine, personal communication). Such lines have considerable practical use. Commercial manufacture of some proteins may be limited by the inability of commonly used cell types to perform some post-translational modifications. The problem would be overcome if established lines of cells normally making the protein were available. □

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Celestial mechanics

THIS fine example of an orrery, or mechanical model of the Solar System, is one of the early scientific instruments on display in the exhibition "Science and Profit in Eighteenth Century London" at the Whipple Museum of the History of Science in Cambridge until 6th December 1985. Orreries, used to teach the astronomy of the Solar System, were not made to scale but illustrate the relative positions and motions of the planets and their moons. This one, made by Geo Adams at Tycho Brahe's Head in Fleet Street, London, is calibrated for the Julian calender, which dates it before 1752. It includes Saturn with its rings and the five moons found between 1655 and 1684, but not Uranus, which was discovered only in 1781. Other instruments in the exhibition include an altazimuth theodolite, made about 1740, a compound microscope improved by tilting the limb for extra comfort, and a reflecting telescope.

Circadian rhythms

Period piece for *Drosophila*

from Kevin Moses and Michael Ashburner

NORMAL male fruit flies sing a courtship song to their prospective mates by vibrating their wings. There is a 20-50 ms interval between pulses of sound-and the length of this interval itself oscillates with a period of 50-60 s. This oscillation is under the control[1] of a genetic locus known as *period*, or *per*, which also plays a part in controlling the rhythm of locomoter activity of the flies. It is one of seven known genetic loci that determine the rhythm of eclosion, that is, emergence from the pupa, in a population of flies. The *per* locus was the first of the seven to be discovered, is still the best characterized and has recently yielded to molecular analysis by two groups, with fascinating, if not altogether consistent, results[2,3].

Genetic analysis of circadian rhythms of *Drosophila melanogaster* began with Konopka and Benzer's isolation of mutations affecting the eclosion rhythm[4]. Mutations of *per* can have a variety of effects on rhythm, which can become shorter than the normal 24 h — for example, 19 h in per^S flies; or longer — for example, 29 h in per^L flies; or arrhythmic — for example, in per^0 flies. Similarly, the 50-60 s oscillation in the period between bursts of love song in normal male flies is shortened to 40 s in per^S flies, lengthened to 80 s in per^L flies and absent in per^0 flies.

Thus, mutant alleles of *per* affect both circadian rhythms and much faster rhythmic activities. By transplanting brains between flies that differ in their genotype at the *per* locus, Handler and Konopka have shown that it is the expression of this gene in the brain that determines the fly's phenotype[5]. This suggests that the fly's behaviour is continuously affected by the expression of the *per* gene, a conclusion supported by studies with a temperature-sensitive per^0 allele (ref.6). The nature of the mutations that affect this rhythm is interesting. A complete absence of *per* function (as seen in a homozygous per^- deletion) gives an arrhythmic fly, but a partial loss of function gives a fly with a long cycle. This is the conclusion from the observation that females that are hemizygous for per^+ have a 25-h cycle.

For their molecular analysis of *per*, the groups from Rockefeller University[2] and Brandeis University[3] have used different starting points. The former cloned *per* by jumping from the previously cloned *Notch* gene, crossing the break points of a deletion that brought *Notch* just distal to *per*. From this starting point, they have characterized about 90 kilobases (kb) of contiguous DNA and by mapping the breakpoints of other chromosome aberrations to the DNA, have defined the *per* locus to a 25-kb interval. On the other hand, the group from Brandeis University microdissected polytene chromosomes to isolate DNA from band 3B1.2, which contains *per*, for cloning. Both groups have characterized transcripts in this region and have confirmed the cloning of *per* DNA by transformation.

Within the 25 kb of DNA that must, on genetic criteria, include the *per* locus, several transcripts have been identified. The two groups differ somewhat in their interpretation of the transcription data. Between the limits of two deletions that bracket the *per* gene the Brandeis group have found four transcripts, and the Rockefeller group three. Perhaps the most startling discovery by the Brandeis group is that the amount of the 0.9-kb transcript varies during the day: it is more abundant at 2 pm than 2 am. Furthermore per^0 flies have a greatly reduced amount of this transcript. The translocation *T(1;4)JC43* provides an interesting mutation of *per* because it acts as a per^0 mutation in eclosion assays but as a per^L mutation in locomotor-activity assays. This translocation has a correspondingly complex effect on transcripts from the *per* region, abolishing a 4.5-kb transcript, reducing the level of 1.1-kb transcript and producing two novel transcripts of 11.5 and 0.9 kb from the *per* region, according to the Rockefeller group[2]. The Brandeis group also finds two new transcripts (which they call 10 and 0.5 kb) and a reduction, but not a complete absence, of the 4.5- and 0.9-kb RNAs. Presumably the 0.9-kb Brandeis RNA and 1.1-kb Rockefeller RNA are the same species.

Unfortunately, the transformation data do not help to define the function of the *per* gene transcripts because the DNA used either did not include sequences that code for the cycling 0.9-kb RNA[7] or included the equivalent DNA but also the DNA that codes for the 4.5- and 1.0-kb RNAs[8]. The ambiguity results from the fact that all of the transformants remain mutant, with longer rhythms than normal. Whether this is due to a failure to obtain correct tissue-specific expression of the introduced *per* DNA, or whether the DNA lacks sequences required for normal *per* function, or is due to differences in the assay of the *per* phenotype, is unclear.

The fact that the two groups disagree as to the details of the structure and function of *per* should not detract from their achievements nor from the great interest in this gene. It is clear that we are now on the way to a molecular description of a gene whose function is required for both circadian and other rhythmic behaviours. □

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Archaeology

Using isotope chemistry to detect prehistoric diets.

from Glynn Ll. Isaac

WHEN archaeologists seek to reconstruct the subsistence systems used by prehistoric hunter-gatherers, they face two particularly intractable problems. The first is to quantify the relative importance of plants versus animals in ancient diets, and the second is that of determining patterns of seasonal movements. Studies on bone composition have the potential to solve the second type of problem, as illustrated by the paper of Judith Sealey and Nikolaas van der Merw on page 138 of this issue[1].

In sites where organic remains are relatively well preserved, it is often possible, by patient study of the animal bones and shells, to rank the relative importance of different animal species as a source of food. Sometimes the same can be done for carbonized plant remains. However, it is seldom possible to gauge the proportions of plants and animals relative to one another. Similarly, while particular remains can sometimes allow archaeologists to say that a site was occupied during one season, it is often much harder to be sure that the site was *not* occupied in other seasons. So, having found a few sites with complementary seasons of apparent occupation, is it safe to link up these observations and infer a patterned seasonal round of movements by the occupants?

During the 1960s and 1970s, the seasonality issue was dramatized by the writings of a Cambridge school of economic prehistorians inspired by Grahame Clark and led by the late Eric Higgs. In effect, Higgs suggested that the most efficient use of many landscapes would have involved sweeping seasonal movements of inhabitants and livestock. The model was (and is) attractive, but how was its applicability to particular pieces of prehistory

to be tested? Did hunter-gathers maximize their foraging? What effects might the packing of several social groups into a landscape have on the feasibility of seasonal movements? These are questions of interest and importance in archaeological modelling of prehistoric ecology, but in very few cases has it been possible to obtain evidence to test intuitive answers.

Fortunately, archaeologists are optimists who continue to pursue their enquiries Micawber-like, and in the last few years they have been rewarded. It has emerged that to some extent humans are what they eat. Diet affects the composition of bone in various ways and subtle signals, indicative of various aspects of the subsistence regime, may be preserved in prehistoric skeletons. For instance, in any given region the calcium to strontium ratio at the time of death can be used to separate pure herbivores from pure carnivores and to establish a scale of mixed diets[2,4]. In addition, the carbon-13 composition of bones can serve as a measure of the relative dietary contribution of C_3 and C_4 plants; for example, the spread of maize into the woodlands of North America has been monitored in that way[5,6]. Nitrogen isotopes, along with carbon isotopes, are providing measures of the relative importance of seafoods and legumes in some ancient diets[7-9].

Thus, in recent years, aspects of stable isotope chemistry and human bone biology have become important in studies of prehistoric subsistence systems, such as that reported by Sealy and van der Merwe in this issue[1]. Their investigation took as its starting point what must surely rank as one of the most sustained and informative archaeological studies of regional hunter-gatherer subsistence and land-use patterns, carried out over some fifteen years by a group at Cape Town University led by John Parkington[10]. It has monitored responses that span a sequence of environmental change starting 20,000 years ago. Amongst other things, Parkington suggests that there existed at some stage a strong pattern of seasonal migration of human groups between the coast and an inland range of mountains. A series of sites with evidence of complementary seasons of occupation make the model plausible and attractive. But is it correct?

Sealy and van der Merwe report an ingenious and painstaking investigation of bone composition, which they use to explore the question of seasonal movement. Extensive measurements of a full range of coastal foods show that they have markedly different carbon-13 abundances from a full range of mountain foods (be they plant or animal). From this, it is possible to predict the isotope content of bones from people on a coastal diet, a mountain diet or the mixed diet that would indicate seasonal migration between the coast and inland mountains. Tested against the predictions, the compositions of bones do not sustain the hypothesis of extensive seasonal migration of the same people between the two habitats.

Although the results hold out promise that bone-composition studies will sometimes provide otherwise unavailable information about prehistoric ranging patterns and the use of food from different ecozones, the findings in this particular case must be seen as provisional. The samples of skeletons from each time period and each zone are small and need enlarging. Careful alternative modelling of various mixed diets will also need to be considered. Nevertheless, the patterns seem consistent enough to be highly suggestive that, attractive though it may have seemed even then, spending summer in the Alps and winter on the Riviera was not always feasible in prehistoric times. □

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Meterology

What limits front formation?

from K.A. Emanuel

FOR many years, meteorologists and oceanographers were perplexed by the tendency of fluid flows to form 'fronts', or near discontinuities, in such properties as temperature, salinity gradients and velocity. These fronts, which occur in both the atmosphere and oceans, seem to defy the general tendency of turbulent flows to smooth out strong gradients of fluid properties. Then, in 1972, B.J. Hoskins and F.P. Bretherton (*J. atmos. Sci.* **29**, 11; 1972) developed an attractive theory which clearly explained the dynamics of front formation and which predicted the formation of real discontinuities in density gradients and velocity. This presented meteorologists with a new problem: why do actual fronts consist of zones of strong gradients, spread out over horizontal distances on the order of several tens of kilometres, rather than of real discontinuities?

It is natural to suppose that turbulent mixing limits the gradients found in real fronts and, until recently, it was assumed that the mature frontal zone represents a balance between the dynamics of front formation and their dissipation by turbulence. Recently, however, I. Orlanski and B.B. Ross (*J. atmos. Sci.* **41**, 1669; 1984) have suggested that frontal collapse may be at least partially limited by processes that do not involve turbulent mixing. Their conclusions stem from the results of numerical simulations of atmospheric fronts, making use of the full equations that describe the conservation of momentum, heat and mass, rather than the approximate forms used in the Hoskins-Bretherton model.

These approximate equations, which can sometimes be solved analytically and which have proved extremely useful in advancing the conceptual picture of front formation, are based on an assumption of cross-front geostrophic balance — that is, forces arising from pressure gradients across the front are assumed to be balanced by Coriolis accelerations acting on the component of wind parallel to the front. This approximation can be shown to be valid during most of the time it takes for density gradients to collapse but it eventually breaks down near the time of formation of discontinuities. When this happens, the cross-front pressure gradient force may be balanced by actual particle accelerations across the front as well as by Coriolis accelerations. The numerical simulations carried out by Orlanski and Ross seem to show that these effects tend to limit rate of front formation, at least, and perhaps also the ultimate amplitude of the cross-front gradient of front-parallel velocity.

As Orlanski and Ross point out, an exact interpretation of the numerical simulations is problematical. It is generally necessary to represent some turbulent mixing to keep the time integration stable, and it is not clear what role this diffusion plays in limiting the numerically simulated fronts. Detailed evaluation of the dynamics of the simulated fronts is also hampered by the finite horizontal resolution of numerical models. (In this case, the horizontal distance between grid points is 61.5 km.)

Finally, it is well known that near discontinuities can form in non-rotating fluids in which the balance in the frontal zone involves no Coriolis accelerations; taken together with present results this implies that, while both purely inertial and purely geostrophic fronts are limited by turbulent mixing, fronts involving both inertial and Coriolis accelerations may be limited by a non-diffusive mechanism.

This non-intuitive idea, if true, presents a startingly different view of the dynamics of mature fronts in the atmosphere and oceans. More work with numerical models and better observations of frontal zones should help assess its validity. □

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Bacterial immunology

Antigenic variation in gonococcal pili explained

from J.R. Saunders

ANTIGENIC variation of surface structures in pathogens is an important mechanism for avoiding host defence mechanisms. *Neisseria gonorrhoeae*, the causative agent of gonorrhoea, is well known for its ability to exhibit interstrain and intrastrain variation in the type of pilus (fimbria) that it produces. Pili — hair-like proteinaceous structures — are important virulence factors in gonococci because they mediate adhesion of the bacterium to mucosal surfaces during the initial stages of infection. Variability in such exposed surface structures goes some way to explaining the success of this bacterium in causing repeated infections in humans. On page 156 of this issue, Per Hagblom and coworkers have shown that pilin, the protein subunit of gonococcal pili, consists of constant and variable regions with the amino-terminus being conserved and the carboxy-terminus containing more-or-less variable amino-acid sequences[1]. They propose that antigenic variation in gonococcal pilins can be explained by chromosomal rearrangements that result from intragenic recombination between a repertoire of cassettes of different pilin genes[1,2].

Gonococcal pili are composed of identical pilin subunits of relative molecular mass 18,000-22,000, with apparent relative molecular mass and antigenic differences depending on the protein strain involved. The 53 or so amino acids at the amino-terminus of the polypeptide are conserved in all pili from *N. gonorrhoeae* and constitute a constant region[1,3]. The remaining 100-110 amino acids that form the carboxy-terminus of the protein are variable and account for the antigenic diversity observed within and between strains. This variable region can be divided into two domains, a semi-variable region that extends between amino-acid residues 54 and 114 and contains many different amino-acid substitutions depending on the pilin examined, and a hypervariable region that extends from residue 115 to the carboxy-terminus and contains deletions as well as substitutions and insertions of 1-4 amino acids in different pilin variants[2]. Immunological and sequence studies suggest that the hypervariable region is itself flanked by short (about 10-residue) regions of conserved amino-acid sequence that centre around the cysteine residues at positions 121 and 151 (refs 1,4). Polyclonal antibodies raised against purified pili are primarily directed at antigenic sites that lie within the disulphide loop formed between these cysteine residues[4]. The constant region seems to contain a much more weakly immunogenic epitope(s) that is common to all gonococcal pili.

Antisera raised against synthetic peptides corresponding in sequence to residues 41-50 in the constant region and residues 69-84 in the semi-variable region of pili from *N. gonorrhoeae* strain MS11 are effective in inhibiting the binding of heterologous gonococci to human cell lines[4]. In contrast, monoclonal antibodies directed against the variable, type-specific hypervariable domain, but not the conserved domain, are able to block binding of purified whole pili from *N. gonorrhoeae* strain P9 to animal cells[5]. These findings suggest that components of both conserved and variable regions are involved in binding to cell receptors because of the way that the mature pilin polypeptide is folded.

Appropriate synthetic peptides, either singly or in combination, might be useful as components of vaccines directed against gonorrhoeae. Such vaccines would work by eliciting antibodies that would prevent adhesion of the pathogen to the epithelial surface. The immunodominance of the variable part of pilin molecules and the many immunologically distinct pili that can be assembled by just one strain of the gonococcus may compromise such an approach.

Variability in piliation of *N. gonorrhoeae* involves two distinct genetic phenomena. In the first, pilus phase variation, pilin genes are reversibly switched on (to produce piliated or P^+ cells) or off (to produce non-piliated or P^- cells) at a high rate. The second process, antigenic variation, involves the generation of biochemical and antigenic diversity in pilus type and is caused by the expression of alternative pilin genes. The two processes are probably closely linked as the transition between P^+ and P^- states is accompanied by genome rearrangements; P^- derivatives that switch back to the P^+ state frequently express pili that are antigenically distinct from those produced by the original P^+ strain[1-3].

The ability to alter piliation status by a phase change is of great advantage to this pathogen because, although they are important adhesions for initiating infection, the pili might render the organism more susceptible to host defence mechanisms once access to tissues has been gained. It is also not yet clear whether the genetic mechanism for antigenic variation requires phase variation to the P^- state as an obligatory intermediate stage. Pili are major surface antigens in the gonococcus and, therefore, frequent changes in their antigenic nature may assist in avoiding host immune responses. Variations in pili may also be associated with differential attachment to, and virulence for, animal cells, suggesting that one role of antigenic variation is to allow sequential adhesion to different cell types during natural infection[5].

The chromosome of *N. gonorrhoeae* strain MS11 contains two expression loci, *pil*E1 and *pil*E2, involved in production of pilus protein and located within about 26 kilobases of each other on the chromosome[3]. When cells are in a piliated state both these genetic loci carry intact pilin coding sequences as well as their own promoters. Phase variation from P^+ to P^- states involves deletion of pilus-coding sequences from one or both of the expressing loci[2]. Deletions in *pil*E apparently involve single or multiple recombination events between directly repeated sequences that lie within these loci. Interestingly, deletion in only one *pil*E gene can result in reduced or no pilus expression despite the fact that the remaining gene is apparently intact and should be functional. This suggests that there is a further mechanism (possibly a *trans*-acting activator) for regulating pilus expression that is itself regulated by the recombinational switching events.

It is not yet clear whether pilin sequences deleted from *pil*E are destroyed, as occurs in yeast mating-type conversion, or whether they are parked in silent, non-expressing loci somewhere else on the gonococcal genome, as occurs in antigenic variation of surface glycoproteins among certain trypanosomes. Certainly the ability of a P^- derivative to switch back to producing homologous pili suggests that the information necessary to produce a specific pilin is conserved. However, a functional expressing gene can be regenerated by recombination between a deleted *pil*E1 and an intact *pil*E2 locus[2]. This may be part of the reason for the duplication of *pil*E in the gonococcal genome, although maximization of pilus expression and the potential to elicit two distinct pilins simultaneously may also be involved.

The chromosome of the gonococcus contains, in addition to the two *pil*E loci, several silent sequences (*pil*S loci) that are partially homologous to the expressed pilin genes but which lack their own promoters[2,3]. One such silent locus, *pil*S1, lies within about 15 kilobases of *pil*E1 and contains sequences that are not colinear with the mature pilin coding sequence.

Sequences corresponding to the variable part of pilin are over-represented in *pil*S by about 15-20 times compared with those encoding the constant part[3]. This suggests that the silent regions contain information necessary for generating antigenic diversity. Furthermore, regeneration of intact pilin-expressing loci during a P^- to P^+ phase change can be explained by gene-conversion events that would result from recombination between a *pil*S locus and a partially deleted *pil*E site[1,2].

What is not yet clear is whether *pil*S1 or some other more remote silent locus is the active donor of DNA sequences corres-

ponding to the variable part of pilin. The enzymatic mechanism that effects the required intragenic recombination events is also unknown. It will be interesting to discover whether specific enzymes exist for this purpose or whether the normal generalized recombination system of the gonococcus is sufficient.

It should be remembered that pili are only one component of a complex armoury of virulence determinants expressed by the gonococcus. One such determinant is principal outer membrane protein II (PII or Op), which also undergoes both a phase change and antigenic variation. Interestingly, *opa*, the structural gene for this protein, is located within 1 kilobase of *pil*E1, although the two genes do not seem to be coordinately regulated nor are gross genome rearrangements involved in the modulation of expression[6].

The genetic mechanisms used by *N. gonorrhoeae* for alteration of pilus type have parallels in other parasites that exhibit antigenic variation and in processes such as the generation of immunoglobulin diversity. Much of the evolutionary success of this pathogen, which has no host other than man, may be attributed to its complex devices for ensuring variation in its major surface antigens. □

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Polar studies

History of an Antarctic lake

from David A. Peel

DESPITE its immense ice-cap, Antarctica is in fact a polar desert where extremes of cold and aridity have created several unusual features. Since the earliest days of exploration, scientists have been fascinated by more than 20 ice-free areas or oases, which are now known to occur where local weather conditions determine that the overall rate of ice sublimation from the surface exceeds the annual rate of snowfall. These areas are completely free of ice providing that local geographical barriers prevent ice flow from a neighbouring area where it is accumulating. Within these dry regions, perennially ice-covered lakes are fairly common, but in many cases satisfactory models of their histories and physical characteristics have yet to be developed. On page 131 of this issue, Hermichen *et al.*[1] present new isotope data from a large freshwater lake in East Antarctica and demonstrate that the present-day lake is the concentrated residue of a body of water originally more than 50 times larger.

The perenially ice-covered lakes of the Antarctic show some unusual chemical and physical properties[2]. Normally highly saline, although basically of freshwater origin, they occur in areas where the mean annual air temperature is around minus 20°C. They are usually covered by a relatively thin layer of ice overlying water which is at least 20°C, and sometimes up to 50°C, warmer than their surroundings. As the occurrence of life depends on the presence of liquid water, the lakes have been a focus of interest for biologists seeking to understand the adaptation of organisms to extremes of temperature[3]. Although the general morphology of the lakes has been understood for some time, they are individually a result of often quite complex histories.

One basic physical characteristic that has a direct bearing on the biological productivity of a lake, the thickness of the ice cover, has been modelled recently[4]. It has long been known that for lakes that are not frozen to the bottom, the ice thickness is relatively constant from place to place despite wide variations in the temperature and chemistry of the water. More than two decades ago it was suggested[5] that solar heating was probably the main source of energy for the Antarctic lakes, with a skin of exceptionally transparent ice at the surface of the lake acting rather like greenhouse glass. Each summer, melt water flows into the lakes beneath the ice, balancing water lost by evaporation from the ice on the surface. Under steady-state conditions, it has been suggested that each winter a thickness of ice freezes onto the base of the floating ice, generating latent heat which balances the loss of sensible heat through the ice cover.

McKay *et al.*[4] have recently developed a simplified model of the energy balance at the surface of Antarctic lakes based on analysis of annual average heat fluxes. The thickness of the ice cover is apparently sufficient to damp out most of the seasonal variations in the fluxes of heat from sources and sinks. Using actual measurements of solar flux and albedo values from one of the best-studied lakes (Lake Vanda, southern Victoria Land), and direct measurements of the typical light-transmission characteristics of the ice, these authors have been able to predict the equilibrium ice thickness as a function of the surface ice ablation rate. They found that this rate of ablation is the prime control of ice thickness; measured ablation rates of around 30 cm per year lead to predicted equilibrium ice thicknesses in the range of 3-4 m, in good agreement with what is observed. Lakes at higher altitudes lie closer to the snow line and suffer less ablation; moreover, the ice is often anchored to the bottom and hence the latent heat contribution from refreezing melt water ceases to be an important factor. In these lakes much greater thicknesses of ice can be maintained under a given air-temperature regime.

Analysis of the stable isotope composition of lake waters has proved to be a valuable tool in deciphering the evolution of present-day water systems[6]. Lake Untersee, the subject of the study by Hermichen *et al.*[1], was first probed by a Soviet team in 1969. The lake has an area of about 10 km^2 and lies in an region of high ablation (about 20 cm per year). Refreezing of melt water supplied by the adjoining glacier leads to an equilibrium ice cover which is 2.5 m thick. It is difficult to envisage how such a large volume of fresh water could have accumulated under present-day climatic conditions (the average temperature is roughly minus 20°C). Both the stable isotope data and the lack of any evidence for modern tritium indicate that the water has originated from the melting of old glacier ice. In this case, the concentration of salts in the relatively saline water now found can be accounted for if the present-day body of water is the residue resulting from the evaporation of a volume originally more than 50 times larger. The new isotope data provide some additional support for this idea. The present-day water is isotopically lighter than the snow that currently falls in the catchment area of the lake. Furthermore, the composition with respect to the Meteoric Water Line (a steady relationship between $\delta^{18}O$ and δ^2H generally observed in atmospheric precipitation, which reflects mainly equilibrium fractionation of the isotopes) is displaced in the direction of preferential depletion of ^{18}O. Both results can be explained satisfactorily if it is assumed that the water has been lost from the lake by sublimation of ice from the upper surface. The isotope composition of the sublimating water is then determined by isotope exchange during the freezing of water where ^{18}O is preferentially deposited. The indications are that this process has been proceeding more or less continuously since a period of warmer climate in the early Holocene. There are signs of biological primary production in Lake Untersee; future studies of organic deposits on the lake bed may help to trace the lake's evolution. □

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Why should plants be evergreen?

SIR — I read with interest Peter D. Moores's article "Why be an evergreen?" (*Nature* **313**, 703;1984). One factor referred to as a possible explanation for leaf longevity was leaf fall as a potential sterilizing agent. It was stated that many evergreens grow in impoverished soils. It has been argued in the past that plants growing in these conditions need to invest more heavily in chemical protection against phytophagous insects since growth on poor soils is slow, increasing vulnerability to attack. Many evergreens appear to be particularly rich in chemicals which defend them from phytophagous insects (terpenes associated with pine, for example). On the other hand, where growth is less costly, the alternative defence against phytophagous insects may be simply to shed leaves synchronously — that is, to adopt the deciduous habit to cleanse the tree annually of pests.

This hypothesis is open to an experimental test. If an evergreen is fumigated to remove phytophagous insects at the same time a deciduous species is coming into leaf, phytophagous insect population growth, measured as insect biomass per tree biomass, should be more rapid on the latter than the former. A favourable result would, however, be indicative rather than conclusive support of the hypothesis.

The hypothesis would be suspect if the maximum insect biomass achieved per tree biomass were higher on the evergreen. The reverse of this result would be consistent with the hypothesis whereas similar maxima would be the least informative outcome (although not inconsistent with the hypothesis).

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Assessing the risk of dioxin exposure

SIR — The health risk of exposure to 2,3,7,8-tetrachlorodibenzodioxin (dioxin) is a subject of considerable international interest which has been addressed recently in *Nature*[1] and *Science*[2,3]. As noted many times, understanding the nature of risk to humans is difficult because of the relative lack of information from epidemiological studies. Because of this, great emphasis has been placed on the results of two papers drawing on the mortality experience of workers exposed to dioxin at the Monsanto chemical plant in Nitro, West Virginia[4,5].

As with many occupational health studies, there are problems with these papers in the definition, and consistency of definition, of exposure. Zack and Suskind[4] report nine cancer deaths in the cohort of 121 workers exposed to tetrachlorodibenzodioxin in a specific trichlorophenol process accident, occurring in 1949. These nine deaths (Table 2 in the paper) give details of the individual's year of birth, year of hire by Monsanto, year of death and the cause of death. In the second paper, by Zack and Gaffey, published three years later[5], there are lists of death from cancer amongst workers at Monsanto who are described as having been exposed to the herbicide 2,4,5-T. Workers exposed to 2,4,5-T were also exposed to varying concentrations of dioxin and were classified on the basis of having contracted the sentinel indicator disease chloracne. Zack and Gaffey report 25 deaths from cancer in the controls, workers not exposed to 2,4,5-T. This is referred to in Table II of the paper[5].

In comparing the two papers, some men are listed as exposed to dioxin in one paper, but as not exposed in the other. In Table 2 in the Zack–Suskind paper, four cases are described as exposed to dioxin, whereas in the Zack–Gaffey paper, Table II reports the same four being not exposed (to 2,4,5-T and dioxin). Cases 1,2,5 and 7 in the Zack–Suskind Table 2 are those listed as being exposed to dioxin whereas in Table II of the Zack–Gaffey study, the same individuals are described (lines 6,7,9 and 22 of table) as being non-exposed. Judith Zack, an author on both papers, has reported that these individuals are one and the same[6].

In addition, a number of exposed individuals may not have been included in the second Monsanto study. In Monsanto records, there are some 19 individuals who died of circulatory disease or cancer whilst in employment at the company, and who meet the criteria for inclusion in the exposed group. No reason is given for not including these cases.

Determining exposure in the context of the Nitro plant is clearly difficult, since conditions may have produced general exposure to much of the work force. Those men exposed to the chemical in the 1949 trichlorophenol process accident are said to have been seriously exposed, on the basis of developing more severe chloracne, than the men exposed to dioxin in the course of making 2,4,5-T over several decades at Nitro. It may be the case that some of the more severe chloracne cases were the workers exposed to dioxin in the trichlorophenol process accident. However, there was a similar spectrum of chloracne incidence in both groups according to a study in 1953[7]. Of the 117 cases of chloracne determined in 1953 in individuals exposed to dioxin as a result of the 1949 accident, 10 workers had left the company, 80 were said to have recovered from the skin disease, 24 had a mild form of chloracne, and 3 had moderately severe disease. In other workers exposed during 2,4,5-T manufacture, 97 additional cases of chloracne were reported as having occurred, 51 of these cases were regarded as being clear of the disease, 36 were said to have a mild form and 3 to have moderately severe skin disease. Seven of the 97 men had left the company. Thus the spectrum of chloracne in the two groups seems similar. Another reason why it is inappropriate to separate these two exposed groups comes from information from a former manager of the Nitro plant[8]. In his deposition to court, Durland reported that some of the same workers were involved both in the clean-up after the trichlorophenol reactor accident and in making 2,4,5-T.

It may not be appropriate to classify degrees of dioxin exposure solely on the basis of chloracne as an indicator of relatively acute high-dose exposure to certain chlorinated hydrocarbons, including dioxin[9]. Other health effects — notably serious diseases of long latency, such as cancer — may be expected to result from lower and more chronic exposure[10-13]. Using one outcome variable to predict others is not advisable. Occupational studies usually use work histories, biological monitoring, or environmental measurements to determine, or approximate, exposure of subjects.

In view of the importance of the Monsanto cohort for determining human health risks of dioxin, it is our view that data from the company need to be reassessed. The *total* cohort of workers exposed to dioxin at Monsanto should be considered as a whole without making a distinction, as apparently done by Suskind, Zack and Gaffey, between workers exposed to dioxin in the trichlorophenol process accident or when making 2,4,5-T.

It may be that the overall number of deaths — from cancer, circulatory disease or other causes — is no greater than an appropriate control cohort. Data from a recent study by Moses *et al.*[14] on this same population suggests that there is an excess of cardiovascular-related deaths[15].

The epidemiological picture at Monsanto remains confused. We believe that more information will only be established by re-examining all of the data, and, given international concerns over dioxin, that this needs to be done urgently.

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Dosage compensation and X-chromosome inactivation

SIR — It is now common practice to use the terms dosage compensation and X-chromosome inactivation interchangeably. It is argued here that this equation is technically incorrect and, more importantly, that it obscures a nontrivial facet of mammalian physiology. The proposition is that in flies and mammals, dosage compensation allows males to survive their monosomy for the X chromosome. In mammals, X inactivation is then a device that prevents females from expressing a tetrasomic level of X-chromosome gene product. That is, X inactivation allows females to survive dosage compensation. If we call X inactivation dosage compensation, we are overlooking something in males. That something is dosage compensation.

The idea of dosage compensation and its definition originated with H.J. Muller in his studies of *Drosophila*[1]. In the context of the issues of his day, Muller saw dosage compensation from a selectionist perspective concerned with optimal phenotypes. In this view, dosage compensation was the mechanism by which selection has achieved equality of expression of sex-linked genes in single-X males and two-X females. In the modern context, however, we are forced to add an element to the Mullerian view: dosage compensation not only equilibrates male to female phenotypes but also — and perhaps primarily — equilibrates a male's single X to his two sets of autosomes. The point we seem to overlook is that a *Drosophila* male (or the heterogametic sex in any organism with a chromosomal sex determination system) is aneuploid and aneuploidy is deleterious.

We know from an extensive study of aneuploidy in *Drosophila*[2] that any heterozygous autosomal deficiency greater than about 3% of the haploid genome is a lethal condition. On the other hand, a *Drosophila* male is deficient for a whole X chromosome (about 20% of the genome). The issue, then, transcends his similarity to his sister. He requires dosage compensation for his survival. In *Drosophila melanogaster*, dosage compensation is effected by the elevation of the rate of transcription of the lone X in males relative to either of the X chromosomes in females[3-5]. More to the point, mutations in *Drosophila* which fail to hypertranscribe the X chromosome in males are male-specific lethals[6].

Aneuploidy in mammals, as in *Drosophila*, is deleterious. Mammalian males, like *Drosophila* males, have a congenital aneuploid condition for a considerable fraction of their genomes. We infer that dosage compensation in mammals, as in *Drosophila*, balances the expression of the single X chromosome in males to the autosomal complement; that is a male's single X is hypertranscribed relative to his autosomes. Hypertranscription in *Drosophila* males presents no problem for *Drosophila* females, as they transcribe their two X chromosomes at a basal rate balanced to the autosomes. However, in a species in which X hypertranscription is a property of all X chromosomes in either sex, the female has become, in a formal sense, a hyperploid. Since hyperploidy is also a deleterious condition, she would require a second system to achieve X to autosome balance. One option is to inactivate one of the two X chromosomes.

If mammalian X chromosomes are hypertranscribed in the interest of male survival, X-chromosome inactivation must be dosage-compensation compensation in the interest of female survival. This inference may be of some heuristic value in that it implies the existence in mammals of a class of genetic elements which function to modify X-chromosome expression and to which we might want to give some thought.

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High time for psychoimmunology

SIR — The term "psychoimmunology" was coined by Solomon[1] to describe his early studies of the effects of behaviour on immune function and on susceptibility to certain experimentally-induced disease processes. More recently, "psychoneuroimmunology"[2] has been used to refer to studies of the neuroendocrine mechanisms mediating the effects of behaviour on immune function — and vice versa. But in a News and Views article, "Psychoimmunology before its time", John Maddox questions the explanatory potential and, by implication, the heuristic value and practicality of pursuing such research[3].

Despite an erroneous characterization of what is "psychosomatic", the article correctly implies that much of the impetus for studying interactions between the central nervous system (CNS) and the immune system derives from observations that psychosocial factors influence the development and progression of disease. To phrase this as a "familiar theme in literature", however, is to impart a scientific triviality to such phenomena. In fact, there is a voluminous scientific literature that documents the integration of mind and body. To justify this scepticism, Maddox points out that the mechanism for such phenomena is unknown, but this does not mean it is unknowable — or that the phenomena are less real. In an attempt to account for such phenomena, then, it is quite reasonable to hypothesize that changes in immune function may mediate the effects of psychosocial factors on the development and/or progression of some pathophysiological states. Such a hypothesis is tenable, however, only if it can be shown that the CNS plays some role in the modulation of immunity.

In referring to a *kind* of connection that is made *plausible* by several unconnected observations, the article begrudgingly acknowledges a functional relationship between the CNS and the immune system. This is not the common knowledge that it is implied to be. That it is accepted at all is, in large part, a result of psychoneuroimmunological research conducted over the past 10 years. The scepticism expressed in the article, however, is not directed at the experimental findings of psychoneuroimmunology. It is fabricated, instead, out of unreferenced claims attributed to "psychoimmunologists": we know of no serious investigators who "talk as if there is no state of mind which is not faithfully reflected by a state of the immune system". The hypothesis that a behavioural state may have immunological consequences, however, cannot be dismissed as easily. After all, there are neurophysiological and neuroendocrine consequences to behaviour and accepting a link between the CNS and the immune system, it would be reasonable to expect that influences are exerted in the opposite direction. Analyses of the interactions between behaviour and the immune system are not a traditional part of either immunology or the behavioural or neurosciences, but perhaps they should be.

Space does not permit us to do more than point to the article's superficial analysis of the studies by Laudenslager *et al.*[4] and Schleifer *et al.*[5], the evaluations of research based on the time consumed and its cost, the false analogy between the (unreferenced) claims of psychoimmunologists and attempts to link states of mind with the development of cancer, or the difficulty of conducting psychoneuroimmunological research and the implication that the results obtained are neither reproducible nor significant. The basic flaw in the argument is revealed by what is claimed to be a crucial unanswered question: if a person's affective response to some event is capable of influencing immune function, why, the article asks, would such a person be more likely to die of a heart attack or even an automobile accident?

This question is not crucial; it is not even relevant. The relationship between an affective state and immune function may be

quite independent of the relationship between that affective state and other physiological processes and, in any case, depends upon a myriad of other biological and environmental factors. Furthermore, such a question could follow only from the underlying assumption that behaviourally-induced alterations in immune function represent the single mechanism by which psychosocial factors influence disease. We know of no psychosomaticist or psychoimmunologist who would accept such a proposition and we cannot imagine how such a hypothesis could be derived from the available literature.

There are probably those in every field whose exuberance outdistances their data. One is not required to share their exuberance, but one is obliged to consider their data. One can only wonder, then, why anyone would want to throw out the baby with the bathwater. Connections between the CNS and the immune system are now being uncovered. Whatever forces were operating to set immunology apart, recent data suggest that much could be learned by studying immunoregulation as part of an integrated network of adaptive processes including behaviour. If not now, when?

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JOHN MADDOX WRITES — My article was a protest against those psychoimmunologists who attribute as yet unwarranted importance to the immune system (as distinct from other physiological systems) among the physiological correlates of behaviour. My statement that "people may be driven by adversity into decline is a familiar theme in literature" was not intended to demean psychoimmunology but, rather, to illustrate that the phenomena it seeks to account for have long been recognized.

My reference to heart attack and road accidents rather than, say, infection among the causes of death of those who have been psychologically shocked was intended to raise the precise question Ader and Cohen answered by saying that physiological systems other than the immune system may be involved.

I see nothing objectionable in the moderate statement of Ader and Cohen. Specifically, I can readily accept that there are many immunological consequences of, say, bereavement, although I do not believe that the tendency towards untimely death among bereaved spouses can be attributed primarily (or even at all) to the immune system (which is not to say that that may not be an attractive speculation).

Underlying this dispute (which seems to me not to be a substantial disagreement) is an important question. These reductionist days, it is proper to seek to identify the mechanisms by which states of mind cause physical illness. The immune system is one candidate, perhaps the most obvious after the adrenal hormone complex. But we all know that Freud's place in intellectual life rests in part on his careful documentation of the phenomena of psychosomatic illness, and on his explanation in terms of the unconscious, nowadays a somewhat old-fashioned concept.

So what if further investigation should show that the nervous system, and especially that part of it within the skull, plays an important part in the causation of psychosomatic illness? Such a development would no doubt be hailed as an important step towards Freud's rehabilitation, as a prophet if nothing more.

The point of this speculation, in the present context, is not to say that that is how it will turn out, but simply to suggest that the less obvious fields for explanation may ultimately be more productive. Much of the abuse that reductionists have recently attracted stems from their weakness for the kinds of explanations which happen to be at hand. □

An unexpected result in classical electrostatics

SIR — A recent discussion of the structure of snowflakes (W.S. Mortley, *Nature* 313, 638; 1985) mentions the possible role of electric forces. In this and similar problems, establishing the least energy configuration appears vital. I would like to draw attention to a surprising result, which, to my best knowledge, has not been mentioned earlier.

What will be a minimum-energy configuration of a system of N equal point charges q placed inside a circle?

At first glance the answer is obvious: the Coulomb repulsion will arrange all N charges at the vertices of a regular polygon inscribed into the circle (configuration A). This statement seems to be so obvious that, apparently, nobody bothered to verify it by a direct calculation of the total electrostatic energy of the system (W). However, such calculation, which can be easily performed on a programmable pocket calculator, shows, rather surprisingly, that this is true only for $N < 12$. In units q^2/R the total Coulomb energy W is equal to 48.57568 for $N = 11$ and 59.80736 for $N = 12$. An alternative configuration (B), which is also compatible with the total symmetry of the system, has $N - 1$ charges equally spaced along the circumference and one 'extra' charge at the centre of the circle. For $N = 11$, $W(B) = 48.62450$ (that is, greater than $W(A)$), but for $N = 12$, $W(B) = 59.57568$, that is, less than $W(A)$. The non-equality $W(B) < W(A)$ also holds for $N > 12$ (I verified this numerically to $N = 400$, but very likely it is true for any $N > 11$).

The above result means that starting from $N = 12$, the second configuration B (with one charge expelled to the centre of the circle) takes over from A as the minimum-energy configuration. In other words, it will be energetically beneficial for the system of N equal point charges confined in a circle to 'expel' one charge to the centre of the circle (if $N > 11$). Similar effect (the 'spontaneous ejection' of one charge to the geometrical centre) will also be likely to happen for N charges inside the spherical surface ($N > ?$). This may lead to some modification of usual theorems of electrostatic stability which claim that at the state of the equilibrium all charges in the conducting body are always located on the surface. The above example clearly illustrates that this is not necessarily so.

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Spider pheromone 'packets'?

SIR — The scanning electron micrograph (SEM) of the pustuliform organ of the spider *Holcolaetis vidua* Lessert (Salticidae) reproduced in *Nature* of 7 March (p.17) shows spherical bodies on the surface. It is suggested that the spheres may be 'packets' of pheromone secreted by the pores, on the assumption that the organ is pheromone producing[1].

In reality the SEM shows that the spheres are not associated with the pores but occur on the pustuliform organs in proportion to the surface area occupied by the organs, about 25%. The implied low volatility of the spheres seems inconsistent with pheromonal function. The mean diameter of the spheres of 1.3 μm, size distribution, aggregation and general appearance are consistent with the spheres coating the chorion of spiders' eggs of all families, including salticids, that I have examined[2]. These spheres may be found on the surface of spiders newly emerged from egg cocoons[2] and the brood guarding behaviour of salticids may expose the adults to such contamination.

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Scientific Corespondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

A multichannel seismic study of lithospheric flexure across the Hawaiian–Emperor seamount chain

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Existing models assume that the thickened crust beneath seamounts is the result of a surface volcanic load flexing an elastic plate. New results suggest that flexed oceanic crust beneath the Hawaiian–Emperor seamount chain is underlain by a 4-km thick deep crustal body. We intepret the body as a deep crustal sill complex associated with the tholeiitic stage of volcano building along the chain.

PLATE tectonics is based on the concept of a strong rigid lithosphere that overlies a weaker asthenosphere[1]. The principal evidence for the existence of a strong lithosphere on long time-scales ($>10^6$ yr) comes from studies of the way in which it responds to surface loads such as late glacial lakes[2], river deltas[3] and oceanic islands and seamounts[4]. One of the best-studied loads[5–11] is the Hawaiian–Emperor seamount chain in the Central Pacific Ocean. This volcanic load is suitable for study because: (1) it is located in the interior of a plate away from the complexities of plate boundaries; (2) it is one of the largest loads ($\sim 10^{18}$ kg) on the Earth's surface; (3) its tectonic setting is reasonably well known, enabling the relationship between load age and the thermo-mechanical properties of the oceanic lithosphere to be examined.

It has been known since the earliest gravity measurements[12] that the Hawaiian Islands are associated with large-amplitude free-air gravity anomalies that cannot be explained by local models of isostasy and require some form of regional compensation to account for them. Venning Meinesz[4] and Gunn[5] showed that gravity data over the islands are consistent with the flexure model of isostasy. According to their model, the Hawaiian Islands represent a load on the surface of the oceanic lithosphere which responds in a similar manner to an elastic plate overlying a weak fluid substratum. Walcott[7], Watts and Cochran[8], Watts[10] and Kunze[11] have shown, using this model, that the best fit to the gravity data is for an effective flexural rigidity of the oceanic plate in the range 5×10^{29}–5×10^{30} dyn cm, which corresponds to an effective elastic thickness, T_e, in the range 17.8–38.3 km.

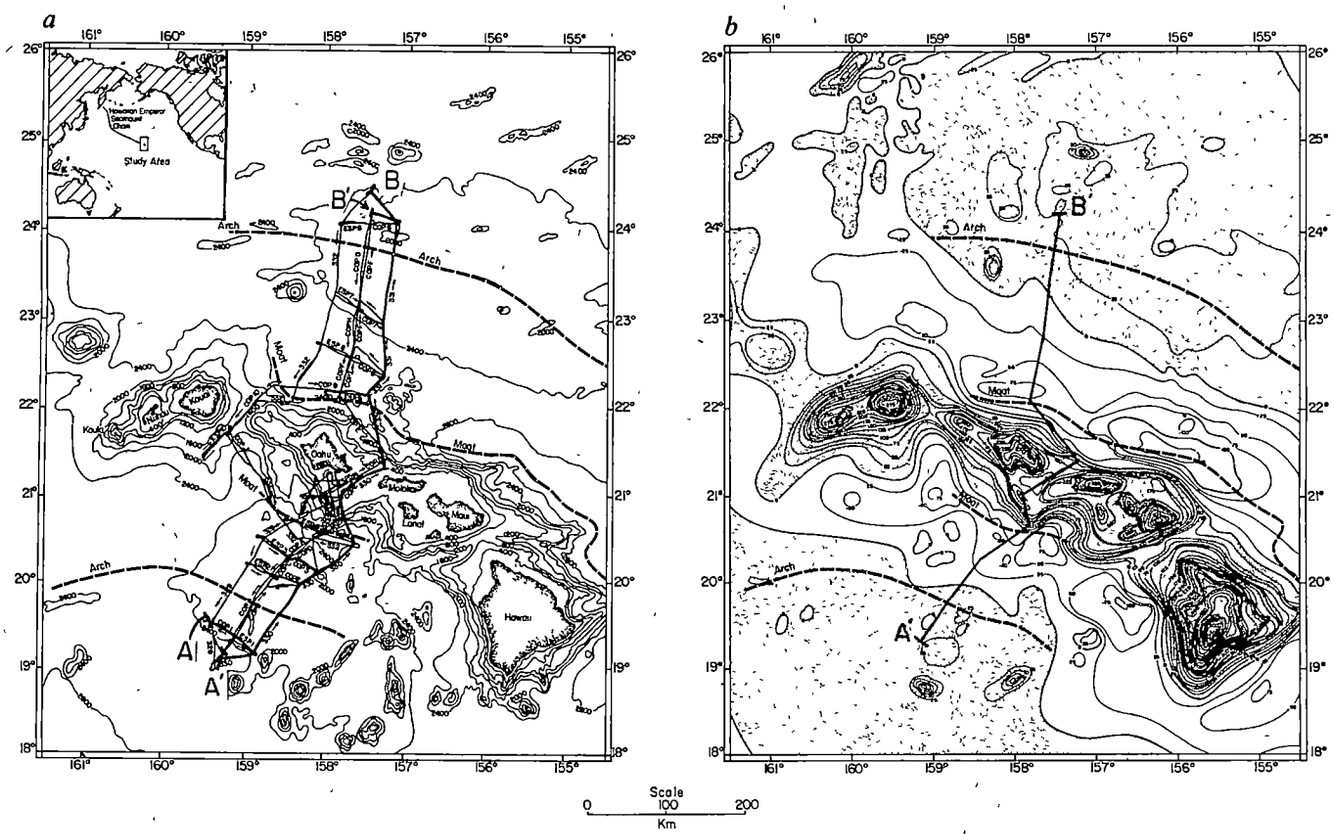


Fig. 1 Location map of the two-ship multichannel seismic experiment, *a*, Bathymetry; *b*, free-air gravity anomaly. In *a*, the contour interval is 400 fathoms (1 fathom = 1.8288 m) and is based on Chase *et al.*[34]. Fine track lines, location of individual CDP, COP and ESPs; heavy line, location of ESP 1/COP 1 (Fig. 3) and CDP 330/CDP 331 (Figs 1, 2, profile AB); A'B', end points of the free-air gravity anomaly and bathymetry profile connecting ESP mid-points In *b*, the contour interval is 25 mgal (1 mgal = 0.001 cm s^{-2}) and is based on Watts and Talwani[35]. Gravity anomalies >25 mgal are stippled. Thick dashed lines in *a* and *b* indicate the location of the Hawaiian arch and moat based on all available bathymetry profiles in the region.

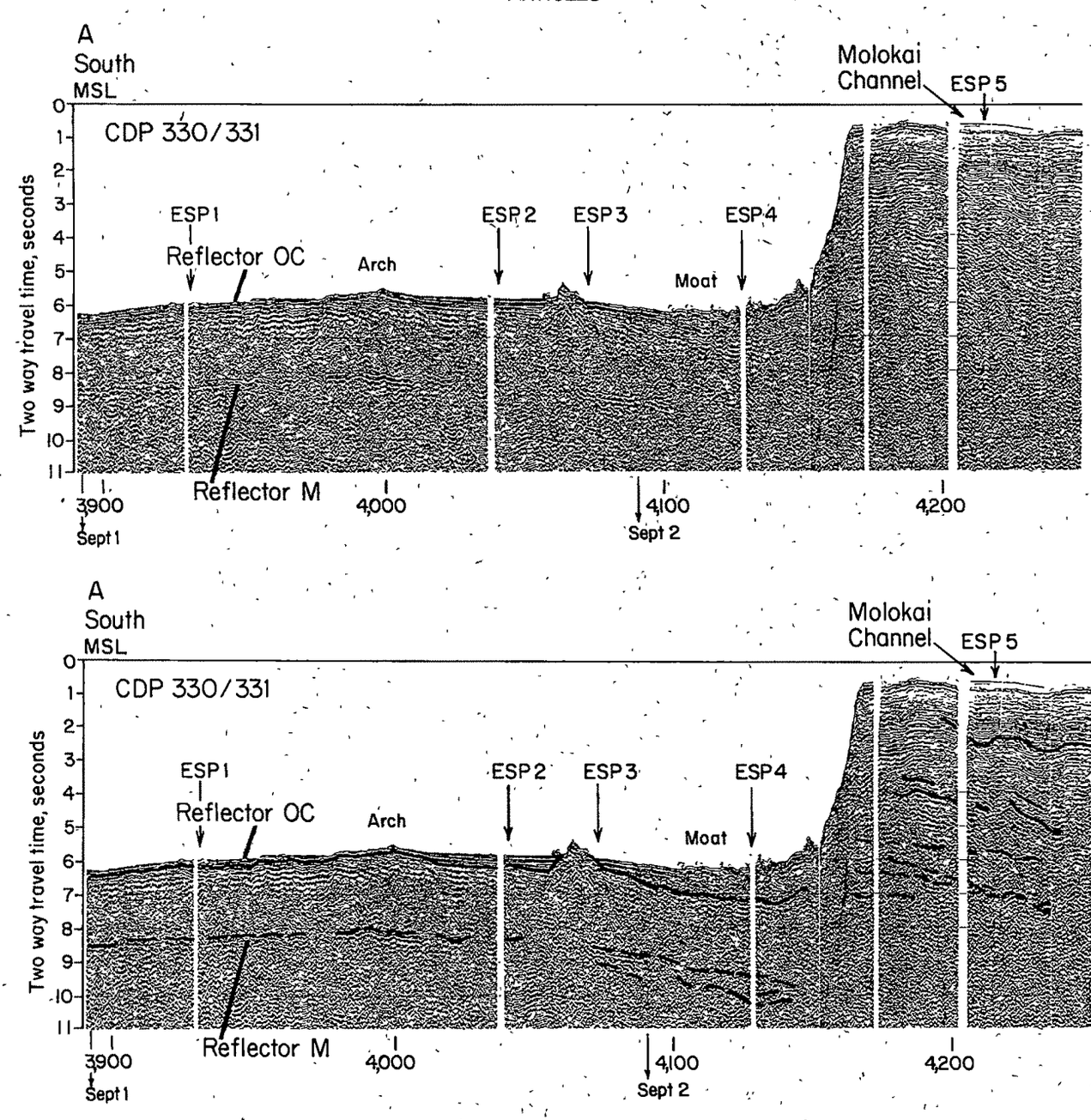


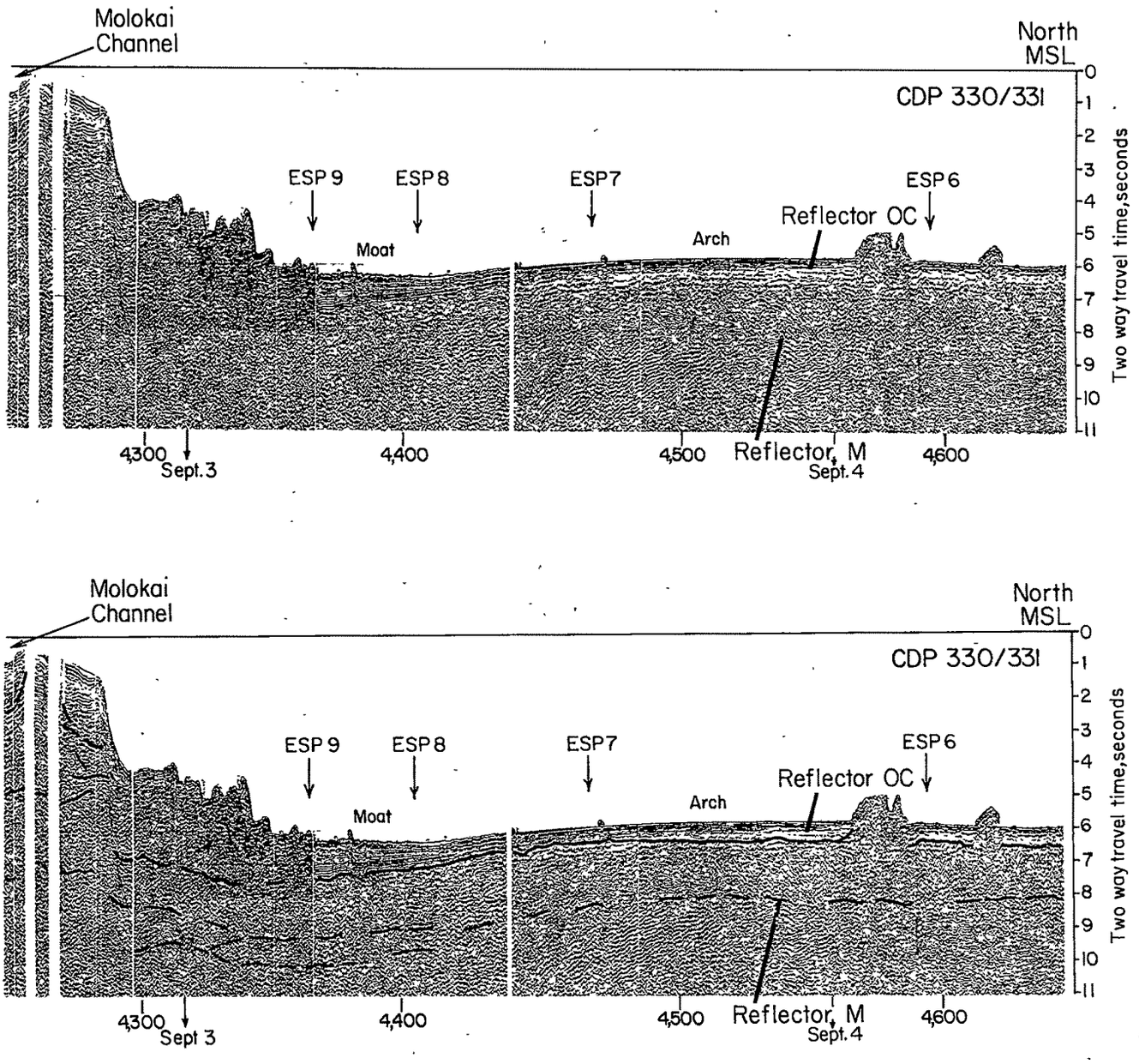
Fig. 2 (Above and opposite) CDP profiles 330 and 331 of the Hawaiian ridge and vicinity (Fig. 1*a*, profile AB). The 48-fold stacked section was acquired with a 3.6-km towed array. The black line on the upper section highlights prominent seismic reflectors. Reflector M at ~8.2 s below the arch is associated with the *M*-discontinuity and is continuous on a scale of ~50 km. Reflector OC is associated with the top of the oceanic crust. The number below the profiles is the accumulated distance in km along the ship track.

The actual value of T_e depends, however, on the type of flexural model that is assumed. For example, Walcott's[7] estimate is based on a model that assumes the plate is fractured beneath the islands, whereas Watts and Cochran[8], Watts[10] and Kunze[11] derive their estimates assuming a continuous plate. Both types of model are in isostatic equilibrium and produce similar shape gravity anomalies to observed profiles. Thus, gravity data have not been able to discriminate unequivocally between different flexure models.

An independent test of flexure is to use seismic data to constrain the predicted deformation of the plate. For example, Walcott[7] predicted that normal thickness oceanic crust is flexed by ~13 km beneath Oahu, whereas Watts[10] and Kunze[11] predicted only ~4 km. If the thickness of the oceanic crust in undeformed regions is 6 km, then these authors imply a depth to the *M*-discontinuity of 23.5 and 14.5 km for the fractured and continuous plate models, respectively.

During the early 1960s, a number of seismic refraction experiments were performed near the Hawaiian Islands, principally in support of Project Mohole (ref. 13). These experiments showed that in the region of the Hawaiian arch the mean thickness of the oceanic crust is ~6.3 km, increasing to ~13.0 km beneath the moat and ridge. Subsequently, Furomotu *et al.*[14] suggested that the crustal thickness is ~19–20 km beneath Oahu, thinning to ~17 km to the north-west beneath Kauai and ~10–14 km to the south-east beneath Hawaii. Unfortunately, these early seismic studies were hampered by poor navigation and drifting receivers and do not provide a suitable data set with which to constrain reliably the flexure model.

The purpose of this article is to summarize the results of a two-ship multichannel seismic experiment over the Hawaiian–Emperor seamount chain near the islands of Oahu and Molokai. A more detailed description of all the data obtained during the experiment will appear elsewhere (U. ten B. *et al.*, in prepar-



ation). The overall objective of the experiment was to define the detailed crustal and upper mantle velocity structure beneath a mid-plate volcanic island chain. We examine here the implications of the seismic experiment for the long-term ($>10^6$ yr) thermo-mechanical properties of the oceanic lithosphere and petrological models for the evolution of mid-plate oceanic volcanoes.

Seismic experiment

During August–September 1982, the Lamont–Doherty Geological Observatory and the Hawaii Institute of Geophysics carried out a two-ship seismic experiment near Oahu and Molokai in the Hawaiian Islands (Fig. 1). The experiment involved two vessels, the *Robert D. Conrad* and *Kana Keoki*, and used the single-ship multichannel seismic reflection profiling technique[15] to determine the configuration of individual crustal layers and the *M*-discontinuity and the two-ship technique[16] to determine the detailed velocity structure of the crust and upper mantle. The *Conrad* was equipped with a 3.6-km long seismic engineering multichannel streamer with 48 active channels and a large-volume (2,500 in^3) high-pressure sound source. *Kana Keoki* was equipped with a single-channel streamer, a large-volume (2,000 in^3) high-pressure sound source and 210 60-pound Tovex charges. Navigation for each ship was by satellite. During the two-ship portion of the experiment, the range between the two vessels was continuously measured by Miniranger and Raydist systems.

The seismic experiment consisted of normal incidence common depth point (CDP) data, constant offset profiles (COPs) and eleven expanding spread profiles (ESPs) obtained across a 600-km long, 100 km wide 'transect' of the Hawaiian ridge centred near Oahu (Fig. 1). The first part of the experiment included a continuous 250-km long COP between the Molokai channel and the Hawaiian arch south of the Hawaiian Islands (Fig. 1*a*). While shooting the COP, the two vessels maintained a constant distance apart of 3.6 km and *Conrad* and *Kana Keoki* alternately fired their airguns each minute. At the end of the COP, the vessels re-grouped for an ESP (Fig. 1*a*, ESP 1). During the ESP, the two vessels separated from each other at a speed of ~5 knots while *Kana Keoki* fired its large airguns each minute and *Conrad* received. At the end of the 65–90-km-long line, the two vessels turned and moved toward each other along the same profile. The ESP was then reshot with the *Kana Keoki* firing Tovex charges on a 10-min schedule. During the ESP re-shoot, *Conrad* fired its airguns each minute (with each 10-min shot missing) so that a CDP profile was obtained along the ESP. On

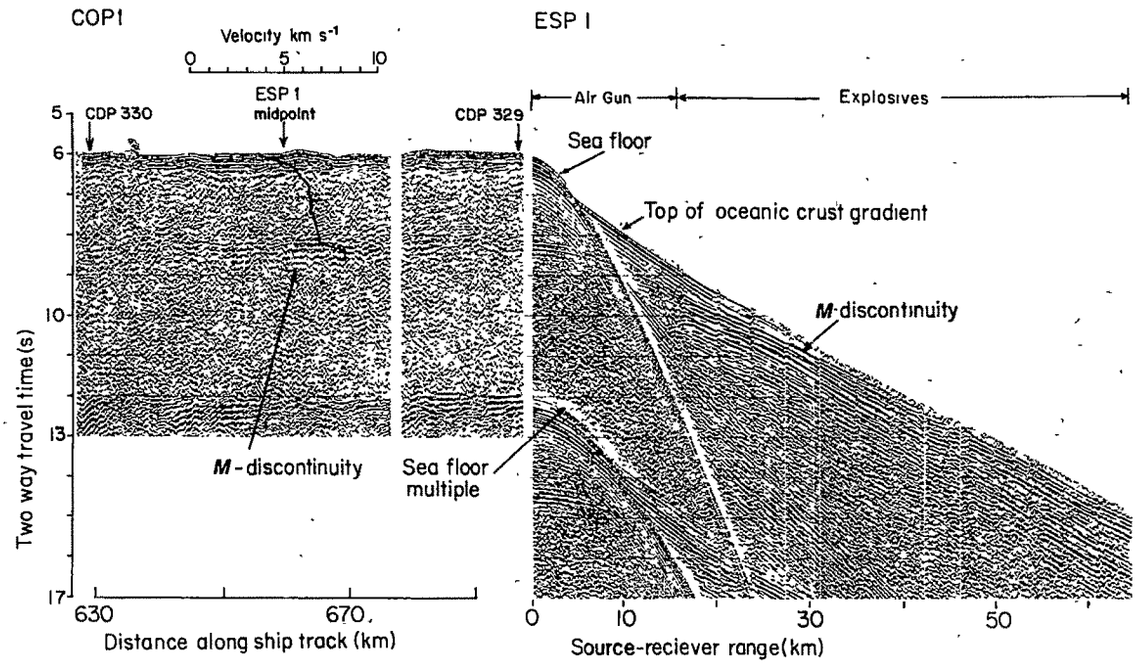


Fig. 3 ESP 1 and COP 1 of the Hawaiian arch, south of the Hawaiian ridge (Fig. 1*a*). The ESP data were obtained as the two ships separated at a constant speed from a common mid-point. At the end-point, the two ships steamed towards each other. During the 'outgoing' part of the experiment *Kana Keoki* fired its air guns and the *Conrad* received. During the 'ingoing' part *Kana Keoki* fired explosives. The ESP data shows a combination of the air gun and explosive record. The COP data were obtained by one ship (*Conrad*) firing its air guns and receiving. The black line on COP 1 represents the best-fitting velocity depth profile, obtained by a τ-p sum of the ESP X-T data.

completion of ESP 1, the vessels regrouped for a COP at a constant separation of 16 km to ESP 2. The COP/ESP sequence was then repeated until the transect had been completed south of the islands. The second part of the experiment was comprised of a similar sequence of COP/ESPs between the Molokai channel and the arch to the north. On completion of ESP 11, *Conrad* began a 1,200-km long CDP profile connecting the ESP end-points. During this portion of the experiment, *Conrad* fired its airguns on a 20-s schedule.

The data acquired during the seismic experiment were processed using techniques described by Stoffa *et al.*[17]. The ESPs were corrected for ship's navigation, the ranges between shot and receiver, shot times and clock synchronization. After demuxing the data, individual seismic traces were sorted and summed in bins of 50 and 100 m. We performed τ-p mapping of the stacked X-T data and carried out a τ-sum inversion (assuming horizontal homogeneous layers) on the τ-p data to yield a velocity-depth function at each ESP mid-point[17]. The solutions from τ-sum inversion were verified by forward modelling of first arrivals using velocity gradients and accounting for spherical losses. Also, the velocity-depth function was compared with the results of semblance analysis of CDP data near some ESP mid-points and the two-way travel time data on the CDP profile that crossed each ESP mid-point. The CDP data were processed using routine methods with some exceptions. Before stacking, velocity filtering was performed on parts of the profiles, each CDP gather was muted and the first and last traces eliminated. This additional processing removed multiples of the sea floor and sub-bottom reflectors and reduced the scattered energy associated with irregularities in each reflector. After stacking and predictive deconvolution, time-varying band pass filtering was applied to all the CDP data.

Results

We present here selected profiles of the ESP and CDP data gathered during the seismic experiment. Figure 2 shows CDP profiles 330 and 331 obtained by *Conrad* on a transect of the Hawaiian ridge between Oahu and Molokai (Fig. 1*a*). The figure shows a strong reflection (reflector M) from the M-discontinuity at ~8.2 s two-way travel time beneath the Hawaiian arch south of the islands and at ~8.0 s north of the islands. In general, the reflector is stronger south of the islands than to the north. The travel time difference between the sea floor and the reflector is ~2.0 s north of the islands and 2.4 s to the south. We interpret these differences as indicating that the oceanic crust is thicker south of the islands than to the north. The travel-time difference between the sea floor and reflector M increases from the arch to the moat indicating an increase in the thickness of the crust beneath the moat. At ~4,070 km (Fig. 2), there is evidence that reflector M 'splits' at a two-way travel time of ~9.1 s. We interpret this split in the M-discontinuity as the result of a deep crustal layer underlying the oceanic crust beneath the moat and ridge. The top of the oceanic crust is characterized by a generally weak reflector (OC) with rough topography underlain by low frequency 'multiples'. Other strong reflectors are associated with the poor-to-well stratified material of the moat infill.

Figure 3 shows ESP 1 (Fig. 1*a*) obtained by *Conrad* and *Kana Keoki* near the Hawaiian arch south of the islands. The right-hand side of the figure shows a line of refracted arrivals which are nearly tangential to the top of oceanic crust and M-discontinuity reflectors. There are departures, however, from straight refraction lines. For example, at ranges of 5–25 km there is a convex downward curvature of the primary refracted arrivals, suggesting a continuous increase in velocity with depth, while at ~27.5 km there is a gap in primary arrivals, suggesting an abrupt decrease in the velocity gradient with depth. The CDP profile (Fig. 3) shows a strong reflector associated with the M-discontinuity between 8.15 and 8.45 s. The solid line at the ESP 1 mid-point on the CDP is the velocity-depth function obtained by τ-p mapping of the X-T data and τ-sum inversion. The velocity-depth function shows a progressive increase from 2.5 to 8.1 km s^{-1}. Abrupt changes in the velocity gradient are associated with the top of the oceanic crust and the M-discontinuity.

The principal results of the CDP data and ESPs 1-9 are summarized in Fig. 4. The velocity-depth data at each ESP mid-point and the depth to prominent reflectors on CDP 330/331 have been projected onto bathymetry profile A'B' (Fig. 1*a*). Figure 4 shows that the total crustal thickness increases from ~6.5 to 6.8 km beneath the Hawaiian arch to ~10.5–12.0 km

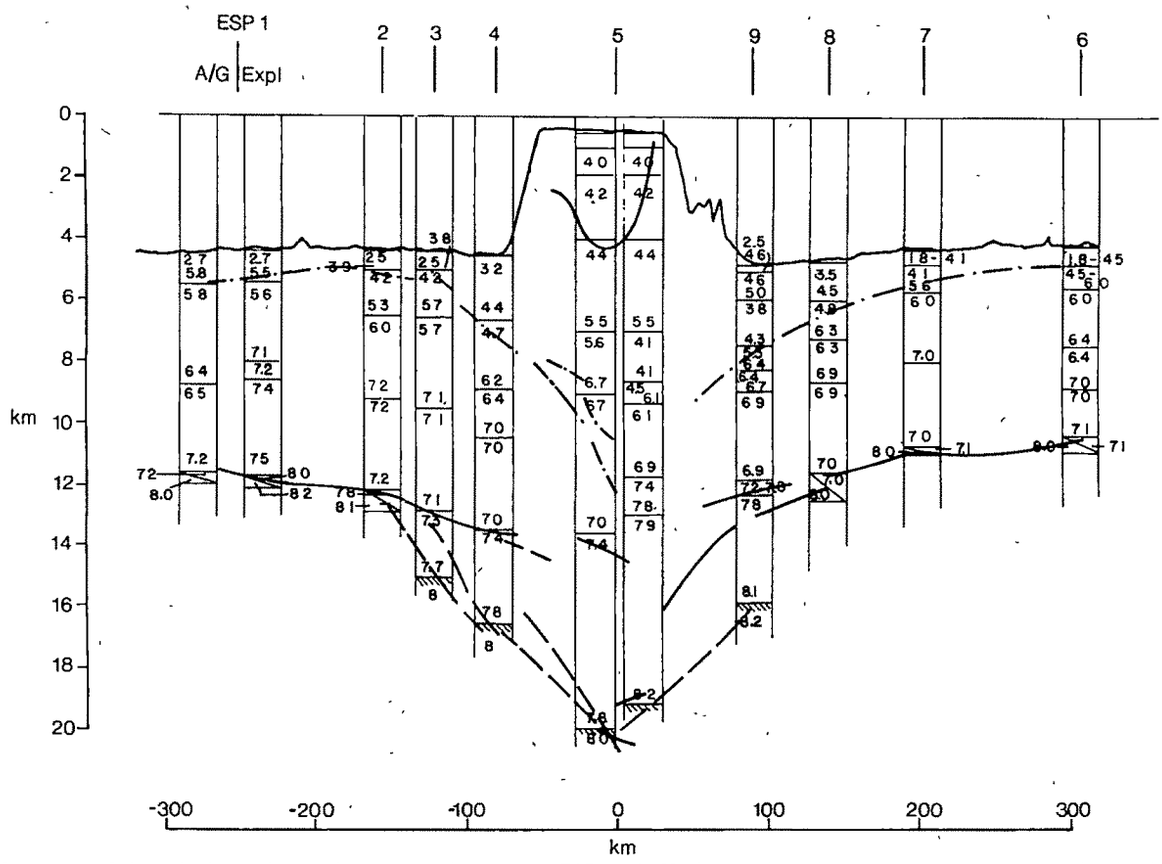


Fig. 4 Summary of the velocity and crustal structure obtained on ESPs 1-9. Horizontal lines in each column represents the depth to a prominent change in velocity with depth. The numbers refer to the velocity (km s^{-1}) immediately above and below the velocity change. The dashed-dot, continuous, and dashed lines represent prominent reflectors in Fig. 2 which have been converted to depth using the ESP velocity data results. These three reflectors are interpreted as corresponding to the top of the oceanic crust, the base of the pre-flexed oceanic crust and the *M*-discontinuity respectively. The ESP data have been plotted at the intersection of each ESP with profile A'B'. Note that two possible solutions are shown for ESPI and ESP5.

beneath the moat, and 18.0-19.0 km beneath the ridge. In the region of the arch, the crustal layer below reflector OC has a velocity which grades from 4.2 to 5.6 km s^{-1} (ESP 1, 2, 6, 7) to ~8.0-8.2 km^{-1}. Below reflector OC in the moat there is a sudden increase in velocity from 7.0 to 7.4 km s^{-1} at depths of 6.5 km below the sea floor (ESP 4) and from 6.9 to 7.2 km s^{-1} at 7.0 km to the north (ESP 9). At ESP 5 on the ridge, reflector OC cannot be as easily identified. Furthermore, the $X-T$ data cannot be interpreted in terms of a single velocity-depth profile. Figure 4 shows two possible solutions for ESP 5, both showing a similar upper crustal structure with velocities increasing from 4.0 to 5.5 km s^{-1}. At greater depths, however, the two solutions differ. The solution, which does not include a low-velocity zone (LVZ) in the upper crust, is characterized by a sudden increase in velocity from 7.0 to 7.4 km s^{-1} at depths of ~13.0 km below the sea floor, whereas the model with a LVZ shows a sudden increase from 6.9 to 7.4 km s^{-1} at depths of ~11.3 km. Above reflector OC, velocities are generally in the range 2.3-4.0 s^{-1}. At ESP 9 in the moat region, there is evidence from arrivals on $X-T$, τ-p and from amplitude data for a LVZ above reflector OC. We interpret this LVZ as the result of a high-velocity lava flow capping low-velocity material of the moat infill.

To constrain better the crustal model, we computed the gravity and geoid effect of the seismic structure and compared it with the observed free-air gravity anomaly profile obtained on *Conrad* and *Kana Keoki* and the geoid anomaly derived from SEASAT radar altimeter data. Because of uncertainties in the seismic interpretation at ESP 5 (Fig. 4), two models for the deep crustal structure of the ridge were considered: one in which the oceanic crust is thinned beneath the ridge (Fig. 5, model A) and one in which the crust is of normal thickness (Fig. 5, model B). Model A is consistent with the solution without a LVZ in the upper crust, whereas model B agrees better with the solution with a LVZ. For the purposes of the gravity and geoid calculation, the velocity-depth data near the arch and moat were generalized to an upper 4.2-6.0-km s^{-1} layer, a middle 6.0-7.1-km s^{-1} layer and a lower 7.4-7.8-km s^{-1} layer. Densities of 2.6, 3.0 and 3.15 g cm^{-3}, respectively, were assigned to these layers using the velocity-density relationships of Christensen and Salisbury[18]. We assigned a density of 2.3 g cm^{-3} for the material filling the moat, a density similar to the average density of vesicular Hawaiian flows[19] and in the range of values normally chosen for sediments. At ESP 5, the volcanic 'core' of the ridge is associated with velocities of 4.0-6.7 km s^{-1}. A density of 2.5-2.7 g cm^{-3} was assigned for the core of the ridge, similar to values normally considered for volcanic flows[19].

Figure 5 shows that there is a good overall agreement between the observed and the calculated gravity and geoid anomalies based on the seismic structure. Gravity and geoid data agree with the suggested thickening of the crust from ~6.5 km beneath the arch to 12.0 km beneath the moat and 18.0-19.0 km beneath the ridge. Furthermore, the data are consistent with the existence of a deep crustal layer underlying oceanic crust beneath the ridge and a portion of the flanking moat. The data cannot, however, distinguish whether the oceanic crust is thinned beneath the ridge or whether it has a similar thickness as beneath the moat and arch. Figure 5 shows that if oceanic crust is normal thickness beneath the ridge a high density body is required beneath it to compensate for the additional low density associated with the increase in thickness of the oceanic crust. The origin of the body is not clear, but gravity modelling suggests that it represents a deep seated extension of the dense volcanic pipe associated with the Koolau caldera on Oahu[20].

Implications

The seismically determined crustal structure of the Hawaiian ridge has implications for both the thermo-mechanical

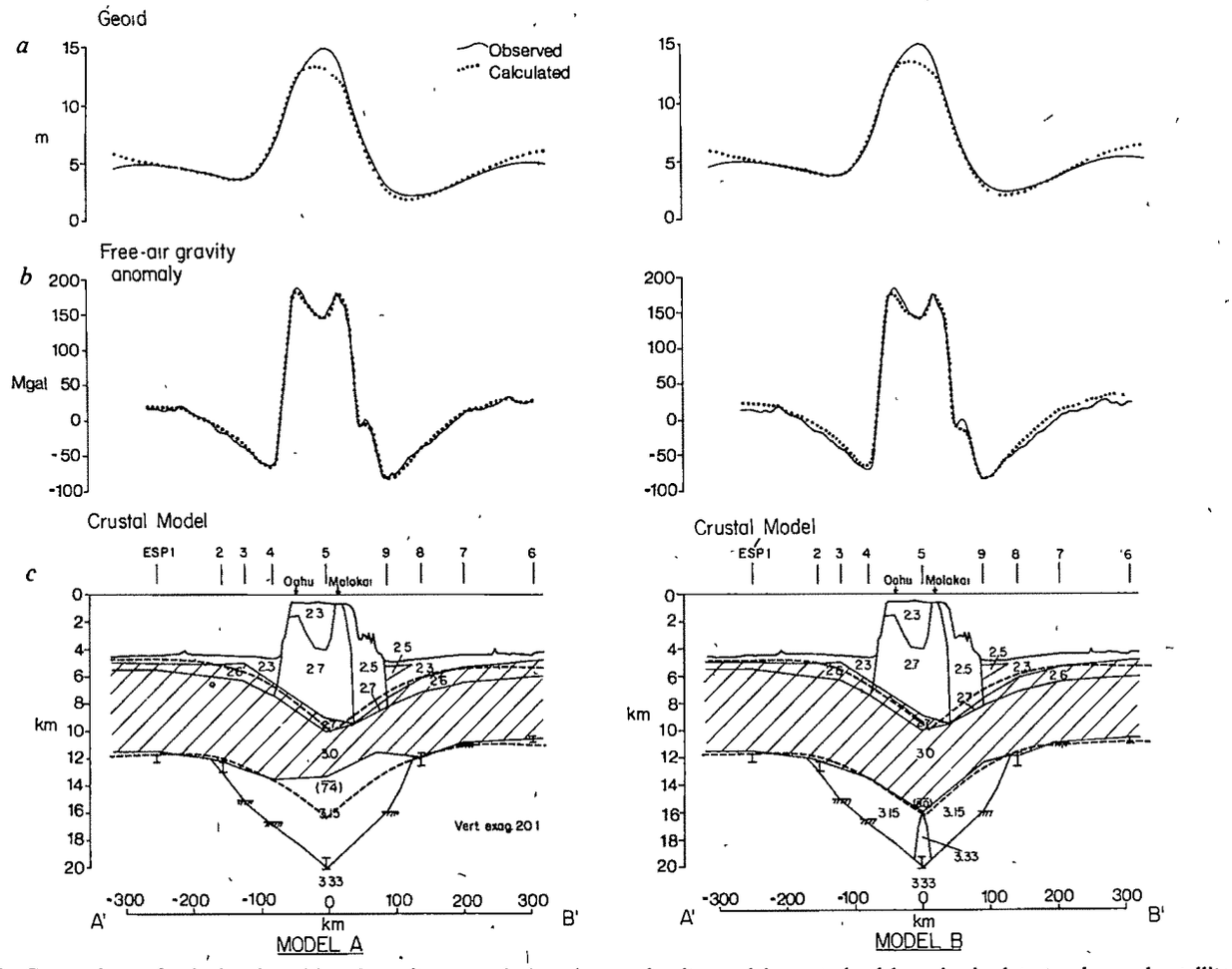


Fig. 5 Comparison of calculated geoid and gravity anomaly based on a density model constrained by seismic data to observed satellite derived geoid and surface-ship free-air gravity anomaly data. *a*, Geoid anomaly obtained by subtracting the geoid derived from a descending track of SEASAT from the GEM 10 Earth model complete to degree and order 12. *b*, Free-air gravity anomaly profile derived from data obtained by *Conrad* and *Kana Keoki* during COP A/COP B and COP E/COP F. *c*, Density model based on velocity-depth data in ESPs 1-9 and the velocity-density relationships of Christensen and Salisbury[19]. The configuration of the models (see text) have been adjusted so as to best-fit the gravity and geoid data and still explain the seismic data. Model A, thinned oceanic crust; model B, normal thickness oceanic crust. Heavy dashed lines are calculated curves based on a flexure model of isostasy with $T_e = 25$ km.

properties of oceanic lithosphere and petrological models for the evolution of mid-plate seamount chains.

The configuration of the oceanic crust near Oahu and Molokai is compared with predictions based on a flexure model of isostasy described in Fig. 5. In the model, it is assumed that the Hawaiian ridge and its associated infill material represent a two-dimensional load on the surface of an elastic plate overlying a weak fluid substratum. Thus, we assume that the only loads that act on the plate are surface loads and that buried loads[21] do not contribute to the flexure. Figure 5 shows that there is a reasonable agreement between the configuration of the upper surface of the oceanic crust and the calculated flexure profile for $T_e = 25$ km. There is also good agreement between the calculated profile and the configuration of the base of the oceanic crust for the model in which the oceanic crust is uniform in thickness beneath the ridge. Figure 5 shows, however, that the agreement is poor for the model in which the oceanic crust thins beneath the ridge. These results, therefore, suggest that beneath the ridge, flexed normal thickness oceanic crust is either underlain by a high density body (model B) or has been altered by densification in its lowest part (model A).

The flexure profile in Fig. 5 is based on a single value of T_e and a fractured plate model. Other values of T_e would be required if different types of flexure model are used. For example, if a continuous rather than a fractured plate had been used, then a lower value of T_e (~ 20 km) would be required. We prefer the fractured plate model (and hence the higher T_e value) because it best explains the shape of the top of the oceanic crust below the moat and the edge of the load. We caution against a too literal interpretation of the T_e value in Fig. 5, however, because it may be possible to construct other models (for example, a model in which T_e decreases gradually from beneath the arch to the ridge) that could explain the seismic, gravity and geoid data (Fig, 5) equally well. A value of $T_e = 25$ km can, therefore, be regarded as a 'best fit' to the regional crustal structure of the ridge and hence, a measure of the average response of oceanic lithosphere to volcanic island loading along the Hawaiian-Emperor seamount chain.

Oceanic flexure studies[10] have shown that T_e is a strong function of the age of the lithosphere at the time of loading. Thus, volcanic loads formed on young lithosphere are associated with small values of T_e, whereas loads formed on old lithosphere are associated with large values. There is a good agreement between T_e and the depth to the 450 °C oceanic isotherm based on the cooling plate model. Bodine *et al.*[22] have considered these results in terms of the rheology of oceanic lithosphere based on data from experimental rock mechanics and shown that

$$T_e \simeq 4.3t^{1/2}, \text{ 'dry' olivine rheology} \quad (1)$$

$$T_e \simeq 3.3t^{1/2}, \text{ 'wet' olivine rheology} \quad (2)$$

where t is age of the oceanic lithosphere (Myr) at the time of load emplacement. Using $t = 80$ Myr in equations (1) and (2) gives a predicted T_e of 30–40 km, which is somewhat higher than the value of T_e deduced here.

Although T_e at a volcanic load is a function for the thermal age of the underlying oceanic lithosphere[10], it may not represent the actual temperature structure of the lithosphere because of perturbing effects associated with magmatic processes and/or subsequent thermal events. Crough[23], for example, suggested that the Hawaiian swell is the result of thermal uplift following reheating of oceanic lithosphere by the Hawaiian hotspot. He estimated from bathymetric data that the 80 Myr lithosphere near Hawaii has been reheated to a thermal age of ~30 Myr. Substituting this value in equations (1) and (2) gives a predicted T_e of 18–23 km, which is similar to T_e estimated here. However, Sandwell[24] computed the response of a moving plate over heat sources with different shapes and has shown that the peak in the temperature distribution may be displaced ≤20 Myr downstream of the source. The age of the volcanic load near Oahu and Molokai is only ~2–3 Myr, so that the T_e estimated here cannot be used to constrain the reheating hypothesis. Thus, the strongest evidence for the swell remains the exponential form of its subsidence.

Although the extent of lithospheric reheating is uncertain, it is clear that a flexure model of isostasy with $T_e = 25$ km can explain the overall configuration of the oceanic crust near Oahu and Molokai. The model cannot, however, account for the 7.4–7.8 km s^{-1} velocity layer which seismic, gravity and geoid data suggest underlies flexed oceanic crust beneath the islands. This deep crustal layer is ~4 km thick, 200 km wide and generally symmetric below the centre of the ridge.

It is known from petrological studies that the Hawaiian volcanoes erupt lavas of different and distinct chemical compositions during their evolution. According to McDonald[25], the main stage of volcano building consists of large amounts of tholeiitic lavas which erupt rapidly (≤ 1 Myr; ref. 26). Following the main stage, the volcano usually erupts small amounts of alkali basalts. Finally, after a few Myr of quiescence, minor amounts of nephelinic lavas may erupt from satellite vents. The exact nature of the source region of the tholeiites and alkali basalts is not known[27,28] but seismicity studies near Hawaii[29] suggest that magma transfer associated with the tholeiitic stage of volcanism extends to depths of at least 50–60 km. The nephelinic lavas are considered to be more primitive and to have originated from an even deeper source.

Seismic studies provide constraints on the velocity and density structure of the crust and upper mantle beneath a volcano and hence are of importance in developing petrological models. For example, seismic data[30] suggest that Mount Etna is underlain by a deep seated magma chamber.

From a consideration of all the seismic data obtained during the experiment, we believe that the most probable interpretation of the deep crustal body underlying flexed oceanic crust beneath Oahu and Molokai is that it represents a deep crustal sill complex. In particular, the body represents 'frozen' intrusions of tholeiitic composition into upper mantle rocks that were trapped in their ascent from a deeper source during the late phase of the tholeiitic stage or during the following period of quiescence. Such a model of a crust–mantle 'mix' agrees with seismic data showing that the deep crustal layer is intermediate in velocity between that of lower crust (gabbro) and upper mantle (peridotite) rocks. It is also in agreement with evidence[31,32] which shows an absence of a deep crustal body below Kilauea and Mauna Loa on Hawaii. These volcanoes seem to be in the early phase of the tholeiitic stage and, therefore, would not be expected to be underlain by such a sill complex.

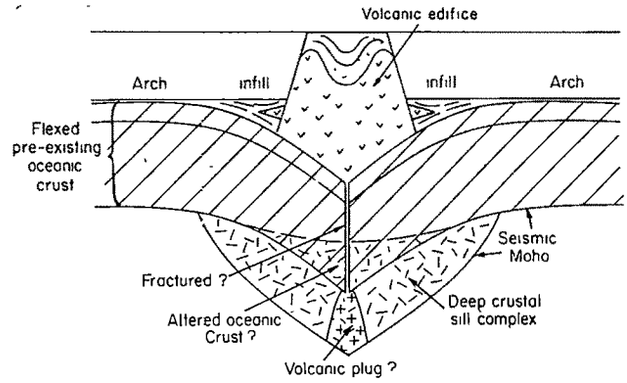


Fig. 6 Schematic model of the crustal structure of the Hawaiian ridge and vicinity based on the seismic, gravity and geoid data discussed here. The model has been generalized from models A and B in Fig. 5.

Figure 6 shows a schematic model for the deep crustal layer beneath Oahu and Molokai that explains the geophysical data and is consistent with current petrological knowledge. In proposing this model, we have eliminated (on density arguments) the possibilities that the deep layer represents either a magma chamber of basic picritic magmas which become ponded at or near the crust/mantle following their ascent through the mantle[33], or dunites and plagioclase-bearing pyroxenites that formed as early cumulates in a fractionating tholeiitic magma chamber[28].

The deep structure of oceanic islands and seamounts, however, is still too poorly known and other explanations may be proposed as more data become available. The seismic experiment described here was the first of its kind to be carried out over a mid-plate seamount chain. We believe that multichannel seismic studies of other seamounts offer particular promise of better understanding the origin of mid-plate volcanism in the future.

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Novel type of crust produced during continental rifting

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A normal oceanic crust cannot be generated after continental crust splitting of rifts filled by a thick pile of young sediments. The magma released by the rising mantle is trapped mainly in these sediments as basalts sills and plugs. The sediments are themselves metamorphosed by the heat flux from the underlying mantle. A metasedimentary crust is formed in direct contact with an abnormal mantle ($V_p \simeq 7.5$ km s^{-1}) produced by partial serpentinization.

THE transition from continental to oceanic crust in a rift may occur in a number of different ways. One model involves the stretching of the continental crust to a thickness comparable with that of the oceanic crust, followed by a transition to this new oceanic crust. This model has been documented by the study of the Atlantic Ocean passive margins[1,2]. Another model predicts a brittle behaviour of the continental crust which would be progressively invaded by mafic dykes, its extension being that of the accumulated thickness of the dykes, and would finally yield without stretching along the mechanically weakened zone of dyke intrusion, passing more abruptly to oceanic crust[3,4]. Coleman[5] describes an intermediate behaviour of dykes and igneous bodies with crustal extension and multiple intrusions which seem to produce locally a complete disruption of the continental crust on the Arabian side of the Red Sea.

In these models, the nature of oceanic crust is little questioned, despite geophysical records which are not always those of typical oceanic crust. It is also implicitly accepted that an oceanic crust can be formed whatever the sedimentary filling of the rift. Is it possible to form a normal oceanic crust, just after the splitting of continental crust, in a trough filled with kilometres of wet sediments? Saunders *et al.*[6] have shown that in the Guaymas Basin of the Gulf of California, where the sedimentation rate can exceed 1,200 m Myr^{-1}, the oceanic layers 1 and 2 are partially intercalated, the young sediments being intruded and metamorphosed by many doleritic and gabbroic sills. Here, I show that in such environments the situation may be even more radical with a total absence of layer 3 and a modified upper mantle lying directly below metamorphosed sediments invaded by basaltic intrusions. Thus, I define a new type of crust, the metasedimentary crust, transitional between the continental and oceanic crusts.

I will discuss geophysical evidence from two well-known rifting environments (Salton Trough at the north end of the Gulf of California, and the southern Red Sea) and from the western Pyrénées where, starting from a similar situation during the Cretaceous[7], it is now possible to observe the deep parts of the crust and the upper mantle peridotites, from mountain building data. The study of these peridotites supports my interpretation. Finally, I suggest an answer to the problem of the crust or mantle nature of the 7.2–7.5 km s^{-1} layers often found in non-typical oceanic lithosphere at depths between crust and mantle.

Salton Trough

The Imperial Valley–Salton Sea subsiding rift corresponds to the transition of the oceanic crust of the Gulf of California to the continental crust of southern California. This rift is opening in a system of large dextral strike-slip faults limited to the north-east by the San Andreas fault. It is rapidly subsiding with the Pleistocene Colorado River delta formations now at depths of 3.7 km below the Salton Sea and 4.8 km below the Mexican border[8].

In the trough below sedimentary layers, Fuis *et al.*[8] define a basement ascribed to deeper sediments metamorphosed in the greenschist facies (Fig. 1). This is based on the geophysical properties of this zone and on thermal measurements in deep wells, the extrapolation of which fix the top of the basement at 300 °C for a depth of 6 km. Basaltic intrusions occur in this basement. At a depth of ~12 km, one passes into the sub-basement through a 1-km thick transition zone. This sub-basement is ascribed to a mafic layer (gabbros and/or amphibolites) representing the oceanic crust on behalf of a P-wave velocity $V_p = 7.2$ km s^{-1} and a modelled density of 3.1 (Fig. 1). The 7.2 km s^{-1} velocity, however, is a lower limit; velocities of 7.5–8.0 km s^{-1} have also been reported. A recalculation of the same data gives an average velocity of 7.5 km s^{-1}. Here I reinterpret the sub-basement as anomalous mantle rather than mafic crust.

Fitting a P-wave velocity of 7.5 km s^{-1} and a density of 3.1 with an oceanic lithosphere formation can be done by a comparison with experimental data on ophiolite specimens or from a direct comparison with *in situ* geophysical data in the oceanic lithosphere. Ascribing to the upper sub-basement pressure–temperature (P–T) conditions of ~4–5 kbar and 400–600 °C, the appropriate comparison should be done with a young crust rather than with a nascent or a mature oceanic lithosphere.

Measurements on gabbros and metagabbros at 4-kbar confining pressure and room temperature give a wide range of velocities with mean values respectively of 7.14 km s^{-1} and 6.92 km s^{-1} for a few west American ophiolites[9]; data from Oman[10] or from the Bay of Islands[11,12] do not differ significantly. Correction for an assumed temperature of 500 °C would lower these values by ~5% (ref. 13). Density measurements on gabbros and metagabbros at normal P–T conditions on the same specimens as above give 2.95 and 2.92, respectively. Data from the

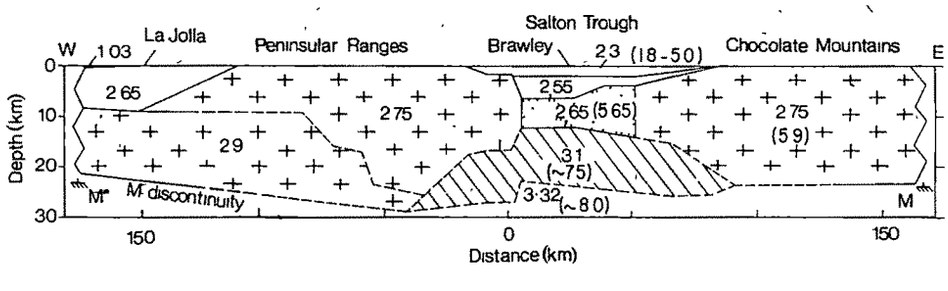


Fig. 1 Cross-sections through the Salton Trough integrating seismic (velocities in bracketed numbers) and gravimetry (plain numbers) data. Dots, metasedimentary basement; crosses, continental basements; oblique hatches, sub-basement (after Fuis *et al.*[8]). The 7.5 km s^{-1} velocity adopted for the sub-basement is derived from ref. 8 and from recalculated velocities using their data.

Table 1 P-wave velocities in layer 3 and upper mantle of young lithospheres

	Reykjanes[38]	Iceland[39]	Galapagos[40]	EPR* 10° S[40]	EPR Siqueiros[41]	EPR 12° N[42,43]	EPR 14° N[44]	Gulf of Aden[45]	Kenya[46]
V_p in layer 3 km s^{-1}	6.5	6.5	6.5	~6.8	7.0	6.8–7.2	6.8–7.0	6.3	
V_p in upper mantle km s^{-1}	7.3–7.4	7.2	7.5	7.5	7.8	8.0	7.75	7.2	7.3–7.5

* EPR, East Pacific Rise.

Table 2 Comparisons between Salton Trough P-wave velocities and other estimates

	Salton Trough sub-basement 4 kbar/500 °C	Gabbro metagabbro 4 kbar/500 °C	Layer 3 young crust	14% Serpentinized peridotite 4 kbar/500 °C	Upper mantle young lithosphere
V_P km s^{-1}	~7.5	6.57–6.78	6.3–7.2 (6.7)	7.34	7.4–8.0 (7.5)
D g cm^{-3}	3.1	2.85–2.88 ≤3.08		3.12	

Figures in parentheses represent average values from Table 1.

Bay of Islands and Oman show comparable densities with lower gabbros reaching densities of 3.15. Recalculating these densities for $P = 4$ kbar and T = 500 °C with compressibility and thermal expansion coefficients of 1×10^{-6} bar^{-1} and 24×10^{-6} deg^{-1}, respectively, would lower these densities by ~0.07, bringing them in the range of 2.88 and 2.85, with the maximum density lowered to 3.08.

An ultrasonic compressional wave measured along the main structural directions on a peridotite with 14% serpentine at 4 kbar gives an averaged velocity of 7.63 km s^{-1} (ref. 14). The results are similar using Christensen's[15] analytical data: 7.6 km s^{-1} for a peridotite (2/3 olivine, 1/3 enstatite) with 15% serpentine. Using the temperature derivative calculated for a comparable peridotite[16], one finds for $P = 4$ kbar and $T =$ 500 °C, a velocity of 7.35 km s^{-1}. The density measured on the same specimen was 3.2, which after the pressure and temperature correction, becomes 3.12.

P-wave velocity data for young oceanic lithosphere and rifts have been compiled in Table 1. When possible, the data for age zero have been avoided. Velocities in the upper mantle are commonly anisotropic, in which case they have been averaged.

Table 1 indicates that anomalous mantle velocities ~7.5 km s^{-1} are common below young oceanic crust where they are ascribed to the strong temperature effect on this velocity. With ageing, these velocities would increase to the more common 8.2 km s^{-1} value[17]. Table 2 shows that a peridotite with 10–20% serpentine fits equally well the data for Salton Trough.

Considering the fact that in the Salton Trough a source of water is found in the overlying sediments undergoing metamorphic reactions, I suggest that the upper part of the sub-basement and the transition zone are composed of partly serpentinized peridotites. The serpentinization decreases downward partly because it is difficult for the water to penetrate deep into the peridotites, but principally because the upper temperature limit for serpentinization (500 °C) is met at shallow depth in the sub-basement. At higher temperatures, tremolite–chlorite and talc can be present, contributing to the thermal effect and maintaining low velocities.

Southern Red Sea

Although the geophysical data are older and less tractable, a comparable situation with the Salton Trough seems to exist in the southern Red Sea, south of the 17° parallel. The magnetic anomaly pattern suggests the existence of oceanic crust in the central and southern Red Sea[18,19]. This is confirmed for the central Red Sea by seismic refraction data[20]. There, material with a mean seismic velocity of 4.3 km s^{-1} and thought to be evaporites, overlies a layer of mean velocity 6.6 km s^{-1}, thought to be oceanic crust.

In the southern Red Sea, seismic refraction and gravimetry profiles[21] and structural reconstitutions[22] in the 50–100-km-wide central trough define sedimentary layers 2–5 km in thickness with velocities from 2.75 to 4.48 km s^{-1}, overlying directly a 7.16–7.31 km s^{-1} domain. These overlying layers are evaporite sediments[22] associated with mafic formations. Seismic reflection profiles[23] suggest the occurrence of volcanic strata and plugs in the sediments. There is a general consensus that the underlying domain is mantle[22–24] whose anomalously low velocities are related to the locally high thermal gradient. Similarly, low mantle velocities have been reported from the adjacent areas[24] (Afar, Danakilh, Jibuti, Gulfs of Tajura and of Aden).

The abundance of mafic intrusions in the southern Red Sea and along its margins can account for the magnetic anomalies mentioned above and do not necessarily imply the presence of an oceanic crust that is not supported by the available geophysical record. Equating the 7.2 km s^{-1} basement with anomalous mantle, we conclude that this mantle is directly overlain by a sedimentary cover with mafic sills or flows and plugs. New seismic refraction profiles would be necessary to test this interpretation.

Western Pyrénées

During the Pyrenean orogeny, small and deep sedimentary basins, possibly analogous to those described above[7] have been squeezed, making it possible to obtain some information on the nature of their substratum.

These sedimentary troughs were filled by flysch during their opening. Thus, 3–6-km-thick flysch was accumulated rapidly in the western Pyrénées where the troughs are best developed. After a period of Triassic continental rifting followed by a relative quiescence, the rifting was reactivated during the Upper Jurassic–Lower Cretaceous[25], with the formation of the troughs under consideration. The precise mechanism for their opening is still unknown. The troughs may open by a pull-apart mechanism related to the essentially sinistral motion of the Iberia block with respect to Europe[7], or there may be a period of extensional tectonics within Iberia, followed by the strike-slip motion and the compression responsible for the mountain building event[26]. The first model seems to better explain the larger opening observed in the western Pyrénées. The content of the troughs has been metamorphosed. Temperatures as high as 600 °C were attained locally during the Albian–Cenomanian phase of the rifting[27]. During the subsequent compressional phase, they were still high enough to produce metamorphic effects.

In the eastern Pyrénées, the continental crust was probably only attenuated during stretching[28]. The mainly calcareous sediments from the basins contain small mafic and lherzolitic bodies, the latter associated with granulite slivers derived from lower

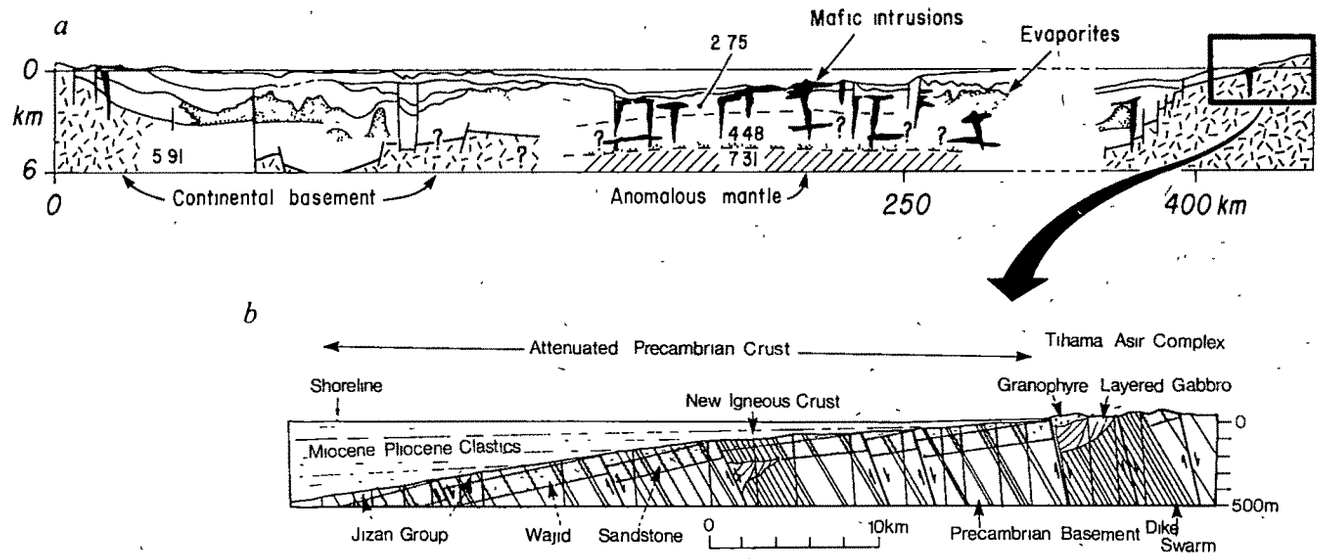


Fig. 2 Cross-section through *a*, southern Red Sea (near 17° parallel) and *b*, Saudi Arabia passive margin, illustrating the contrast in mafic magmatic activity: in the Red Sea trough, mafic sills, plugs and flows and along the passive margin, local creation of oceanic crust (ophiolites). *a*, Modified from Mulder *et al.*[23] by adding main seismic refraction results[21] and presumed deeper mafic intrusions in the 4.48 km s^{-1} layer; *b*, reproduced from Coleman[5].

continental crust and modified at depth by the thermal effects of the lherzolites[29]. These numerous lherzolite bodies are remarkably fresh with a plastic deformation indicative of large strain in the $T \approx 1{,}000$ °C range, but locally down to 650–700 °C (ref. 30), which we ascribe to their ascent.

In the western Pyrénées, the continental crust was probably disrupted[26–28]. This is suggested by the nature of the troughs and the large gravity anomaly. The small peridotite massifs upthrust during the orogeny are more harzburgitic (F. Boudier and F. Cordellier, personal communication). They are also largely serpentinized (lizardite), essentially at low temperatures and in static conditions, but with relicts of a high temperatures earlier stage in the greenschist facies (antigorite–tremolite–chlorite). The mafic intrusions are mainly ophites which are metamorphosed tholeitic dolerites, intrusive within the Triassic evaporites in remarkably abundant and large (kilometre-sized) sills and plugs, and alkali-basalt sills and plugs related to the Albian rifting. The ophites are considered to be related to the Triassic period of rifting[31] because they do not intrude younger formations. But this may result from the location of Triassic evaporites at the bottom of the troughs or to their mechanical properties; the ophites could be related to the Cretaceous rifting as suggested by other authors[32].

Discussion

A new analysis of the geophysical data on the Salton Trough and southern Red Sea rifts leads to the conclusion that after splitting of the continental crust a new lithosphere can be created with a metasedimentary section directly above the mantle formations and without a normal oceanic crust. The existence of this metamorphism is deduced from heat-flow data[8]. It is also imprinted in the Red Sea evaporitic sediments in contact with the Zabargad Island intrusion of hot spinel-feldspar lherzolites[33]. The metasediments are intruded by mafic sills and plugs[23]. In the Red Sea, there is a striking contrast between the style of mafic magmatism in the continental crust (Fig. 2*b*) and in the evaporitic sediments of the trough (Fig. 2*a*).

The Mesozoic Pyrenean troughs seem to represent a similar situation, at least in the western part where the continental disruption was probably complete, a stage not attained in the eastern part. This interpretation is supported by the comparative study of the intruded peridotite bodies.

The scenario proposed for the western Alps lherzolites[34] can be applied to spinel lherzolites from the eastern Pyrénées, which are very similar. They would be derived from a diapiric asthenosphere uprise below a continental rift. Because of a particularly slow ascent rate in relation to a presumed slow (<1 cm yr^{-1}) opening rate, the partially molten asthenosphere meets the thermal conduction front of the lithosphere during its ascent at ≈30 km, where the melts carried so far start crystallizing[34]. This explains the comparatively fertile character of the lherzolites, their equilibration in the spinel-lherzolite field, the limited amount of known associated magmatism and the low temperature characteristics of their subsequent plastic deformation, which records their further ascent and possibly the first stages of the rift closure. It has been proposed recently[35] that this lherzolite diapir has disrupted the continental crust and intruded through the rift sediments. This interpretation, proposed in the case of feldspar-lherzolites[34,36], seems unlikely for the spinel lherzolites considered here.

In the western Pyrénées, the occurrence of harzburgitic lherzolites is symptomatic of the creation of oceanic lithosphere[34] and of substantially larger spreading rates there than in the east[36]. In Boillot's model[26], it is possible to estimate the opening rate at ≈1.3 cm yr^{-1}, which is sufficient to generate an oceanic lithosphere in terms of the amount of melting (20%) and of the harzburgitic nature of the residual mantle[36]. However, the oceanic mantle would be directly overlain by metamorphosed sediments, as suggested by the absence of oceanic crust fragments associated with the harzburgite bodies. The large and numerous ophite intrusions could represent the mafic products generated by the mantle melting, but this would imply that they are not Triassic (see above). The extensive serpentinization of the harzburgite bodies, contrasting with its scarcity in eastern lherzolites, would be partly achieved at high temperatures by water contamination from the overlying metasediments and partly during and after the squeezing up of the peridotites through the trough's formation. This is recorded by the serpentinization sequence, showing first a high temperature alteration (antigorite–tremolite–chlorite), followed by a low temperature one (lizardite).

Such a partial and early serpentinization of the mantle section beneath metamorphosed sediments may explain the seismic and gravimetric data obtained in the Salton Trough[8]. In particular, the upper sub-basement could correspond there to a peridotite with 15–20% serpentine.

Conclusions

Based on the data presented here, I propose that in certain circumstances a new type of crust can be created during the rifting of a continent. During the disruption of the continental crust, this new crust would be composed of a thick sedimentary

sequence, metamorphosed at its base in the greenschist or the amphibolite facies depending on its thickness and be invaded by sills and plugs of kilometre-sized mafic intrusions. The immediately underlying anomalous mantle ($V_p \simeq 7.5$ km s^{-1}) would be composed of hot peridotites and/or of partially serpentinized peridotites. The latter interpretation agrees with the one proposed by Hess[37], in which a lower oceanic crust is formed by serpentinized peridotites. The formation of this special crust would require conditions in which the creation of a normal oceanic crust would be impossible. Such conditions could occur when the transition from continental to oceanic lithosphere occurs in a trough filled with a thick pile of young sediments, a situation opposed to that of a starved rift[1]. The basaltic magma released by the underlying mantle diapir into the immature sediments does not meet conditions to create a volcanic cap below which a plutonic section can fractionate. On the contrary, as already proposed by Saunders *et al.*[6], the magma reacts with these sediments and is frozen in them as sills and plugs, thus contributing to the metamorphism of the sediment. Dolerite and gabbro sills material and metamorphosed sediments have been recovered by DSDP drilling in the Gaymas Basin of the Gulf of California[6] which, as mentioned above, seems to correspond to an appropriate environment.

It may be of interest to contrast the situation on the Arabian edge of the Red Sea and in the trough itself (Fig. 2). On the edge, the disruption of the continental crust with a little and old sedimentary cover leads directly to the creation of typically ophiolitic segments of oceanic crust, whereas in the trough where young and wet sediments have accumulated, the geophysical record does not show the existence of such oceanic crust but suggests a direct contact between sediments and serpentinized mantle.

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Heritable formation of pancreatic β-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes

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Following the transfer into fertilized mouse eggs of recombinant genes composed of the upstream region of the rat insulin II gene linked to sequences coding for the large-T antigen of simian virus 40, large-T antigen is detected exclusively in the β-cells of the endocrine pancreas of transgenic mice. The α- and δ-cells normally found in the islets of Langerhans are rare and disordered. Well-vascularized β-cell tumours arise in mice harbouring and inheriting these hybrid oncogenes.

INSULIN, a polypeptide hormone involved in the control of carbohydrate metabolism, is synthesized and stored in the β-cells of the endocrine pancreas, from which it is released into the bloodstream in response to various signals. The endocrine pancreas is comprised of isolated islands or islets, which are dispersed throughout the larger mass of the exocrine pancreas; the islets consist of four major cell types, α, β, δ and PP, which synthesize the hormones glucagon, insulin, somatostatin and pancreatic polypeptide, respectively[1,2]. Rat and human insulin genes have been cloned and analysed[3–13]. There are two non-allelic rat insulin genes that differ in nucleotide sequence and in the number of introns; the rat insulin I and II genes have one and two introns, respectively[5,6]. The two genes share a region of considerable sequence homology in the 5′ flanking region, extending ~300 base pairs (bp) upstream of the point of transcriptional initiation[12]; the human gene is 75% homologous over the first 240 bp of this region[8]. Expression of hybrid genes using the 5′ flanking regions of both the human insulin and rat insulin I genes occurs when they are introduced transiently into cultured cells derived from an insulinoma[11].

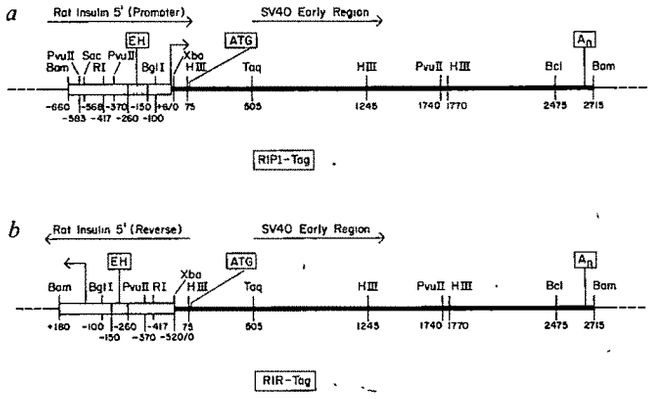


Fig. 1 Structure of recombinant insulin/large-T antigen genes. The plasmids pRIP1-Tag (*a*) and pRIR-Tag (*b*) consist of the coding information of the SV40 early region fused to sequences derived from the 5′ flanking region of the rat insulin II gene. Boxed regions denote insulin gene flanking DNA and the thick solid line refers to the SV40 early region. The cap site where insulin gene transcription initiates is indicated by an L-shaped arrow, and the associated transcriptional enhancer element[11] (EH) is shown (the insulin gene promoter, which lies between them, is not indicated). The points of SV40 large-T antigen translation initiation and transcription termination are indicated by boxed ATG and A_n respectively. The *Bgl*I site in SV40 was converted to an *Xba*I site using an oligonucleotide linker, and the *Xba*/*Bgl*I to *Bam* fragment comprising the SV40 early region[24] was linked to two different orientations of the insulin gene flanking region. This fragment of the SV40 early region includes the early mRNA cap sites, but lacks all known components of the SV40 early promoter. For *RIP1-Tag*, an *Xba* linker was inserted into the *Dde*I site at +8 bp relative to the point of initiation of insulin gene transcription, and the *Bam* to *Dde*/*Xba* fragment from −660 to +8 was combined with the *Xba*/*Bam* fragment of the SV40 early region and inserted as a *Bam* linear into a derivative of pBR322 that lacks the R1 site, and includes a *lacUV5* promoter in place of the R1 to *Bam* fragment of pBR. For the plasmid pRIR-Tag, which carries the insulin flanking region in the opposite orientation, a *Dde* site at −520 was converted to an *Xba* site, and the *Bam*/*Xba* fragment extending from +180 nucleotides past the cap to −520 bp upstream was combined with the structural gene for large-T antigen and inserted into the same plasmid vector. The two hybrid genes were similarly prepared for microinjection, except that for *RIR-Tag*, the *Bam* insert was first purified from the plasmid vector by gel electrophoresis and electroelution, followed by passage over a DEAE-Sephacel column and pRIP1-Tag was linearized using *Sal*I. The DNAs were extracted with phenol, chloroform, precipitated in 70% ethanol, resuspended in 10 mM NaCl, 5 mM Tris *p*H 7.4, 0.1 mM EDTA and passed through a 1-ml spin column of Sepharose CL6B, after which the DNA was diluted to concentrations of ~75 copies per pl for pRIP1-Tag/*Sal* or ~20 copies per pl for the *RIR-Tag*/*Bam* insert. The DNAs were injected into fertilized one-cell embryos essentially as described[14,16], except that the injected embryos were cultured overnight to the two-cell stage before being re-implanted into the oviducts of pseudopregnant females. The F_2 embryos were derived from matings of B6D2F1 (C57BL/6J × DBA/2J) males and females, obtained from the Jackson laboratory.

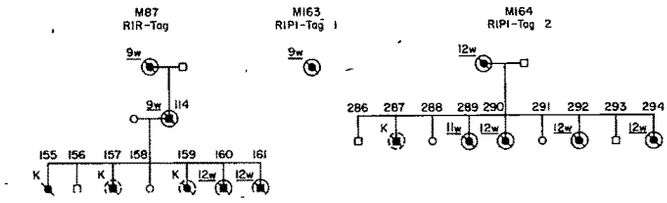


Fig. 2 An early pedigree of insulin/large-T antigen transgenic mice. A partial pedigree analysis is presented, to examine the heritability of the phenotype of premature death and islet hyperplasia. Females are indicated by open circles, males by open squares and transgenic mice by solid circles and squares. The circled slash denotes premature death, with the age at death shown in weeks (w). A slash alone with a superscript K indicates the animal was killed and the dotted circle around a killed mouse denotes evidence of islet hyperplasia following histopathology. (M155 was killed but not examined for hyperplasia.) Each original transgenic mouse is assigned a genotype consisting of the name of the gene that was injected and acquired followed by a number which distinguishes it from the other independent mice carrying the same gene. Therefore, progeny of a particular mouse can be readily identified as such (for example, all mice of genotype *RIP1-Tag2* are progeny of the original transgenic mouse 164).

Fig. 3 (Opposite) Histology and pathology of normal and insulin/T-antigen transgenic mice pancreas. *A*, Cresol violet-stained sections of a normal pancreas and a pancreas derived from a transgenic mouse bearing solid tumours. *a*, Normal (non-transgenic) pancreas with a single islet in the centre and an adjacent blood vessel (×50). *b*, Part of a solid tumour (one of those pictured below) near two normal-sized islets (at the top) in M384 of the *RIP1-Tag2* line (×50). *c*, A normal-sized islet from a non-transgenic mouse (×200). *d*, A normal-sized transgenic islet (from *b*) (×200). In all cases, exocrine cells show purple cytoplasmic staining with somewhat deeper-coloured nuclei, whereas endocrine cell nuclei stain dark violet with minimal cytoplasmic staining. Note the denser packing of nuclei in all sizes of islets derived from the transgenic mouse relative to the normal mouse islet. Compare the normal islet in *a* with the two small islets near the top of *b* and the higher magnifications in *c* and *d*. The extent of cytoplasmic staining observed in the exocrine tissue varies with the length of time the sections were stored before treatment with cresol violet. *B*, Comparison of a whole pancreas from a normal mouse (*a*) with that of M384 (*b*), showing several vascularized solid tumours in the transgenic pancreas (×25).

Methods. Sections (14 μm) were taken on a cryostat from pancreas flash frozen in liquid nitrogen, stored at −70 °C and then mounted in tissue Tek OCT embedding medium and sectioned at −20 °C on to gelatinized microscope slides. The sections were fixed for 10 min in 4% paraformaldehyde in phosphate-buffered saline (PBS), washed in PBS and air dried. Sections were then rehydrated in PBS, treated in filtered cresol violet for 2 min, washed for 5 min in two changes of 70% ethanol, then washed twice for 2 min each in 90% ethanol, 100% ethanol and xylene and then mounted under coverslips in Gurr Fluromount. The cresol violet solution consists of 0.25% (w/v) thianin that had been dissolved by heating in 200 mM hydroxyacetate plus 26 mM NaOH and stored at 50 °C.

Here I seek to address aspects of insulin gene expression by the introduction of recombinant insulin genes into the mouse germ line, using microinjection of DNA into fertilized mouse embryos. This technique has been used previously to examine the control and consequences of expression of a number of genes[14–23], including two oncogenes. The complete simian virus 40 (SV40) early region, including the viral transcriptional enhancer and promoter, is not expressed at detectable levels in normal tissues of transgenic mice harbouring it, but choroid plexus tumours heritably arise, and these tumours produce high levels of large-T antigen[19]. The cellular proto-oncogene c-*myc*, when linked to the long terminal repeat of mouse mammary tumour virus, is expressed in a number of tissues, including mammary cells, and two lines of mice heritably develop breast tumours[20].

The recombinant oncogenes used here consist of regulatory information associated with the rat insulin II gene linked to protein coding information for the oncogene SV40 large-T antigen (Tag). The rationale for using such a hybrid is threefold: (1) to examine the ability of the insulin sequences to mediate correct tissue- and cell-type specific expression in the β-cells of the endocrine pancreas, using a readily identified and distinguishable marker protein (a viral antigen); (2) to examine the consequences to the organism of tissue-specific expression of an oncogene, in particular the possibility that SV40 large-T antigen might induce β-cell proliferation and consequent oncogenesis; and (3) to assess the prospects that oncogenes can be used to facilitate the establishment of stable cell lines from rare cell types specifically expressing such fusion genes. This report describes analyses pertaining to the first two of these questions.

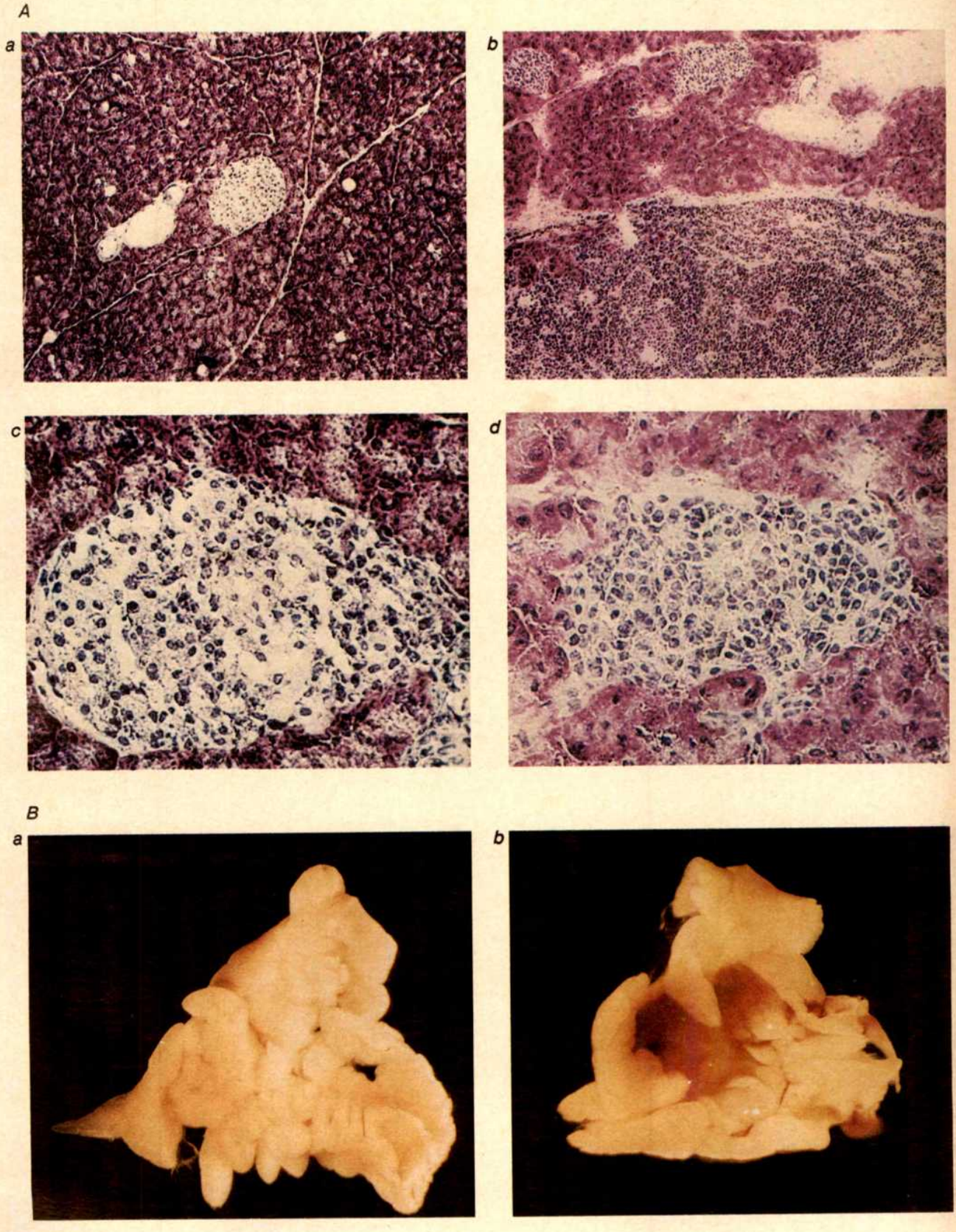


Fig. 3

Hybrid gene constructs

Two hybrid genes were constructed to combine DNA 5′ to the coding sequences of rat insulin II gene with the large-T antigen structural information. This 5′ flanking region includes the insulin gene promoter as well as a transcriptional enhancer element that stimulates initiation of transcription in cultured insulinoma cells but not in fibroblasts[11]. The first configuration, denoted *RIP1* (rat insulin promoter 1) carries the insulin control region aligned to promote transcription of the large-T antigen gene (Fig. 1). The two genes were linked together in regions corresponding to the 5′ untranslated portion of each messenger RNA. The resulting hybrid gene, *RIP1-Tag*, thus comprises a complete transcription unit with 660 bp of sequence located 5′ to the point of transcriptional initiation of the insulin gene linked to protein coding and termination information from the early region of SV40.

The second configuration, denoted *RIR-Tag* (rat insulin reverse), includes 520 bp of DNA 5′ to the insulin gene and the first 180 bp of the transcribed portion of the gene. In this hybrid, the promoter element for insulin gene transcription is inverted with respect to the coding information for SV40 large-T antigen, and, therefore, does not comprise a fusion in the 5′ untranslated regions of the two genes, but rather includes an inverted promoter and enhancer region linked to the *Tag* structural gene, as shown in Fig. 1. Both hybrid genes lack the SV40 early promoter and transcriptional enhancer element, and each includes protein coding information for SV40 small-T antigen, as well as for large-T, because the two proteins are derived from the primary transcript of the SV40 early region by differential splicing of overlapping introns[24].

The two fusion genes were injected into fertilized one-cell mouse embryos, which were then inserted into the oviducts of pseudopregnant female mice and allowed to develop. Four transgenic mice were produced from the *RIP1-Tag* injections and one from the *RIR-Tag* injections. The apparent heterozygous copy number (per diploid genome) of the acquired DNA ranged from ~1 for mouse 87, which carries *RIR-Tag*, to ~5 for mouse 163, which carries and is genotyped as *RIP1-Tag1*, and 5 for mouse 164 (*RIP1-Tag2*). Two of the founder mice (165 and 168) were mosaic for the acquired transgene, using the criteria of infrequent transmission to progeny and lower apparent copy number in the founder than in its progeny. The non-mosaic progeny of M165 (*RIP1-Tag3*) have copy numbers of ~25, whereas those of M168 (*RIP1-Tag4*) have ~12 copies per diploid genome. In each case of multiple copies, genetic and biochemical analysis indicates that the genes are heterozygous and integrated in a single location (data not shown).

Phenotype of transgenic mice

During the initial breeding analysis of the insulin large-T antigen transgenic mice, a phenotype of premature death emerged. The three original non-mosaic mice died at 9-12 weeks of age. Two of these transmitted the transgene to offspring before dying, whereas the third (M163) did not. Surprisingly, this phenotype appeared with the *RIR-Tag* gene, which has the insulin promoter inverted with respect to the large-T antigen coding region, as well as with the *RIP1-Tag* gene, in which the insulin promoter is aligned to transcribe the large-T antigen gene.

The phenotype of premature death proved to be heritable and co-segregated with the acquired hybrid gene; the partial pedigree in Fig. 2 illustrates this observation. The penetrance is virtually complete in the early generations. The analysis that follows deals primarily with the progeny of the two original non-mosaic mice that transmitted—M164 (*RIP1-Tag2*) and M87 (*RIR-Tag*). Progeny of each were killed and subjected to pathological and biochemical analysis. The only obvious abnormal pathology consisted of hyperplasia of the islets of Langerhans, determined following standard histochemical staining of tissue sections. Solid tumours were observed in a few of these mice. Figure 3*a* shows representative pancreas sections of a normal mouse and of a transgenic mouse (M384, *RIP1-Tag2*) that carried visible tumours; both were ~12 weeks old. Sections were stained with cresol violet, which distinguishes the endocrine islets from the surrounding exocrine tissue. Sections from M384 showed normal-sized islets, enlarged (hyperplastic) islets and solid tumours. All stained with similar characteristics, and were identifiable as islet-like regions by comparison with normal islets. Note, however, that all islet-like areas in the transgenic mouse appear more densely packed with cells than do the normal mouse islets, demonstrated by comparison of Fig. 3*A c* and *d*.

Analysis of the mice listed in Fig. 2 shows that all transgenic mice in these two lineages have the phenotype of islet hyperplasia (if killed) and/or premature death. Before their sudden death, the mice seem normal in appearance and activity. The pedigree stops at the generation during which the mice were placed on a high sugar diet to counteract indications that they were hypoglycaemic, which is a probable consequence of islet hyperplasia, and a possible cause of sudden premature death. Subsequent generations, maintained on this diet, now live somewhat longer and heritably develop solid tumours of the pancreas between 10 and 20 weeks of age. An example of a pancreas containing several tumours is shown in Fig. 3*B*. These tumours eventually comprise a significant fraction of the mass of the pancreas and are highly vascularized. Individual tumours can reach a size of 5 mm in diameter, with up to five or six separate tumours visible in a pancreas. There is no indication that the tumours metastasize other organs.

Cell-type specificity

To evaluate the observed hyperplasia of the islets of Langerhans, I examined normal and transgenic mouse pancreas for expression of both SV40 large-T antigen and polypeptide hormones characteristic for the major cell types. Thin sections were prepared from fresh frozen pancreas obtained from two transgenic mice (M384, *RIP1-Tag2*, and M306, *RIR-Tag*) carrying visible pancreatic tumours as well as from a normal mouse. The sections were analysed by immunostaining using antisera to glucagon, insulin and somatostatin and each visualized with rhodamine-conjugated second antibody to identify the α-, β- and δ-cell types of the islets. Large-T antigen was identified using both a polyclonal antiserum and monoclonal antibodies, each visualized by horseradish peroxidase-conjugated second antibody. Figure 4*a* shows representative immunostains of normal (non-transgenic) islets for the presence of insulin, large-T antigen, glucagon and somatostatin. The islets are comprised predominantly of α- and β-cells with fewer δ-cells; no cells react with antibodies to large-T antigen. Controls using second antibodies alone show no specific staining (not shown).

The islet tumours (Fig. 4*b*, *c*) in contrast, seem to consist of solely of insulin-producing cells, identifiable as β-cells by immuno- and histochemical staining. Glucagon- and somatostatin-producing cells are rare if not completely absent from the tumours. Characteristic localization of large-T antigen to the nucleus is observed in the islet tumour cells; it is clearly absent from adjacent exocrine cells. Evidence from both polyclonal serum (shown) and monoclonal antibodies (not shown) indicates the same pattern of large-T antigen expression. By these criteria, the islet tumours are essentially pure populations of proliferating β-cells. Occasional clusters of α-cells, perhaps remnants of an islet, can be detected on the edges of a tumour. Thus, both hybrid oncogenes specifically induce proliferation of the β-cells of the islets of Langerhans, eventually resulting in the appearance of pure β-cell tumours. In comparison, naturally occurring insulinomas are characteristically a mixed population of both β- and δ-cells, producing insulin and somatostatin, respectively[25,26].

Tissue-specific expression

The ability of the insulin control region to mediate tissue-specific expression of the SV40 large-T antigen following integration of the hybrid genes into the mouse germ line was assessed by

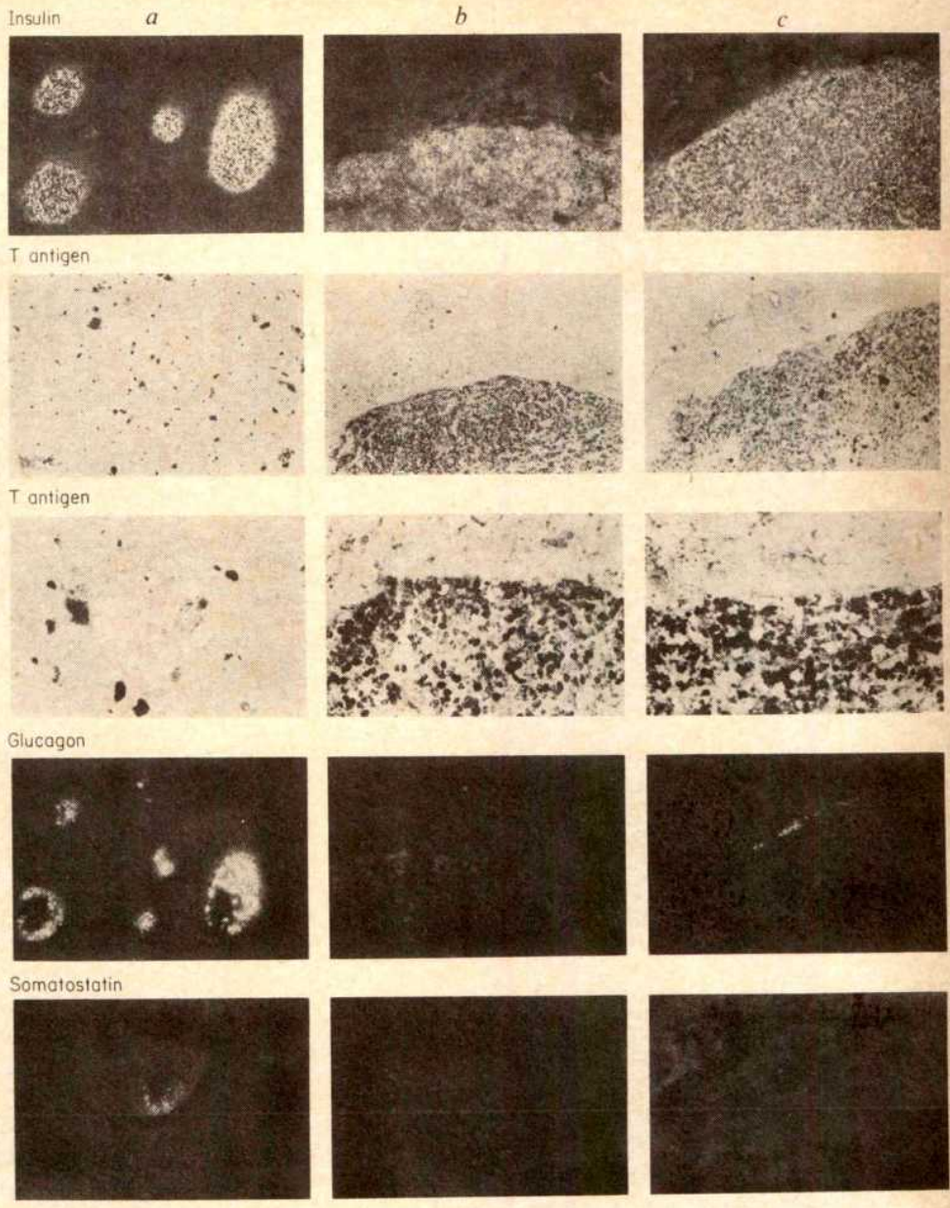


Fig. 4 Analysis of islet cell hormone and large-T antigen expression in a normal mouse and two tumour-bearing transgenic mice. Each column shows an analysis of one mouse: *a*, normal (non-transgenic); *b*, 384 from the *RIP1-Tag2* line; *c*, 306 from the *RIR-Tag* line. In each case, near (but not necessarily adjacent) thin sections were analysed for the presence of the specified antigen by using an antiserum to that antigen followed by a conjugated second antibody to visualize the first. The first row examines insulin, the second and third rows large-T antigen, the fourth row glucagon and the fifth row somatostatin. Magnification is ×50 except for the third row, which is ×200. The use of second antibodies alone gives no specific staining. In *a*, insulin and glucagon plates are of the same cluster of islets, whereas the somatostatin and large-T antigen are of other islets, because that cluster was not present in those sections. The immunostains for the hormones were visualized with rhodamine-conjugated second antibody and epifluroscent illumination, whereas the immunostain for large-T antigen used peroxidase-conjugated second antibody, and was viewed under bright-field illumination. *b*, *c*, Orientation of all the plates is the same. The islet tumour is on the bottom, with adjacent exocrine tissue above it. Column *b* is aligned so the tumour is a centred arc, whereas the plates in *c* have the tumour ascending to the right. The glucagon and somatostatin plates of *b* and *c* are of sections near those used for insulin and large-T antigen; the outline of the exocrine/tumour boundary can be seen in each. The ×200 plates of large-T antigen also show an exocrine/tumour boundary, in which peroxidase staining occurs in nuclei in the tumour tissue but not in the exocrine tissue.

Methods. 14-μm sections were taken as described in Fig. 3 legend, except that for the large-T antigen immunostaining the sections were not fixed, but rather air dried on gelatinized slides. The hormone immunostains were performed on paraformaldehyde-fixed sections as in Fig. 3. Sections were rehydrated in Net-Gel (150 mM NaCl, 5 mM EDTA, 50 mM Tris *p*H 7.4, 0.25% gelatin, 0.02% NaN_3, 0.05% NP40), then treated for 30 min in blocking solution (45% Net-Gel, 45% Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 1% bovine serum albumin (BSA), 0.5% NP40). The sections were then incubated overnight at room temperature in the first antibody, in a binding solution (blocking solution without BSA), washed several times for 30 min in Net-Gel, incubated with second antibody in binding solution for 3 h at room temperature, and again washed in several changes Net-Gel. The sections treated with rhodamine-conjugated second antibody were taken through graded alcohol into xylene and mounted under coverslips in Gurr Fluromount+2% diazabicyclooctane. The large-T antigen sections were visualized with horseradish peroxidase-conjugated second antibody, which was developed by treatment for 10 min in 0.25 mg ml^{-1} diaminobenzidine, 3 mg ml^{-1} nickel sulphate, 0.003% H_2O_2. The slides were then washed several times in Net-Gel and mounted. The antisera, sources and dilutions are as follows: guinea pig anti-porcine insulin (gift of R. Santerre) 1:5,000; rabbit anti-large-T antigen (a gift of D. Lane) 1:2,000; rabbit anti-human glucagon, Dako A565, 1:1,000; rabbit anti-human somatostatin, Dako A566, 1:1,000; horseradish peroxidase-conjugated swine anti-rabbit IgG, Dako P217, 1:200; rhodamine-conjugated goat anti-guinea pig IgG, Capel 2207-0081, 1:200; rhodamine-conjugated sheep anti-rabbit IgG, Capel 2212-0084, 1:200.

protein blotting analyses, using monoclonal antibodies that specifically recognize large-T antigen. Figure 5*a* presents a comparison of pancreas from several mice, including one normal (non-transgenic) mouse, several insulin/large-T antigen transgenic mice, and one carrying a collagen/large-T antigen fusion gene (M410) which therefore lacks the insulin gene sequences (D.H., unpublished results). M287 and M289 are siblings in the *RIP1-Tag2* lineage (see Fig. 2); M287 had large solid tumours whereas M289 did not, which illustrates the difference in levels of expression observed in mice of the same age and genotype with and without solid tumours. M277 contains immunoprecipitable large-T antigen, demonstrating that the *RIP1-Tag3* lineage derived from the founder M165 also expresses the fusion gene. Large-T antigen is not detectable in a normal (F_1) pancreas or in the transgenic mouse (M410) that lacks the insulin gene regulatory region. The *RIR-Tag* transgenic mouse 155 does not show expression in the blot demonstrated here, but other analyses show a low level of large-T antigen. The *RIP1-Tag4* lineage, exemplified here by M274, does not show detectable expression of large-T antigen by protein blotting. However, pancreatic tumours occasionally arise in this line, which indicates that the *RIP1-Tag4* insertion can express.

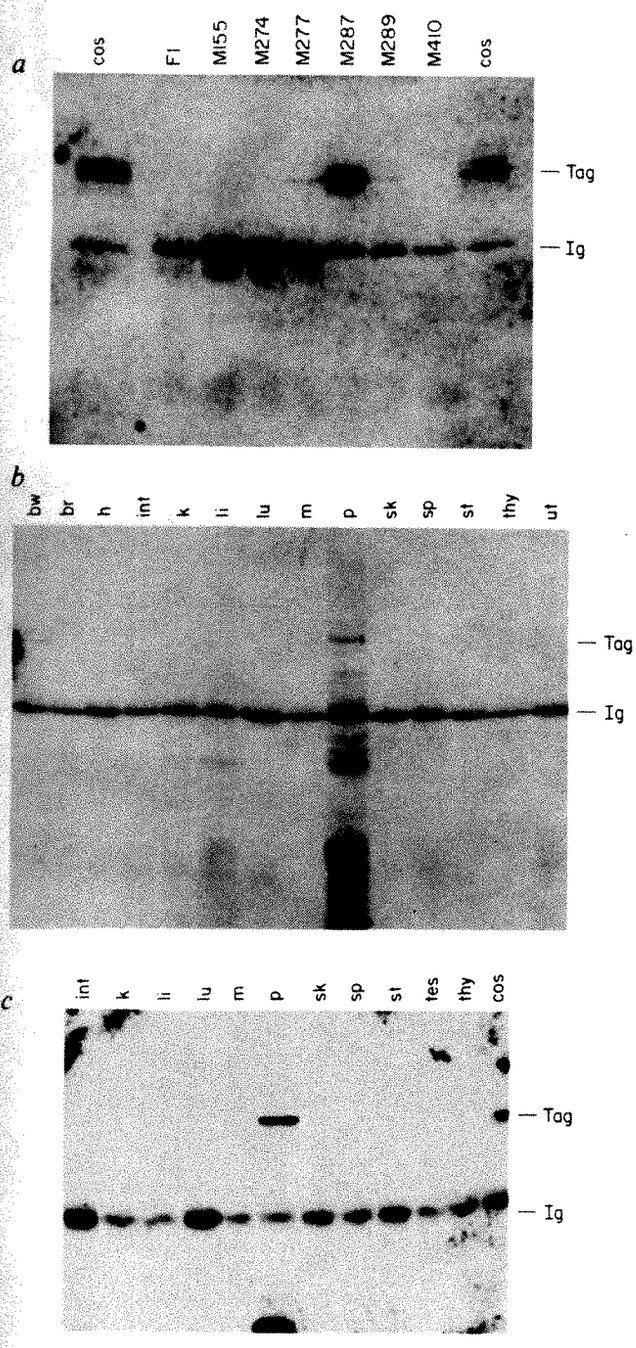


Fig. 5 Tissue specificity of transgene expression. The presence of SV40 large-T antigen in tissues was evaluated by protein blotting[39,40], in which tissue homogenates were immunoprecipitated with a monoclonal antibody (PAb 419) (ref. 41) to T antigen, fractionated on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose by electroblotting and visualized with a second radiolabelled monoclonal antibody (PAb 416), which recognizes a different epitope on the protein[41]. *a*, Pancreas from several mice are compared. F_1 is a non-transgenic mouse, M155 an *RIR-Tag* transgenic, M274 is *RIP1-Tag4*, M277 is *RIP1-Tag3*, M287 and M289 are from siblings from the *RIP1-Tag2* lineage, with M287 having visible tumours and M289 only hyperplastic islets, and M410 is a mouse harbouring a type I collagen/SV40 large-T antigen fusion gene. To provide a control of authentic large-T antigen, total protein from a lysate of the SV40-transformed monkey cell line cos A2 (ref. 42) was immunoprecipipitated and similarly treated, except that only 5% as much protein was used compared with the amount of pancreas protein. *b*, *c*, Tissue specificity of large-T antigen expression in the *RIP1-Tag2* (*c*) and *RIP1-Tag3* (*b*) lineages. Equal concentrations of each tissue were examined: bw, body wall; br, brain; h, heart; int, intestine; k, kidney; li, liver; lu, lung; m, muscle, p, pancreas; sk, skin, st, stomach; tes, testes; thy; thymus; ut, uterus. A separate analysis indicates that no large-T antigen can be detected in body wall, brain or heart of M287 (not shown). The radiolabelled antibody PAb416 sticks to the vast excess of immunoglobulin protein used in the immunoprecipitation, thus generating a band of relative molecular mass 55,000 in all samples. This band is further identifiable as IgG using either radiolabelled protein A or rabbit anti-mouse IgG.

Methods. Tissues were flash frozen in liquid nitrogen, stored at −70 °C, and then homogenized in 50 mM Tris *p*H 7.2, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM dithiothreitol, 1% NP40, 100 μg ml^{-1} DNase I, 50 μg ml^{-1} RNase A, 75 μg ml^{-1} phenylmethylsulphonyl fluoride. Protein concentrations were determined using the Bio-Rad protein assay reagent. 500 μg of tissue protein or 25 μg of cos cell protein was incubated overnight at 4 °C with 100 μl of tissue culture supernatant containing the monoclonal antibody PAb419, and then precipitated with 100 μl of 3% protein A-Sepharose (Pharmacia) for several hours. The immunoprecipitate was fractionated on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose by electroblotting overnight, incubated first in a blocking solution (Fig. 4) for 12 h and then in a binding solution (Fig. 4) containing 10^5 c.p.m. ml^{-1} of PAb416, which had been radiolabelled with ^{125}I using the chloramine-T method. The blots were washed in Net-Gel and exposed with intensifying screens onto Kodak XAR film.

The tissue specificity of fusion gene expression was evaluated further by comparing a number of different tissues from two mice (Fig. 5*b*, *c*), progeny of the two founders M164 and M165. Both show tissue-specific expression of SV40 large-T antigen, as only pancreas is producing immunoprecipitable protein that co-migrates with authentic large-T antigen. Some degradation of the antigen in pancreatic tissue is observed routinely; it is not clear whether this represents a normal situation in the β-cells or is simply a consequence of the protein isolation procedure. Analysis of a mouse from the *RIR-Tag* lineage also shows expression only in the pancreas, as does a similar examination of the tissues of mouse 163 (*RIP1-Tag1*) (not shown). In a normal pancreas, insulin-producing β-cells comprise only ~1-2% of the total cell mass[1,2]. Therefore, a lower limit can be established for inappropriate expression: either <~1% of the cells in another tissue could be expressing at levels comparable with those in β-cells, or all the cells in an inappropriate tissue could be expressing at levels of <1% of those observed in the islet cells. Within these limits, the expression of both insulin/large-T antigen hybrid genes is tissue specific to the pancreas. Taken together with the immunohistochemical analysis, the transferred insulin sequences are specifying correct expression in the β-cells of the endocrine pancreas.

Penetrance and the onset of oncogenesis

The relationship between large-T antigen expression and the development of β-cell tumours bears on possible mechanisms for oncogenesis. The two previous examples of oncogene expression in transgenic mice give contrasting results[27]. An apparently dormant SV40 early region is activated to express at high levels, and frequently amplified, in the development of choroid plexus tumours[19]. In contrast, a murine mammary tumour virus *myc* fusion gene is expressed in all mammary glands and in several other tissues before oncogenesis, which occurred in only 1 of the 10 mammary glands, implying that a secondary event is required to elicit tumour development[20]. In the insulin/large-T antigen transgenic mice examined to date, a similar pattern emerges. No more than 4 or 5 of the ~100 islets in a pancreas develop into solid β-cell tumours, which are highly vascularized and readily distinguishable from normal pancreatic tissue (Fig. 3*B*). Yet most islets show hyperplasia and all islets examined have increased β-cell density. Furthermore, the β-cells seem to be expressing the hybrid genes well before the development of hyperplasia and tumours, as is demonstrated in an examination of the pancreas from an 8-week-old mouse (M633) in the *RIP1-Tag2* lineage. This mouse exhibited normal pathology and histology, in particular showing no hyperplasia or visible tumours in the pancreas. Thin sections were prepared from the pancreas and stained with antibodies

for insulin and large-T antigen; a representative analysis of a pair of islets is shown in Fig. 6. Large-T antigen is expressing in both of these islets, as well as in all other islets examined in this pancreas. Thus, large-T antigen is expressing in the β-cells of a young mouse before emergence of obvious hyperplasia and solid tumours.

The interpretation of these observations is that the hybrid insulin/large-T antigen genes are expressed in all β-cells, thereby inducing their prolifieration, which leads to initial hyperplasia followed by oncogenic transformation. The development of solid tumours occurs in only a few of the islets, which indicates that additional events are probably necessary to mediate the rapid proliferation and extensive vascularization characteristic of the β-cell tumours. It will be of interest to distinguish between two possible classes of transformation: (1) an islet-specific change which affects most of the β-cells in an individual islet; and (2) a rare cell alteration that then produces a clonal tumour arising from that one altered cell.

Cell–cell interaction

Examination of the distribution of cell types in islets of these transgenic mice shows that islets of every size class (small, normal, enlarged, tumour) are densely packed with β-cells. Furthermore, the α- and δ-cells are disordered and rare. Comparison of the glucagon immunostains of normal islets (Fig. 1) and islets of the young transgenic mouse 633 (Fig. 6) shows that the characteristic peripheral distribution of α-cells has been disrupted and that their total number is reduced. Similarly, the normal polar organization of δ-cells is disordered and the δ-cell number severely retarded. This applies to every islet examined in this pancreas, in analyses that included sections taken to include different anatomical regions. These results suggest that during biogenesis of the islets, the α- and δ-cells are either sterically excluded or specifically suppressed by the altered β-cells.

Conclusions

The results presented here demonstrate that DNA within 520 bp upstream of the rat insulin II gene is sufficient to mediate qualitatively correct tissue- and cell-type-specific expression of hybrid insulin/SV40 large-T antigen genes. Protein blotting analysis of four mice harbouring independent insertions of such genes demonstrates expression only in the pancreas among the major tissues examined. The *in situ* analyses of thin sections from the pancreas demonstrates that this expression is cell-type specific, as large-T antigen is only detected in the insulin-producing β-cells of the islets of Langerhans.

The specification of tissue- and cell-type expression by the insulin gene 5′ flanking region occurs in both possible orientations when it is located upstream of the large-T antigen structural gene. Both tissue specificity and bidirectionality are qualities previously associated with transcriptional enhancer elements using transfection of cultured cells[28,29]. It is not clear whether this flanking region carries an additional real or cryptic promoter element oriented away from the insulin gene, or whether non-specific initiation of transcription is occurring in the *RIR-Tag* hybrid gene. RNA analyses will be necessary to address these possibilities.

There are three phases to the phenotype elicited by these hybrid oncogenes: the development of disordered islets, the subsequent enlargement of those islets into obvious hyperplasia and the formation of solid tumours. In young mice, the islets are of normal size, but densely packed with β-cells. The organization of both α- and δ-cells is disrupted and numbers of each severely reduced, δ-cells being particularly rare. These results suggest that islet cells interact to maintain a proper balance, and the transgenic β-cells effectively reduce α- and δ-cell number and organization by retarding their normal cell development. This possibility motivates a similar examination of the distribution of PP cells in the islets and islet tumours of these mice, because PP cells, like δ-cells, are often found at significant levels in naturally occurring insulinomas.

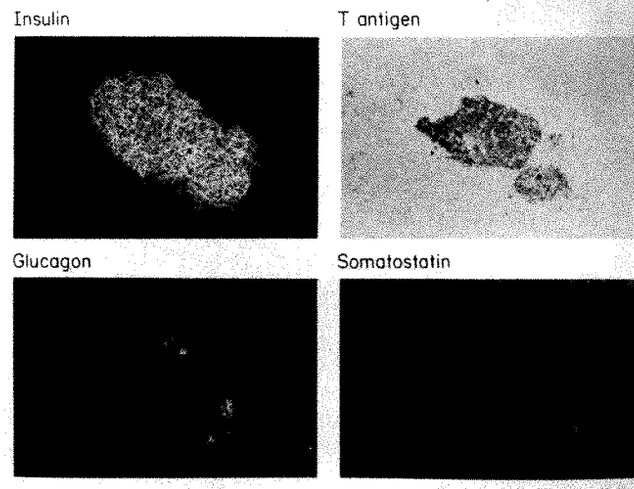


Fig. 6 Immunohistology of islets in the pancreas of a young transgenic mouse. Mouse 633, from the *RIP1-Tag2* lineage, was killed at 8 weeks of age and its pancreas flash frozen in liquid nitrogen, sectioned in a cryostat and analysed for the expression of islet cell hormones and large-T antigen as described in Fig. 4. legend. The two islets shown are representative of all islets in these and other thin sections from this pancreas. The sections used for insulin on large-T antigen are near but not adjacent and the islet sizes have changed somewhat across that distance (×50).

The prospect of disruption in islet-cell interactions is intriguing in view of observations that each of the three hormones influences secretion of the other two by their respective cell types. Somatostatin inhibits the release of both insulin and glucagon, insulin inhibits the release of glucagon and somatostatin, whereas glucagon stimulates secretion of insulin and somatostatin (see, for example, ref. 30). The organization of the endocrine pancreas into separate islands has led to suggestions that the islet cells arise from a common stem cell[31,32]. The character of the alterations in islet composition produced by the insulin/large-T fusion gene should allow more detailed examination of islet biogenesis. If islet cell types are interacting through growth factors, the results presented here suggest that they are probably suppressors of cell growth rather than inducers. Insulin itself is one candidate for such a suppressive growth factor.

The dense packing and disorganization of islets is followed by their expansion (hyperplasia), which seems to be a direct consequence of the expression of large-T antigen. Preliminary analysis indicates that serum insulin levels are elevated (two- to ten-fold), but not in proportion to the increased number of insulin-producing cells (>100-fold), which implies that either normal regulation of insulin secretion remains in effect, or that the β-cell structure, organization and/or vascularization is inappropriate for effective secretion. Isolation of β-cell lines from these transgenic mice should be facilitated by the ability of large-T antigen to establish (or immortalize) primary cells to growth in culture, with large-T antigen synthesis here maintained by the co-expression of the insulin and insulin/large-T antigen genes in β-cells. The availability of such β-cell lines will allow more detailed examination of the characteristics of altered β-cells. We may now examine how islet disorganization is correlated with changes in glucagon, somatostatin and insulin serum levels over a detailed timescale. It seems likely that the mice described in this study suffer from serious alterations in regulation of carbohydrate metabolism and that the explanation of their premature death does not simply result from excessive insulin in their serum, but also involves other factors.

Solid β- cell tumours eventually develop from a few of the hyperplastic islets. Tumour development occurs between 10 and 20 weeks of age, is heritable and has occurred in all progeny of the two lines (*RIP1-Tag2* and *RIR-Tag*) in which mice have lived to this age, and is occurring now in the *RIP1-Tag3* line. The solid tumours are frequent in the sense they arise in every

mouse harbouring these genes, although they occur only in a fraction of the islets. The characteristics of tumour formation suggest that synthesis of large-T antigen is necessary but insufficient to produce that condition in every islet. Large-T antigen is of a class of oncogenes that can both immortalize primary cells and release (or transform) cultured cells from their normal growth controls, such as contact inhibition and dependence on serum growth factors[24]. Yet, *in vivo* SV40 virus will produce tumours in mice only after a long latent period; oncogenesis seems to be related to the efficacy of the immune response and the presence of large-T antigen on the cell surface as well as in the nucleus[33–35]. There is no evidence of an immune response to hyperplastic islets, nor of large-T antigen on the surface of β-cells; it remains to be established whether the frequency of solid tumour formation is associated with an escape from immune surveillance. A similar hybrid gene, composed of the 5′ flanking region of the rat elastase gene linked to the coding information for SV40 large-T antigen, produces tumours of the exocrine pancreas in transgenic mice (R. Brinster and R. Palmiter, personal communication), which further indicates that T-antigen is oncogenic in mice. Given that large-T antigen synthesis is the primary event, there are other potential secondary alterations that may be necessary to induce the development of solid β-cell tumours. These include activation or inactivation of growth factors or receptors, or the cooperation of another oncogene[36,37]. It is well established that nascent tumours cannot exceed certain size constraints without becoming vascularized; thus the induction of angiogenesis[38] is probably one (if not the only) secondary event necessary for solid tumour development. Biochemical and further histochemical comparisons of the islets before and after the predictable development of tumours may provide insight into these possibilities.

The ability to examine the pre- and postnatal development of the endocrine pancreas as well as heritable oncogenesis, using as markers the hormone gene products of these cell types and the SV40 tumour antigen, will provide a means to examine the consequences of cell-specific oncogene expression and the ontogeny of the effects reported here, as well as to address further aspects of the control of insulin-gene expression. It will be of interest to compare different recombinant insulin/oncogenes (such as *myc* and *ras*) for their effects on islet-cell biogenesis and cell–cell interaction, and on secondary events necessary for the development of β-cell tumours.

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LETTERS TO NATURE

Colour, albedo and nucleus size of Halley's comet

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Halley's comet (1982 i) is now bright enough for broadband photometry by large infrared telescopes[1–3]. We report here on photometry of the comet in the *B* (0.44 μm), *J* (0.55 μm), *V* (1.25 μm) and *K* (2.2 μm) broadband filters during a time when the coma was very weak and presumed to contribute negligibly to the broadband photometry. The *V* − *J* and *J* − *K* colours suggest that the colour of the nucleus of Halley's comet is similar to that of the D-type asteroids, which in turn suggests that the surface of the nucleus is of albedo < 0.1.

The first detection of Halley's comet in the near-infrared was made on 20 December 1984, when the comet was 5.39 AU from the Sun[1]. Our new observations at *J* and *K* were made on February 18.3 and 20.3, 1985 with the 3-metre NASA Infrared Telescope Facility (IRTF) at Mauna Kea, Hawaii, and measure reflected sunlight, not thermal emission from the comet itself. Simultaneously, we obtained *B* and *V* filter data with the 2.2-metre University of Hawaii telescope, also on Mauna Kea. All data were corrected for extinction and system response by comparison with standard stars. Table 1 gives the results of the observations, together with the comet's distances from the Earth and the Sun, and the solar phase angles. The *B* and *V* photometry was done with a 9.5-arc s diameter aperture, with sky measurements of the same field taken after the comet had moved completely away. With the IRTF we used an 8-arc s aperture and a 20-arc s amplitude oscillation of the telescope's secondary mirror to correct for the sky radiation. Both telescopes were set to follow the apparent motion of the comet across the sky. We

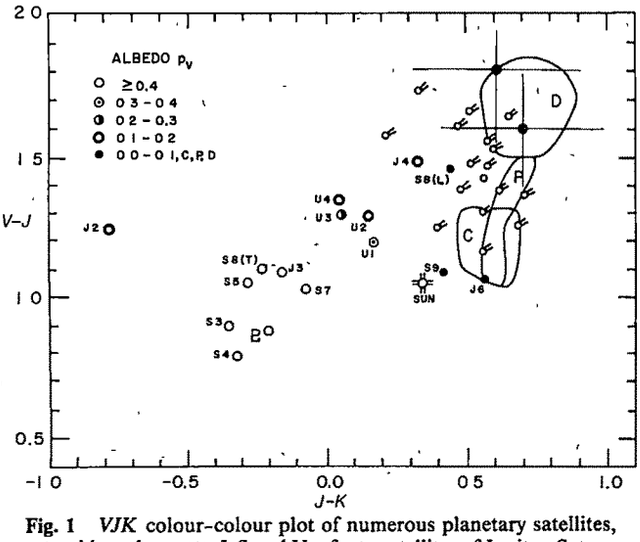


Fig. 1 *VJK* colour-colour plot of numerous planetary satellites, asteroids and comets. J, S and U refer to satellites of Jupiter, Saturn and Uranus, respectively. The fields for C, P and D-type asteroids are shown schematically, based on our data for 6 Cs, 5 Ps and 6 Ds. The two data points with error bars shown in the D field are the results for Halley's comet reported in the present work. This figure is reprinted, with modifications, from ref. 7.

could not see the comet visually through either telescope; it was located by means of a two-body ephemeris (computed by D.J.T.). The 2.2-metre telescope was then peaked on the signal through the *B* filter where the signal was strongest. The IRTF was then directed to the comet by precision offsets from field stars (observed by D.J.T.).

The question of how much light is contributed in the various filter bands by the weakly developed coma around Halley is an important factor in the interpretation of the photometry reported here. On 24 January 1985, a very weak coma was observed in 3-min unfiltered exposures with a charge-coupled device (CCD) on the 2.2-metre telescope (C. R. Chapman, D.P.C. and J. M. Pasachoff). The coma was ~5 arc s in diameter and was also very weak in long exposures (24 min) with a filter centred on the H_2O^+ emission band at 7,025 Å. We do not have a corresponding CCD image of the comet at the time of our February photometry.

The *V* filter used in this work extends from about 5,200 to 6,000 Å (20% transmission points) and misses most of the emission in the C_2 Swan band at 5,150 Å. It includes all of the weaker C_2 band at 5,550 Å. The *B* filter includes the C_3 band at 4,030 Å and C_2 at 4,700 Å. All of these bands are prominent in bright, active comets. The *J* filter includes the CN (0—0) band in very bright comets, such as West (1976 vi)[4]. A preliminary evaluation of spectrophotometric observations obtained in February by A. L. Cochran (personal communication) showed no significant emission of CN or C_2, thus suggesting that our *V* filter measurements are not seriously contaminated by coma emission bands. Similarly, the *J* filter is not expected to be contaminated because of the weakness of the CN (0—0) band. We note that any emission in the *V* filter would serve to lessen the $V-J$ colour. Thus, the $V-J$ values plotted in Fig. 1 are lower limits, and the comet was at least as 'red' as we show.

Table 1 gives the observed magnitudes and $J(1, 0)$, the *J* mag reduced to unit distance from Earth and Sun and corrected to zero phase, assuming a phase coefficient of 0.039 mag deg^{-1}, a value commonly used for asteroids in the *V* filter. We also include a nearly contemporaneous measurement of *J* and *K* from Brooke and Knacke[2]. In the present discussion, the colours $V-J$ and $J-K$ are of most interest. For February 18.3, we find $V-J=1.6\pm0.2$ and $J-K=0.7\pm0.3$. The mean of the three independent determinations of the $B-V$ colour is 0.6 ± 0.1.

The interpretation of these colours in the context of the small bodies of the outer Solar System (OSS) requires a brief discussion of previous work on this subject. In a series of recent papers[5-7], we reported photometry of OSS bodies with emphasis on the colours of ices and soils on their surfaces. On bright objects, various ices (water, methane and sulphur dioxide) and mineral components of soils have been identified by medium-resolution spectrophotometry. Broadband filter observations can be used in conjunction with the spectrophotometry of the brighter objects to establish correlations between *VJK* colours and spectral classes (related to surface compositions). These correlations are assumed to apply to the fainter bodies for which only *VJK* photometry can be obtained at an adequate signal-to-noise ratio with presently available instrumentation. Halley's comet falls into this latter class because of its faintness at the solar distance when we observed it. At much smaller solar distances, the coma could be expected to develop to the point where the nucleus itself is no longer visible and the measurements would refer instead to the material in the coma.

Among small bodies in the OSS we have found a range of *VJK* colours from relatively blue to red, with clumping at the extremes, as shown in Fig. 1. The satellites of Jupiter, Saturn and Uranus (keyed with J, S and U, respectively) are known from spectrophotometry to be dominated by water ice, with increasing ice abundance towards the lower left part of the figure. Satellite J4 (Callisto) has the weakest ice bands in its spectrum. The D, P and C-type asteroids define three fields in the $V-J$ and $J-K$ colour diagram, as indicated by the enclosed areas in the figure.

The trend in the colours shown in Fig. 1 can be interpreted if OSS bodies are composed of mixtures of ice and dark (carbonaceous?) condensates. The surfaces, then, are mixtures of bright ices and dark soil, and the ratio of the two components determines colour and albedo. The ice/soil ratios are determined in turn by geological processes on the bodies. The *VJK* colour diagram is remarkably effective for discriminating between the C and D subtypes of dark materials. The redder D-type (abundant among Trojan asteroids) is isolated in the upper right part of Fig. 1, while the more neutral C- and P-types fall in the right centre.

The C-type asteroids in the outer belt are believed to resemble carbonaceous meteorites containing up to some 20% water. They are black objects with albedos of ~0.04 and flat spectra. The D-type objects have similar low albedo but a more reddish-black colour. Gradie and Veverka[8] concluded that the D-type objects are coloured by organic material representing a lower temperature condensate than the carbonaceous material colour-

Table 1 Photometry of Halley's comet

	Date	r (AU)	Δ (AU)	β	B	V	J	K	$J(1,0)$
Birkett *et al.*[1]	20.5 Dec. 1984	5.39	4.42	2.1°	—	—	18.6±0.1	—	11.6±0.1
Hanner and Tokunaga[3]	18.3 Jan. 1985	5.12	4.30	6.6°	—	—	18.4±0.2	—	11.4±0.2
Brooke and Knacke[2]	17.3 Feb. 1985	4.84	4.45	11.2°	20.22±0.05*	19.50±0.06*	18.5±0.2	17.8±0.3	11.9±0.2
Present work	18.3 Feb. 1985	4.83	4.46	11.3°	20.18±0.05	19.56±0.07†	17.8±0.3	17.2±0.3	10.6±0.3
Present work	19.3 Feb. 1985	4.82	4.47	11.4°	20.08±0.08	19.66±0.09	—	—	—
Present work	20.3 Feb. 1985	4.81	4.48	11.5°	20.32±0.06‡	19.66±0.09	18.1±0.2	17.4±0.2	11.0±0.2

* *B* and *V* measurements at the 2.2-m telescope (by D.J.T.).
† Derived from *B* measured on this night and $B-V$ determined on February 17, 19 and 20.
‡ Average of two measurements: at 0648 UT, $B=20.28\pm0.05$; at 0842 UT, $B=20.35\pm0.06$.

ing the C-types. This would be consistent with formation of the D-type objects at a great heliocentric distance. Gradie and Veverka predicted that the D-type material ought to dominate the silicate component in the outermost Solar System and might comprise the dusty constituent in comets[8]. Our observations of several comets, some of which were active and some of which were not, confirm that comets always lie near the D, P and C-class asteroid colours, as shown in Fig. 1. No comet observed by us has a colour consistent with clean ice, and the majority of them have colours just offset from the D colours. The ices of comets are therefore coloured by dark dust similar in colour to D-class asteroid material, in excellent agreement with the Gradie-Veverka prediction[8] that comets contain D-type dust.

We note that the mean $B-V$ colour of Halley's comet is also consistent with D-type asteroids[9]. Because the comet is near to the brightening curve of an asteroid, and because of the minimal coma and emission in our wavelength regions, we may be seeing the nucleus. If so, the nucleus may have the appearance of a D-type asteroid, with a radius of about 10 km (calculated from equation 2 in ref. 13, using albedo 0.04). Alternatively, the nuclear condensation we observed may have been a swarm of particles. If those particles were macroscopic in size, colouring effects due to scattering by particles smaller than the wavelength of our observations should be minimal, and the albedos of the individual fragments would be of the order of 0.04.

Table 1, particularly the column for $J(1, 0)$, shows that the reduced brightness of the comet was changing. While this could be attributed to activity in a developing coma, rather significant variations in brightness were detected at solar distance 8 AU where no comatic activity is expected[10]. The variations in brightness have been widely considered as evidence for rotation of an irregularly shaped nucleus[11,12], although no unambiguous period has thus far been established.

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Note added in proof: Hughes[14] has estimated the disk and other properties of Halley's comet from a consideration of its performance during the 1910 apparition. He finds that $D^2 p_V = 5.90 \pm 0.33$ km^2, where D is the diameter and p_V is the geometric albedo at the V filter wavelength. The diameter derived by Hughes is approximately half that found by us at a similar albedo.

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On the size of the galactic centre compact radio source: diameter < 20 AU

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The existence of a compact nonthermal radio source at the galactic centre was first suggested by Lynden-Bell and Rees[1] as the possible signature of a black hole. Such a source was detected unambiguously by Balick and Brown[2]. Very long baseline interferometry (VLBI) observations of the structure of this source, hereafter referred to as Sgr A*, have been made for several years[3,4]. In all previous experiments, the visibility amplitudes, measured over a restricted (U, V) range, could be fitted only to the simplest model of brightness distribution, that is, to a circular gaussian. We have re-observed Sgr A* at λ3.6 cm and λ1.35 cm with many baselines, using the more sensitive receivers and VLBI recording terminals now available. These observations set an upper limit of 20 AU (3×10^{14} cm) to the diameter of Sgr A* at λ1.35 cm and reveal for the first time an elongated structure at λ3.6 cm, with the position angle of the long axis almost parallel to the rotation axis of the Galaxy. Sgr A* is unique in our Galaxy, but resembles most closely the compact radio sources at the centre of external galaxies. Observations of the central 4 arc s (0.2 pc) at radio and other wavelengths are best explained by a single massive collapsed object at the galactic centre.

The λ3.6-cm observations were made on 7 May 1983, using six telescopes: Haystack 36-m; National Radio Astronomy Observatory (NRAO) 43-m; Fort Davis 26-m; Goldstone 64-m (DSS14); Owens Valley Radio Observatory (OVRO) 40-m; and Hat Creek (HCRK) 26-m. The MkIII VLBI recording terminals[5] were used with an effective recording bandwidth of 28 MHz. Observations alternated between Sgr A* and the neighbouring calibrator source NRAO530. The absolute flux-density scale was fixed by observations of NRAO530 relative to 3C286 (assumed flux density of 5.4 Jy) with DSS14.

Observations at λ1.35 cm were made on 23 June 1983, using four telescopes: Haystack 36-m, NRAO 43-m, Very Large Array (VLA) phased D-array and OVRO 40-m. The MkIII recording terminal was used with a 56-MHz bandwidth. We calibrated with the H_2O maser source in Sgr B2, ~0.7° away. At the end of each Sgr A* observation, Sgr B2 was observed for 1 min. We have adjusted the scale so that the total flux density of Sgr A* was ~1.5 Jy, a value consistent with the measurement at the VLA during the observations. The size of the gaussian model discussed below is completely independent of the flux-density scale.

At λ3.6 cm, the calibrator source NRAO530 was detected on all baselines, whereas the compact radio source in Sgr A* was detected only on the short baselines formed between the DSS14, OVRO and HCRK telescopes (for visibility coverage, see ref. 6). The visibility amplitudes of Sgr A* (Fig. 1) were fitted to two simple models, namely, circular and elliptical gaussian brightness distributions. The baseline most sensitive to the difference of the two models is DSS14–HCRK because of the relatively large rotation of the baseline. The elliptical model is preferred.

The eliptical gaussian model based on all three baselines has the following parameters: the major-axis (half-power) diameter is 15.5 ± 0.1 milliarc s (marc s), and an axial ratio of 0.55 ± 0.25, with the long axis oriented at a position angle (PA) of 98 ± 15°. From 08:30 to 10:00 UT, the closure phase of Sgr A* on the DSS14–OVRO–HCRK triangle is <5°. This suggests that the elongated source is very symmetrical, but the closure phase is measured over a very limited time.

The present λ3.6-cm results, together with our earlier work[3] spanning 8 yr, set an upper limit of 0.6 marc s yr^{-1} to any change in the observed size (corresponding to a radial expansion velocity of ~16 km s^{-1} at 10 kpc). The combined data on the transcontinental baselines to DSS14 set an upper limit of 0.011 Jy (5σ) at λ3.6 cm for any component of Sgr A* with diameter ≤1 marc s, within a 2 arc s × 2 arc s box centred on RA(1950) = 17 h 42 min 29.3 s, dec.(1950) = −28° 59′ 18.2″.

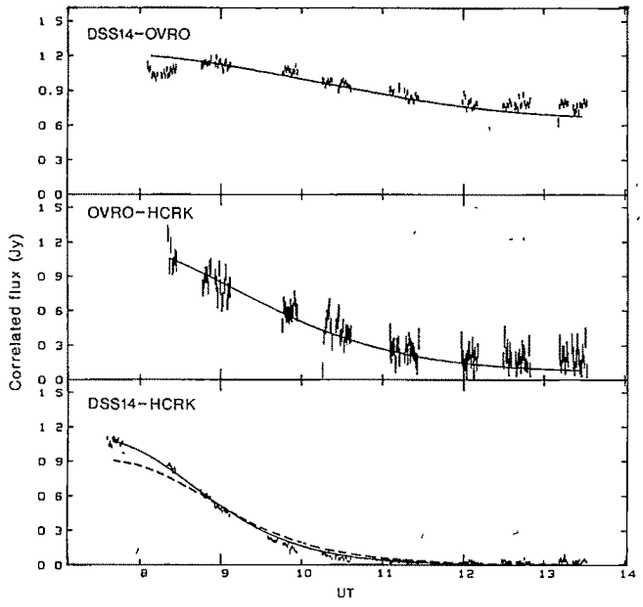


Fig. 1 Fringe amplitudes of the galactic centre compact radio source Sgr A* at λ3.6 cm (7 May 1983). The values beyond 11.00 UT on the HCRK baselines correspond to 2.5σ upper limits to the correlated flux density. The error bars represent ±1σ in the fringe fitting. The solid curves represent the best-fitting elliptical gaussian model visibility (major-axis half-power diameter = 15.5 ± 0.1 marc s, PA = 98 ± 15°, axial ratio = 0.55 ± 0.25). The dashed curve is the circular gaussian model visibility. On the other two baselines, the circular and elliptical gaussian model visibilities are essentially the same.

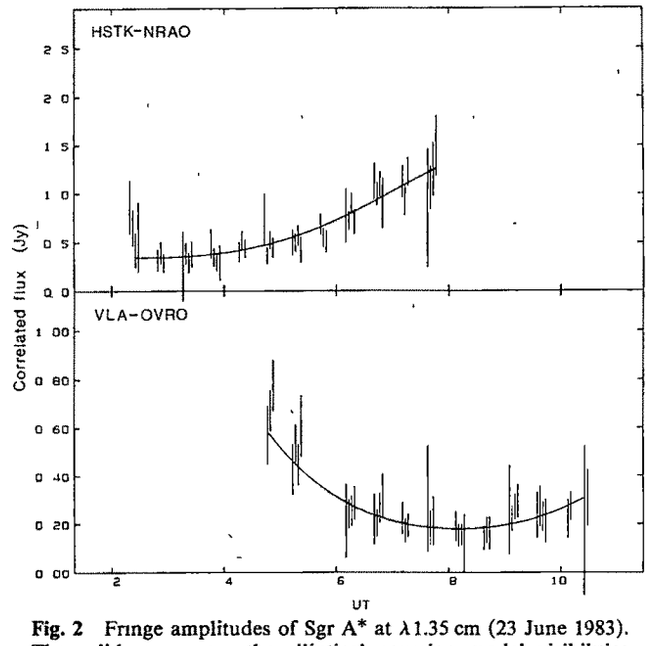


Fig. 2 Fringe amplitudes of Sgr A* at λ1.35 cm (23 June 1983). The solid curves are the elliptical gaussian model visibilities (major-axis diameter = 2.2 ± 0.2 marc s, PA = 87 ± 30°, axial ratio = 0.55 ± 0.5).

The λ1.35-cm data (Fig. 2) are well fitted by a circular gaussian model with a diameter of 2.1 ± 0.3 marc s. An elliptical gaussian fit, starting with the above model, results in a major-axis diameter of 2.2 ± 0.2 marc s, a position angle of 87 ± 30° and an axial ratio of 0.55 ± 0.5. The λ1.35-cm data are thus consistent with the elongated morphology observed at λ3.6 cm, scaled by λ^2, but do not require it.

The wavelength dependence of the angular size of Sgr A* can be derived from these data, the previously published 6-cm data[3] and 13-cm observations[7]. These are the only four wavelengths for which the angular size has been determined from visibility data with a range of variation exceeding a factor of two. All observations are mainly sensitive to the east–west brightness distribution. The sizes at λ = 13, 6, 3.6 and 1.35 cm are 203, 49.0, 15.5 and 2.1 marc s, respectively. Although these observations were obtained at different times, there is no evidence for time variations of the angular size. The exponent of the power-law dependence of the size on wavelength is 2.0 ± 0.1.

The λ^2 dependence of the source diameter could be attributed to self-absorption by internal thermal electrons[3,8], or ascribed to angular broadening of the intrinsic source size resulting from scattering by interstellar electrons[9]. The observed elongation of Sgr A* does not preclude the scattering interpretation, as a symmetrical elongated structure can be caused by scattering within a confined layer of anisotropic turbulence[10,11]. Asymmetric scattering from anisotropic turbulence in the interplanetary medium near the Sun has recently been observed (B. Rickett, personal communication). A brightness-distribution map of Sgr A* could resolve this ambiguity, because a complicated source structure would preclude interstellar scattering. Under either interpretation, the λ1.35-cm source size of 2.1 marc s (~20 AU) is an upper limit to the intrinsic source size of Sgr A*. Unless there is an abrupt change in the λ^2 dependence of the observed size for λ < 1.35 cm, the true size is ≤1.5 marc s.

If the observed size is dominated by scattering, the upper limit to the source-expansion rate observed at λ3.6 cm may not apply to the intrinsic expansion. However, even in the scattering case, the small λ1.35-cm size places an upper limit on the intrinsic source expansion of 0.26 (2.1/8) marc s yr^{-1}, or ~6 km s^{-1}, for the intrinsic source size not to exceed the scattering angle. Therefore, measurements at the two wavelengths imply an upper limit to the intrinsic source expansion velocity of <16 km s^{-1} averaged over 8 yr.

The observed parameters of Sgr A* are summarized in Table 1; its properties are similar to those of compact radio sources in galactic nuclei and quasars[12], but at a much lower luminosity. The physical conditions of the radio source implied by the observed properties have been discussed previously[8,13]. The extremely small size indicates that the scale of the underlying energy source must approach stellar dimensions. However, models of the radio source invoking stellar objects, such as a young supernova remnant, a pulsar[9], pulsar-driven wind[13] or a binary stellar radio source[8], are inconsistent with the observations[3]. Furthermore, there is no other radio source with comparable strength to Sgr A* found in the central five square degrees of the galaxy (D.C.B. and R. Sramek, unpublished results). Sgr A* is a unique radio source in the Galaxy. On the other hand, it resembles closely a weaker version of the nuclear radio source in external spiral galaxies, such as M81 (NGC3031; ref. 14) and M104 (NGC4594; ref. 15).

Problems of confinement of the radiating particles[13] and the stability of the source size would require the emission to originate in a steady wind or jet. The observed elongation of the source that is almost parallel to the rotation axis of the Galaxy (PA ~ 122°) is possible supporting evidence. Further observational constraints on the nature of the radio source must await the construction of a brightness-distribution map.

Table 1 Observed properties of the galactic centre compact radio source

Source size	<20 AU (~3×10^{14} cm)
Wavelength dependence of size	$\lambda^{2.0}$ ($\lambda \geq 1.35$ cm)
Position angle of elongation	98 ± 15° (λ = 3.6 cm)
Axial ratio	0.55 ± 0.25 (λ = 3.6 cm)
Upper limit to source expansion	<16 km s^{-1}
Flux density variability ($\Delta S/2S$)*	≤0.2 (1975–83)
Spectral index (ref. 29)	~0.25
Turnover frequency (ref. 29)	≥89 GHz
Radio luminosity	~2×10^{34} erg s^{-1}
Brightness temperature	>7×10^8 K (λ1.35 cm)

* ΔS, peak-to-peak variation; S, mean flux density; ref. 28 and J. van Gorkom, K.Y.L. and M. Claussen, unpublished results.

The central few square degrees of the Galaxy are full of interesting and energetic phenomena on many size scales[16-19], in the middle of which lies the unique Sgr A*, coincident to within 0.5 arc s of IRS16 centre. IRS16 may be the primary source of the $\sim 10^7\ L_\odot$ of infrared and ionizing radiation from the centre[20-22]. A $3\times10^6\ M_\odot$ point mass within 0.5 pc of the centre is suggested by the latest Ne II observations (E. Serabyn and J. H. Lacy, in preparation). Furthermore, a large velocity ($\pm 750\ km\ s^{-1}$) outflow[23,24] and, possibly, a compact source of positronium[25] are also identified with the central 0.2 pc ($\sim 5\times 10^{17}$ cm, or ≤ 4 arc s).

Although the luminosity and ionization over the 10^{20}-cm scale of the galactic centre may be explained by a recent burst of star formation[26], the activities within the central 5×10^{17} cm require an alternative source of energy, such as a massive collapsed object in a quiescent state of low-level accretion[27]. The unique compact radio source and the other phenomena at the centre would be natural consequences of the presence of such an object.

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Middle atmospheric NO and NO_2 observed by the Spacelab grille spectrometer

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Almost 20 years after Nicolet[1] suggested the presence of nitric oxide in the upper atmosphere as a source of D-region ionization by solar Lyman α-radiation, this trace species was first observed[2] by the resonance fluorescence method; almost 10 years later, its vertical distribution was observed in the stratosphere by means of balloon-borne infrared absorption spectrometry[3]. Since then much emphasis has been placed on odd nitrogen compounds in the stratosphere, partly because of their role in controlling the ozone abundance. Many measurements have been made of NO, NO_2, HNO_3 and NO_3 in the stratosphere with inferences on the N_2O_5 abundance. Meanwhile, several determinations of NO in the mesosphere and in the lower thermosphere[4-10] have used resonance fluorescence and mass spectrometry[11]. We report here the first observation of NO from the low thermosphere down to the low stratosphere by instrumentation similar to that used previously on board balloon gondolas, but on this occasion the observation platform was Spacelab I, which gave access to higher altitudes. As before[12], NO and NO_2 were observed simultaneously.

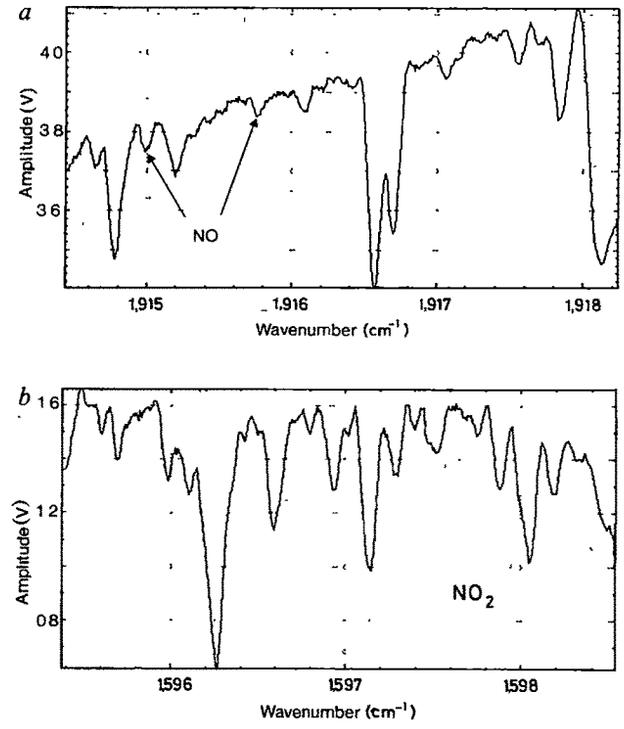


Fig. 1 *a*, Solar spectrum recorded at sunlight-tangent altitude equal to 86 km. Two NO absorption lines are indicated; all the other spectra lines are attributable to solar absorption features. *b*, NO_2 absorption spectrum recorded at sunlight-tangent altitude equal to 26 km. In this spectral region, the solar spectrum is a continuum. Most of the spectral features are attributable to nitrogen dioxide. In *a* and *b*, amplitudes are in arbitrary units and the origin of the scale is the level of total absorption for the solar radiation.

The infrared grille spectrometer on board the space shuttle during the first Spacelab mission has been described elsewhere[13]. During this mission, one Earth-limb solar occultation run was devoted to the observation of NO and NO_2 in the respective wavenumber ranges, 1,914.5-1,918 cm^{-1} and 1,595-1,598.5 cm^{-1}. The corresponding wavelengths were selected in the sixth and fifth orders of the grating by two interference filters, one for each of the two liquid nitrogen temperature-cooled detectors.

This observation took place over the southern Pacific Ocean on 1 December 1983, at sunrise after the very short nights of the high southern latitudes in their late spring. For the 20- and 100-km tangent-altitude locations, the solar depression angles did not exceed 0.3° and 1.4° respectively, during the course of the day. The geographical coordinates of the tangent points of the solar rays in the atmosphere were respectively 68° S, 129° W and 67° S, 118° W. Thus, the observations took place in almost full daylight; almost all the odd nitrogen was in the form of NO in the mesosphere and NO, NO_2 and HNO_3 in the stratosphere, according to our present knowledge of atmospheric photochemistry. NO and NO_2 spectra (examples are shown in Fig. 1*a*, *b*) were recorded every 2 s and the measured equivalent

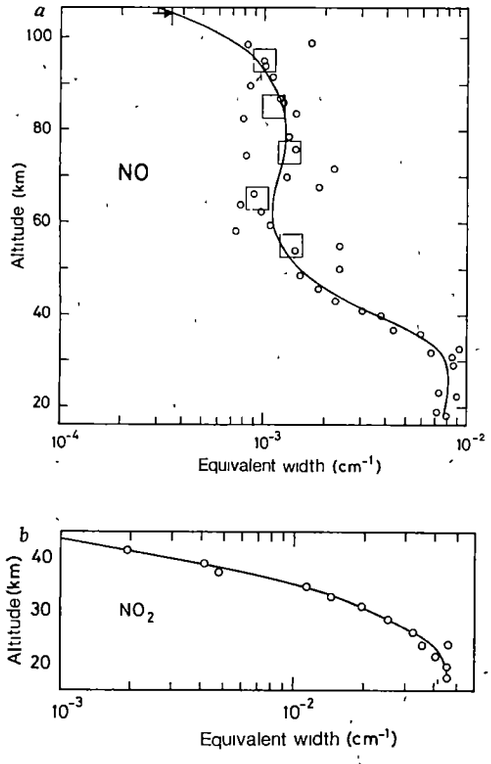


Fig. 2 Equivalent widths versus grazing-sunlight altitude for *a*, the NO absorption doublet at 1,914.99 cm^{-1} (the squares represent five kilometres averages) and *b*, the NO_2 multiplet centre at 1,597.15 cm^{-1}.

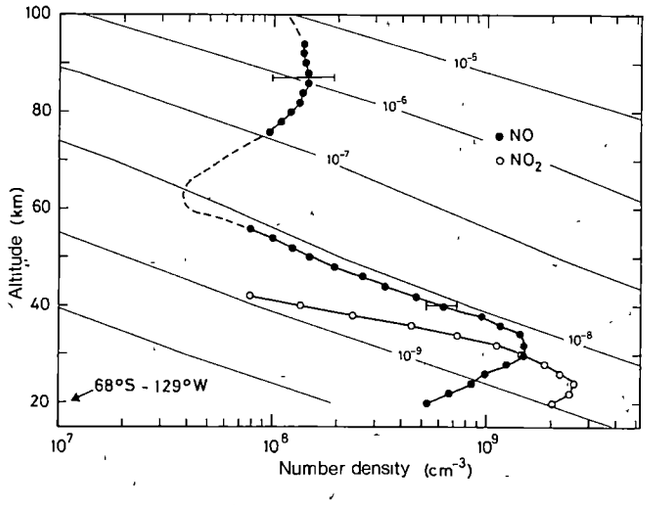


Fig. 3 Number densities of NO and NO_2 versus altitude (1 December 1983). The near straight lines indicate constant volume mixing ratios from 10^{-10} to 10^{-5}. Error bars are related to the scattering of the measured equivalent widths.

widths versus tangent heights in the atmosphere are shown in Fig. 2*a*, *b*.

The data, represented by the smooth curves in Fig. 2*a*, *b*, were invented using the molecular-line parameters of Rothman *et al.*[14] in the 'onion peeling' inversion method, which leads to the vertical distributions of NO and NO_2 shown in Fig. 3. Above 98 km altitude, the scanned wavelength intervals broadened to observe broader band solar spectra. The 1,914.99-cm^{-1} NO line region was observed again at 105 km tangent altitude where the NO signal was too weak to be detected. From 55–75 km of

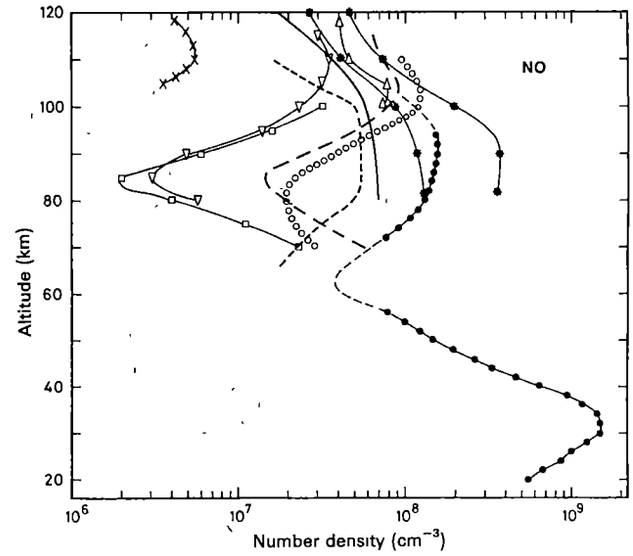


Fig. 4 Nitric oxide number densities presented in this paper are compared with the results of other authors. Barth[2] (——); Meira[4] (—·—·—); Tisone[5] (– – – –); Witt *et al.*[6] (○); Backer *et al.*[8] (□); Thomatsu-Iwagami[7] (▽); Thomas[9] (×), Trinks *et al*[11] (△); this work (—·—·—); Massie[10] (*—*, sunrise).

altitude a dashed line represents inverted NO number densities, so low that the slant absorption must be attributed to NO at higher altitudes. Hence, at this altitude the NO abundances can only be given tentatively and probably represent an upper limit.

The stratospheric portion of the observation is characterized by a high signal-to-noise ratio for the absorption lines. Hence, the uncertainty on the volume concentrations is small and the values, which will be discussed elsewhere, are in the range of previous observations. Our results are compared in Fig. 4 with other observations in the mesosphere and in the low thermosphere. Although our results are in rather good agreement with other observations at the highest altitudes, the differences become very large at 85 km, particularly with the vertical profiles exhibiting a minimum volume concentration at that altitude. The shape of the vertical profile compares as well as possible with the results of Barth[2] and Tisone[5]. The only other absorption measurements to compare with our data are those of Massie[10], based on OSO-8 observations of the (1.0) δ band at 182.94 nm, which are in good agreement.

Slight modifications of our new data may be necessary if further refinement were to alter significantly the orbit parameters of the shuttle during the first Spacelab mission. More observations will be possible when the instrument is flown in a mission dedicated to atmospheric studies. When this happens we should gain understanding of the the distribution of NO in the mesosphere, which shows a disagreement between the NO abundances derived from different observation methods. This has important consequences for modelling neutral and charged-particle abundances in the upper-middle atmosphere and ionospheric D and E regions.

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Measurement of surface currents in Liverpool Bay by high-frequency radar

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The backscattering of high-frequency radar echoes from the sea surface can yield useful information about ocean waves and surface currents[1–3]. The Rutherford Appelton Laboratory Ocean Surface Current Radar (OSCR) system is based on the CODAR system[4] but differs significantly in that it uses a beam-forming receive antenna to determine the direction of the backscattered signals instead of using the CODAR direction finding system. OSCR derives a radial component of surface velocity by measuring the shift in recorded spectra associated with advancing and receding waves. In this survey of surface currents in Liverpool Bay, using only OSCR, measurements were made first for 7 days from the Lancashire coast followed, 1 month later, by similar measurements from the Wirral coast. These were combined to compute current ellipses for the predominant M_2 tidal constituent. Spatial distributions of the ellipse parameters agree well with values obtained from a numerical model and from moored current meters. This new observational technique should provide data on the fine structure of near-shore surface currents for use in studies of sediment transport and pollutant dispersal.

The handicap of having only one OSCR unit was overcome by deploying it in an area where tidal currents predominate. Tidal measurements from two separate deployments could be combined to produce a stereoscopic image. Liverpool Bay was selected as a suitable site because, in addition to the convenient shoreline geometry (Fig. 1), the region is characterized by strong tidal currents flowing over complex bottom topography, thus producing horizontal structure on a scale commensurate with the resolution of OSCR. Despite the influence of major estuaries, the tidal forcing is sufficient to overcome vertical stratification. Moreover, harmonic analysis of tidal elevations in this region indicates the predominance of three major constituents, namely M_2, S_2 and N_2. This allows a reasonable resolution of the primary M_2 constituent from a data set as short as 25 h.

The computing system installed in OSCR at the time of this experiment limited data acquisition to measurements along just four beams per hour. Hence, the net duration of data recorded for each beam was restricted to between 1 and 4 days. Measurements were made over 14 beams each 6° wide; resolution along each beam consisted of 20 bins each 1.2 km long. Previous preliminary trial results[5] have indicated that, using an integration period of about 10 min, current measurements accurate to 1 cm s^{-1} can be obtained.

A simplified tidal analysis was performed by correlating (1) the time series $Z(t)$ for tidal elevations at Liverpool (predicted from harmonic constants) with (2) the time series $U(t)$ for the radial current component in a specific bin. A phase lag δt was introduced between these time series and the error $\varepsilon(\delta t)$ determined for the relationship

$$\varepsilon(\delta t) = \sum_{t=1}^{N} [(U(t) - \bar{U}) - \alpha(Z(t+\delta t) - \bar{Z})] \qquad (1)$$

where N is the total number of relevant observations, $\bar{U}$ and $\bar{Z}$ are means of the respective time series and the coefficient α was calculated by the method of least squares to minimize ε.

In this manner, the value for δt_{min} (within the range −12 h to +12 h) corresponding to the smallest overall value of ε was determined. The value δt_{min} was then assumed to represent the phase lag of current relative to elevation for the predominant M_2 constituent. Similarly the amplitude of the M_2 constituent of tidal current was assumed to be given by the product of α (corresponding to δt_{min}) and the M_2 amplitude for tidal elevation. The accuracy of this analysis was aided by the quiescent meteorological conditions with winds <10 knots for 80% of the time.

At each intersection point of the two beam sets shown in Fig. 1, the elliptical pattern of surface tidal currents for the M_2 constituent was calculated by linear interpolation of the respective current components in the nearest two bins. The component parts of Fig. 2 indicate the spatial distribution of the four parameters concerned: (1) amplitude of the major axis of the ellipse; (2) direction of the major axis; (3) phase or timing of the occurrence of maximum current relative to the lunar transit; and (4) ellipiticity, with positive values indicating anticlockwise rotation. Figure 2 also indicates comparable values

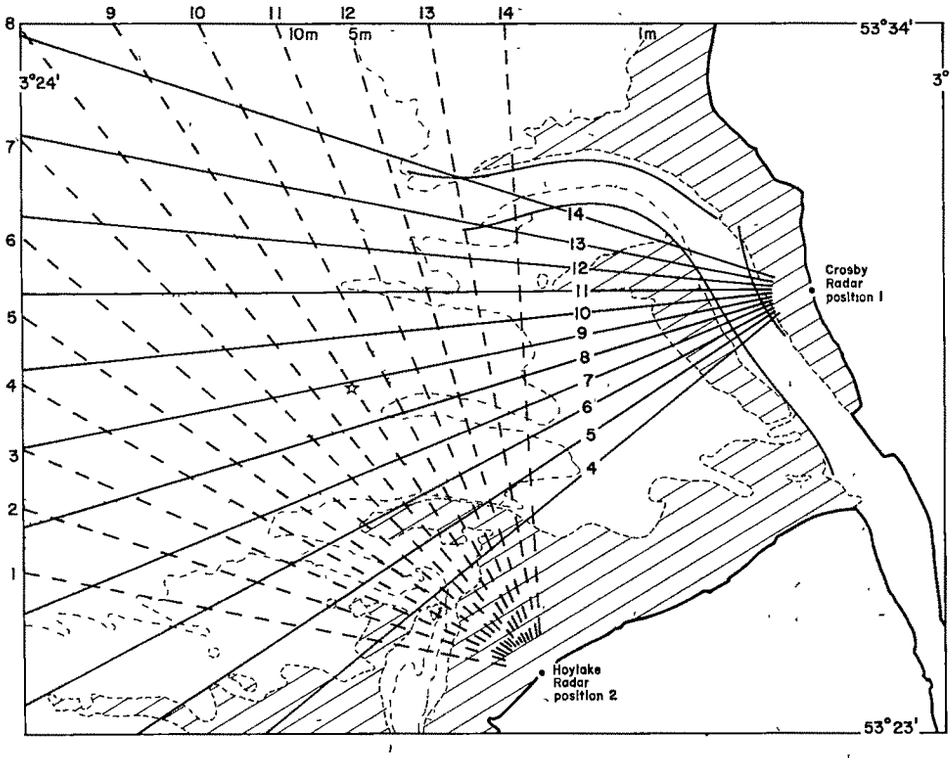


Fig. 1 Liverpool Bay showing centre lines of radar beams. ☆, Location of moored current meters.

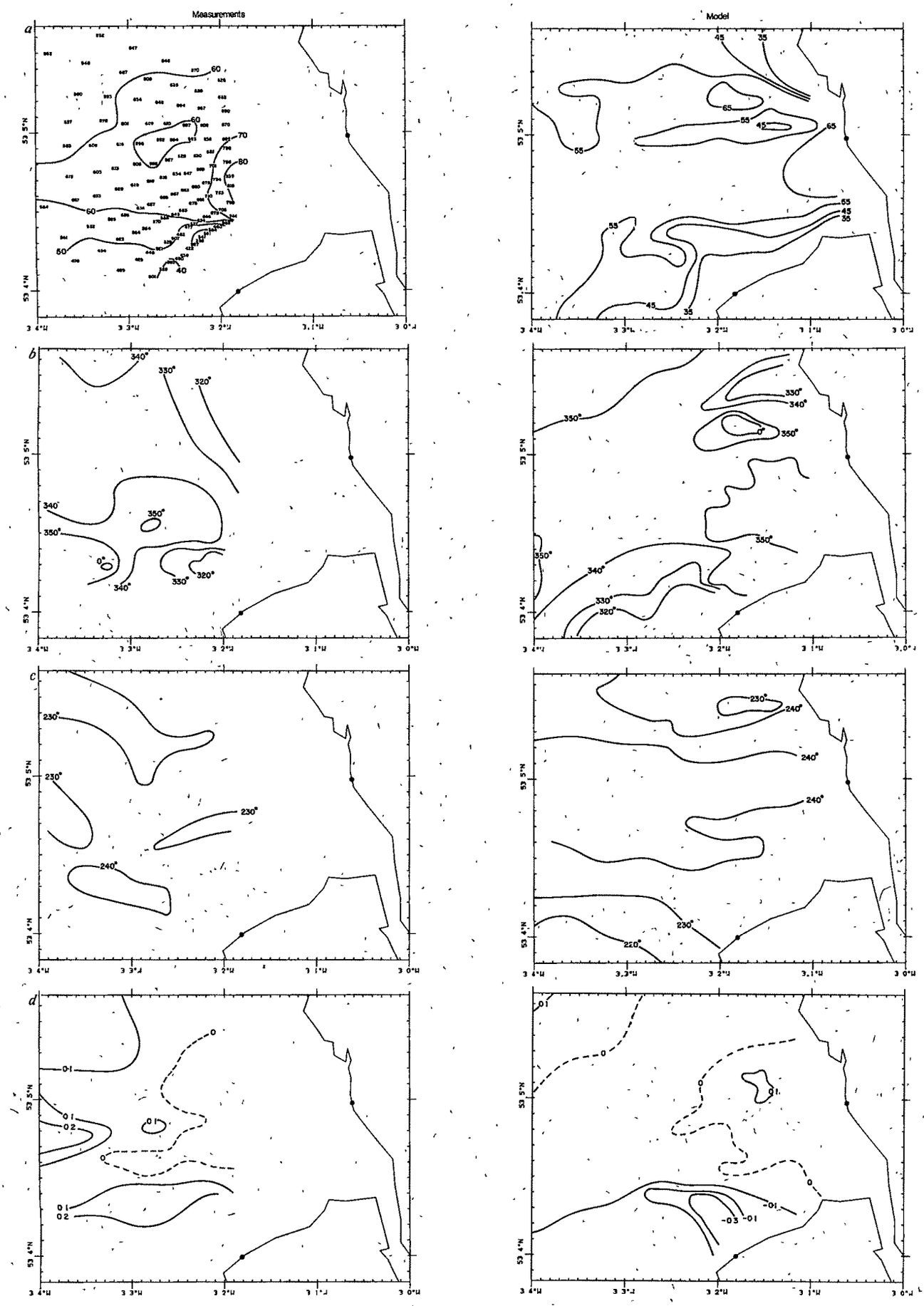


Fig. 2 M_2 current ellipse parameters from surface measurements by high-frequency radar (left), and vertically integrated numerical model (right). *a*, Amplitude of the major axis in cm s^{-1}; *b*, direction of major axis, measured anticlockwise from the east; *c*, phase, time of maximum current after lunar transit at Greenwich; *d*, eccentricity, minor axis/major axis, +ve anticlockwise rotation.

Table 1 M_2 tidal ellipse properties at 53° 28.3'N, 3° 15.4'W

	Amplitude (major axis) (cm s^{-1})	Direction* (deg)	Phase (deg)	Eccentricity (+ve anti-clockwise)
Numerical model	58	350	243	−0.02
Current meters (1)	54	351	237	−0.02
(2)	50	356	231	−0.04
High-frequency radar (surface)	61	342	232	+0.03
High-frequency radar corrected to mid-depth value	55	344	229	+0.08

* Measured anticlockwise from due east.

obtained from a numerical model based on a 1-km rectangular grid. In the model, the tides are specified at both the northern and southern limits of the Irish Sea and comparison between tide gauge recordings and model computations for M_2 elevations in Liverpool Bay indicates agreement to within 2% in amplitude and 7° in phase. Figure 2*a* is reproduced to a larger scale to illustrate the actual values of current amplitude obtained from the radar observations. The spatial coherence indicated by these values suggests that the precision of the radar measurements is of the order of 1 cm s^{-1}, thus substantiating theoretical estimates. Moreover, the agreement between spatial patterns from the radar measurements and the model establishes the accuracy of this instrument.

The surface amplitudes indicated by the radar are larger than the depth-mean values calculated in the model. This tendency is predicted from theoretical analysis of current structure[6,7]. Thus, for a position in the centre of the survey region, Table 1 indicates M_2 ellipse properties from (1) the numerical model, (2) two current meters, (3) the high-frequency radar surface measurements and (4) the high-frequency radar measurements corrected to reflect conditions at mid-depth. (The current meter deployments formed part of the OSCR evaluation experiment.) These latter corrections were carried out by resolving the ellipse into clockwise and anticlockwise components and assuming the current structure shown in Fig. 5 of ref. 6. Relative to surface values, the current ellipse at mid-depth exhibits a smaller amplitude, phase advance, increase in anticlockwise eccentricity and the direction may veer in either sense[7].

Table 1 also indicates that for the two current meters, located just 500 m apart, amplitude may vary by 10% and both phase and direction by 5°. Thus, allowing for the inherent spatial variability in current ellipse properties, both vertically and horizontally, the small discrepancies shown in Table 1 between the ellipse properties measured by the high-frequency radar and those obtained elsewhere appear entirely explicable. Likewise, the agreement between the high-frequency radar measurements and the numerical model shown in Fig. 2*b*, *c* and *d* for direction, phase and eccentricity respectively is within the limits of expected variability.

The residual circulation in Liverpool Bay is influenced by tides, winds and density gradients and hence may be highly variable in time. To construct a residual flow pattern from the present radar data involving velocity components measured on separate days up to 5 weeks apart must be of questionable validity. However, an examination of the residual current data was made both as an illustrative example of the potential use of dual OSCR systems and to indicate the order of magnitude of surface residuals even if the spatial distribution proved incoherent. Residual currents R for each bin were obtained from the expression

$$R = \bar{U} - \alpha\bar{Z} \qquad (2)$$

where $\bar{U}$ and $\bar{Z}$ represent the means calculated in equation (1) and the value of α corresponds to $\delta t_{\min}$. Then for each intersection point of the two beam sets, a residual current vector was calculated using linear interpolation between the respective

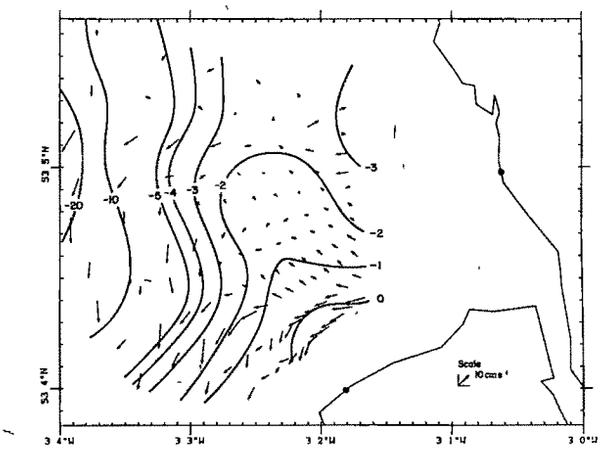


Fig. 3 Residual circulation measured by high-frequency radar. Streamlines are in units of 10^3 m^3 s^{-1}.

residual current components in the nearest two bins. Figure 3 shows these observed residual current vectors together with superimposed streamlines. The streamlines are based on the assumption that the surface residuals persist uniformly throughout the water column. The streamlines were constructed using the intersection of beam 9 from Hoylake and beam 5 from Crosby as a zero datum. The spacing of the streamlines was then determined along each beam from Hoylake by calculating the total flow orthogonal to each beam relative to a datum along beam 5 from Crosby. These datum values for beam 5 (Crosby) were initially determined in similar fashion by calculating flow orthogonal to the beam.

The results shown in Fig. 3 suggest residual currents at the surface of up to 19 cm s^{-1} with a mean value of about 5 cm s^{-1}. The maximum currents occur in the deeper water on the western boundary of the survey region. In this region, a strong southerly flow is indicated involving a flow of 20×10^3 m^3 s^{-1}, this latter circulation may be compared with a freshwater discharge of the Mersey of $\sim10^2$ m^3 s^{-1} and a net flow through the Irish Sea of about 60×10^3 m^3 s^{-1}.

Over the shallower regions in the eastern section, the residuals are much smaller and form an anticlockwise gyre. In the south-western section, a pronounced westerly circulation is suggested. Residuals measured by the moored current meters averaged over the period 1 April to 17 May 1984 were 5.6 cm s^{-1} towards the south-southwest 4.3 cm s^{-1} eastwards. The latter may reflect the proximity of this mooring to the knoll shown in Fig. 1 while the former value reflects assymetry in the direction of flood and ebb currents of some 10°. The magnitude of these currents is of the same order as the surface residuals shown in this region in Fig. 1, while the variation in their directions illustrates the limitations of conventional instruments for measuring residuals in regions of this kind.

Despite extensive efforts to measure the residual circulation in Liverpool Bay[8] there is no clear pattern against which to evaluate Fig. 3.

One residual flow component, seemingly overlooked in these earlier studies, is the Stokes drift arising from variation in total water depth over the tidal cycle. For the predominant M_2 constituent, a tidal elevation $\hat{Z}\cos\omega t$ combined with a tidal current $\hat{U}\cos(\omega t - g)$ produces a residual flow $\frac{1}{2}\hat{U}\hat{Z}\cos g$. Using the distribution of M_2 currents shown in Fig. 2 with the $\hat{Z}$ value of (295 cm, 318°) we calculate a net flow over the survey region due to Stokes drift of 10×10^3 m^3 s^{-1} directed southeastwards towards the Mersey (the direction corresponds to the net propagation of tidal energy). The velocities associated with this flow range from 2 cm s^{-1} in the deepest water to over 10 cm s^{-1} in the shallower southeasterly region. This Stokes transport must be counterbalanced by an Eulerian residual current component. The latter requirement may partially explain the strong westerly flow observed in the southern region of the radar data. However,

both observations and theory suggest that residual circulation in areas of the kind considered differ significantly between surface and bed and such considerations are beyond the scope of the present paper.

The residual circulation thus forms a coherent pattern with current magnitudes comparable with theoretically derived residual flow components. Most excitingly, high-frequency radar offers the possibility of measuring the residual circulations (at the surface) in such regions where conventional instruments have proven ineffective.

This initial evaluation of OSCR has confirmed the accuracy of the instrument and provided unique data on both tidal and residual circulation in Liverpool Bay. Further, more rigorous, determination of the accuracy of OSCR measurements is hindered by the absence of comparable independent measurements, the present results suggest a significant advance over the levels of accuracy cited in earlier high-frequency radar measurement[9-11].

Using the two OSCR units now available, similar surveys in other coastal areas can, in as little as 7 days, produce current data which may well supplant conventional data sets obtained over many years of observations. For the first time, the fine detail of spatial structure of surface currents can be measured. This should improve our theoretical understanding of the circulation within bays, around headlands, sand banks and so on, and thus be of direct benefit in studies of sediment transport, dispersal of pollutants or ship navigation.

The OSCR system was developed at the Rutherford–Appleton Laboratory under the direction of J. W. King; other members of the development group, F. D. G. Bennett, D. Eccles and K. Slater, participated directly in the Liverpool Bay experiment. Current meter observations were analysed by J. Howarth (IOS Bidston). We thank other staff from Bidston who participated in the measurement programme. D.K.R. is in receipt of a postgraduate studentship from the NERC.

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Lake Untersee, a first isotope study of the largest freshwater lake in the interior of East Antarctica

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Perennially ice-covered lakes partially bounded by glacier ice are common in some Antarctic coastal regions[1] but very rare inside the continent. Here we describe hydrological studies on the largest freshwater lake of interior Antarctica, Lake Untersee (71°20′ S/13°30′ E, surface area 10 km², maximum depth >79 m)[2], showing that the lake arose from a melt-water pond during climatic optimum periods in the Holocene. At present, the studied water body is thermally, hydrogeochemically and isotopically homogeneous because of thermal convection during the austral summer. Lake Untersee is fed throughout the year by underwater melting of the adjoining glacier ice. Isotope data suggest a permanent ice cover during its existence. The drainless lake is constantly losing water through sublimation on the surface of the more than 2.5-m-thick ice cover. The salt content suggests that the present water body is the remainder of an amount of melt water at least 50 times as great.

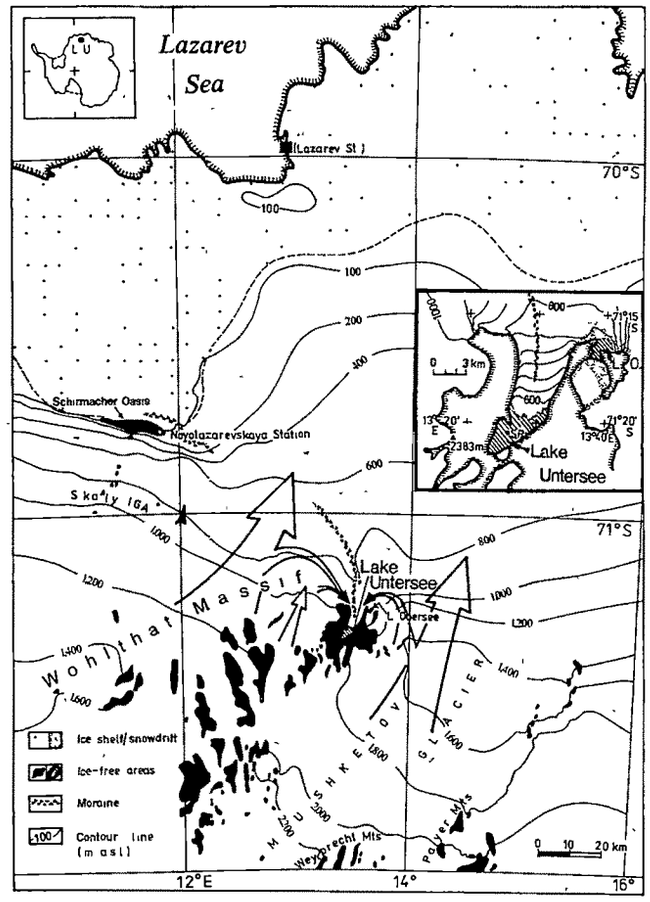


Fig. 1 Sketch map of the working area with locations mentioned in the text The arrows show the main glacier flows feeding Lake Untersee. L.U., Lake Untersee; L.O., Lake Obersee; S.P., sampling point.

On the northeastern margin of the mountains in central Dronning Maud Land, pilots of the German Antarctic Expedition in 1939 discovered two large dark ice planes under which liquid water was presumed to be present—Lake Untersee and Lake Obersee[3] (Fig. 1). Then, in 1969, a Soviet group explored Lake Untersee and proved the existence of a large water body under a 2.5-m-thick ice cover[2].

In March 1982 our group first implemented a hydrological sampling programme with the following results: (1) At the end of the summer, the water body sampled at depths of 5, 10, . . . , 40, 44 m was homogeneous down to the bottom (44 m), with respect to temperature ($T_{water} = (+0.45 \pm 0.05)$ °C), isotope composition ($\delta^2H_{water} = (-255 \pm 5)$ ‰, $\delta^{18}O_{water} = (-35.0 \pm 0.5)$ ‰) and salt content (sum of ions) ($M_{water} = (330 \pm 10)$ mg l^{-1}). Probably this remarkable homogeneity (within analytical error) results from convection, induced by the greenhouse effect of the ice cover, as known from lakes of the Schirmacher Oasis[4]. (2) The ice cover (interval 0–1.3 m) is characterized by $\overline{\delta^2H}_{ice} = (-250 \pm 5)$‰ and $\overline{\delta^{18}O}_{ice} = (-34.0 \pm 0.5)$ ‰. (The techniques of sample preparation and isotope analysis are described by Mühle *et al.*[5]. Isotope data refer to Standard Mean Ocean Water (SMOW).) (3) The tritium content of the water is below the detection limit, that is, <0.5 tritium units (TU). For comparison, large lakes of the Schirmacher Oasis, fed with melt water from old ice as well as from recent snow patches, have tritium contents of 6–8 TU (ref. 6). (4) Lake water is of sodium sulphate type with an extremely low magnesium content (Table 1, Fig. 2). (5) There are signs of primary production in the lake. As already observed[2] the bottom

is at least partially covered with an organic layer a few millimetres thick.

No continuous record of meteorological data is available, but the recent climate of the Lake Untersee region may be compared with that of the South Victoria Land dry valleys[7]: mean annual temperature of ~−20 °C and high summer aridity producing an annual sublimation rate of >200 mm of ice. In March 1982 no appreciable thawing was observed near the lake. The great difference in altitude between the lake surface (620 m above sea level (a.s.l.)) and that of the glaciers south of Lake Untersee (1,500–2,000 m a.s.l.) gives rise to strong catabatic winds.

Under recent climatic conditions the formation of such a large water body cannot be expected. Therefore, and because of the impossibility of increased endogenous heat flux, the origin of Lake Untersee must be sought in Holocene climatic optimum periods. The elucidation of the palaeoclimatic conditions of this area is the aim of investigations on organic deposits (fossilized stomach oils of petrels)[8] and ice-cores[9].

Relics of strandlines of boulders above Lake Untersee[3] indicate a former higher level of the lake and probably represent a pause in icesheet retreat (compare ref. 1). The salt content of the lake water is high compared with that of Antarctic snow and ice from locations at similar distances from the coast or similar altitudes (see Table 1)[10–19]. Marine aerosols (mainly Na^+ and Cl^-) and chemical weathering of rocks (mainly Ca^{2+} and SO_4^{2-}) are the principal sources of salts. Presumably, the local weathering solutions have a high Ca/Mg ratio because of widely distributed anorthosites (CaO/MgO > 50) in the Lake Untersee area.

For water-balance studies, chloride, which enters the lake exclusively as deposited marine aerosol via melting glacier ice, seems to be the most suitable indicator. A comparison of the Cl^- concentration of lake water (43.4 mg l^{-1}) and interior Antarctic precipitations (0.05–1 mg l^{-1}; see Table 1 and refs 10, 12, 13, 15, 19) shows the present-day water body to be the remainder of an at least 50 times larger quantity of melt water.

The isotope data indicate that Lake Untersee has been supplied mainly by underwater melting of old glacier ice in recent times. The hydrogeochemical characteristics of this inflow are determined by the precipitation chemistry of two nourishing areas (see Fig. 1): (1) the area between Richthofen, Weyprecht and Payer Mountains with a surface level of 1,400–2,000 m a.s.l. and deuterium contents of precipitations ranging between about −220 and −300‰ (ref. 20) and (2) the firn basin glacier between the Skaly IGA nunataks and the Wohlthat Massif with δ^2H ~ −200‰ (ref. 9).

If more than 200 mm are removed annually from the ice cover of the lake by sublimation, a corresponding regeneration of the floating ice cover may occur through freezing of water onto its underside during the low insolation season. However, the observed isotope differences $\delta_{ice} - \delta_{water}$ are lower than expected for a thermodynamically-equilibrated ice/water system[21] by ~15‰ (δ^2H) and ~1‰ ($\delta^{18}O$); the same tendency was observed in some lakes of the Schirmacher Oasis[4]. In both cases the ice formation seems to be diffusion controlled: concentration of the heavy isotopes in the forming ice produces a depletion in ^2H and ^{18}O in the adjacent water. If the rate of downward growth of ice is high enough, say 0.1 cm h^{-1}, diffusion, being the only active mixing process within the stably stratified water system (diffusion coefficient $D_{water}(0\,°C) = 1 \times 10^{-5}$ cm^2 s^{-1})[22], is unable to rehomogenize the liquid phase. Thus, the deeper freezing ice equilibrates thermodynamically not with the bulk water of the

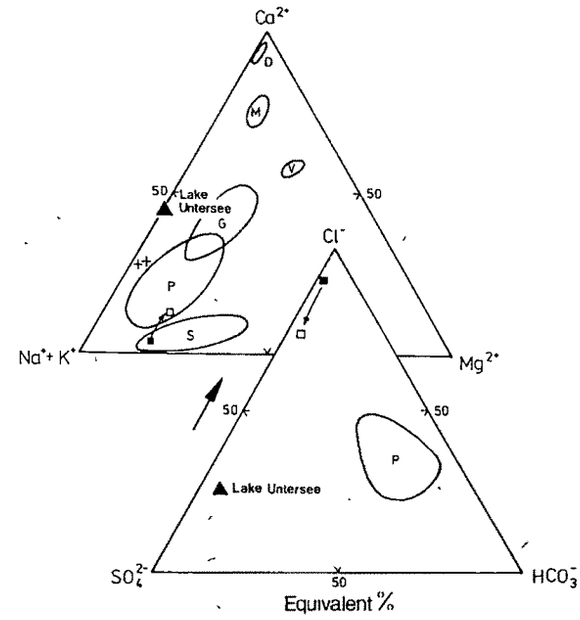


Fig. 2 Chemical composition of Lake Untersee water compared with other Antarctic waters P, Atmospheric precipitation, Schirmacher Oasis, Dronning Maud Land (after ref. 6); D, Don Juan Pond, saline water, G, glacial melt water; M, Lake Miers, fresh water; S, Antarctic snow and ice; V, Lake Vanda, saline water (fields from ref. 28); ■, Antarctic sea water (after ref. 26); □, average atmospheric precipitation over the oceans (after ref. 29); +, snow, Lake Untersee area (see Table 1).

Table 1 Chemical composition of Lake Untersee water and of Antarctic atmospheric precipitations sampled in the working area

Sample	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	SO_4^{2-}	$\frac{Na^+}{Cl^-}$	$\frac{SO_4^{2-}}{Cl^-}$	$\frac{Ca^{2+}}{Mg^{2+}}$
Lake Untersee, average lake water (2.7–44 m)	60.5	4.25	44.5	0.18	16.7	43.4	158.1	1.4	3.6	247.2
Snow*, Lake Untersee area (~1,250 m a.s.l.)	4.0	1.1	1.7	0.15		4.7	4.2	0.8	0.9	11.3
Snow* on the ice cover of Lake Untersee	2.5	0.5	0.95	0.05		2.4	3.1	1.0	1.3	19.0
Freshly fallen snow, Schirmacher Oasis[24]	0.2–2.5	0.07–0.6	<0.05–0.8	<0 05–0.48		1 8–3.6		0.3–0.9		0.4–10
Firn/ice, Law Dome, East Antarctica[25] (distance from coast 110 km, 1,400 m a s.l.)	0.050	0.006	0.004	0.008						0.5
Snow, James Ross Island, Antarctic Peninsula (1,660 m a.s.l.)[13]	0.25	0.009	0.013			0.58		0.43		
Atmospheric precipitation, Mirnyy Station[11] (annual mean)	0.98	0.19	0.057	0 087		1.24	0 38	0.79	0 31	0.65
Sea water[26]								0.56	0.14	0.04

Concentrations (mg l^{-1}) of cations were determined using an atomic absorption spectrometer; those of anions, by standard techniques of water analysis[27].

* Snow presumably altered by sublimation causing unusually high salt contents.

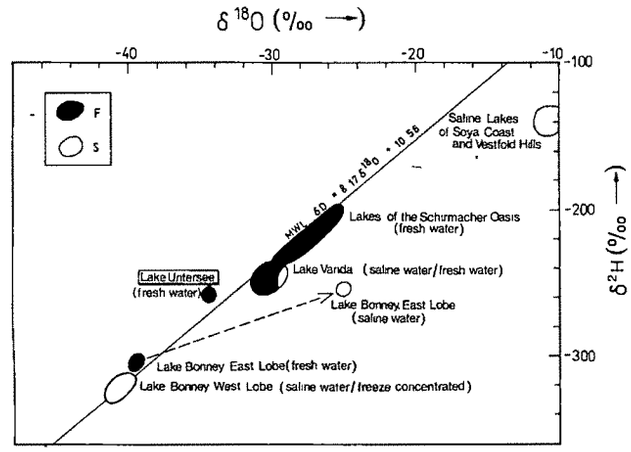


Fig. 3 Position of Lake Untersee water in the $\delta^2H/\delta^{18}O$ diagram (values versus SMOW). For comparison, data[23] of other Antarctic lakes with different evolutions are also shown. Freshwater lakes (type F) are slightly shifted isotopically to the left of the Meteoric Water Line (MWL)[21] by freeze-concentration. Saline lakes (type S) are, as a rule, isotopically shifted well to the right by dominating evaporation-concentration.

lake but with isotopically somewhat lighter water of the boundary layer. Because the sublimation of the ice cover takes place layer by layer without any isotope separation, the heavy-isotope content of the remaining liquid water decreases as the amount of water decreases[21] and it remains at slightly lowered values if input and sublimation are equilibrated[23]. In both cases, an increase in salt concentration is noted.

Lake Untersee has been predominantly or permanently covered with ice throughout its evolution. This is demonstrated by the position of its water isotope data in the $\delta^2H/\delta^{18}O$ diagram (Fig. 3), slightly shifted to the left from the global Meteoric Water Line (MWL)[21] by the isotope fractionation in the water/ice transition. The permanence of an ice cover is also supported by the occurrence of supra-glacial till boulders resting on the ice cover (compare ref. 1). The dominance of evaporation at an ice-free surface would have produced a shift to the right side of the MWL as observed in most of the Antarctic saline lakes[23].

A comparison of the strandline of the Lake Untersee on the basis of air photographs taken in 1939, 1961 and 1982 does not show any relevant change in the shape of the lake. Obviously, its mass balance in this period was approximately equalized.

More extensive work is in progress on the Lake Untersee environment as well as its Holocene evolution.

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A new method for monitoring temporal trends in the acidity of fresh waters

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Acid precipitation and the consequent increased acidity of fresh waters are subjects of widespread concern. Several organizations in the United States, Canada and Europe have established extensive monitoring programmes in an attempt to determine the relationship between the acidity of preciptation and the change in acidity of fresh waters. We contend here that the present strategies for monitoring temporal trends in the acidity of fresh waters will not yield results sensitive enough to detect trends in acidity even on a 10-year timescale. We propose here an alternative scheme based on calculations using precise measurements of dissolved inorganic carbon (DIC) and partial pressure of CO_2 (P_{CO_2}). This scheme eliminates biases inherent in *p*H electrode determinations and minimizes the perturbations in acidity caused by changes in the P_{CO_2} of lake waters associated with biological cycles.

A method that accurately and precisely yields hydrogen-ion activity is needed for the following reasons: (1) Aquatic biota are sensitive to hydrogen-ion activity. Most laboratory bioassay experiments refer to changes in, for example, quantity, distribution and viability of a given species as a function of *p*H. (2) Trace metal mobilization is one of the major effects of lake acidification. Hydrogen-ion activity is an essential parameter in metal speciation calculations. (3) Total alkalinity measurements cannot reliably substitute for measurements of hydrogen-ion activity. The presence of titratable organic anions leads to an overestimate of bicarbonate ion from acid titration data and hence to an underestimate of hydrogen-ion activity at any given P_{CO_2}. (4) The predicted response of a lake to acidification for a given *p*H of rain is complicated by many unknown factors such as dry fallout, soil and sediment neutralization and HNO_3 sequestering. Policies to set anthropogenic emissions of nitrogen and sulphur oxides must be based on sound predictive models derived from accurate empirical data.

The difficulties associated with measurement of *p*H using the standard glass/calomel- or combination-electrode systems have been well documented. In dilute natural waters, electrode determinations are very sensitive to sampling and handling artefacts and to liquid junction potential problems[1-6]. The use of a flow-cell electrode system[5] has to some extent remedied the last problem, although the technique has not yet received widespread application. More serious are interactions of dissolved organic carbon (DOC) and/or suspended matter with the electrodes, which cause systematic errors of up to 0.5 *p*H units[2-4].

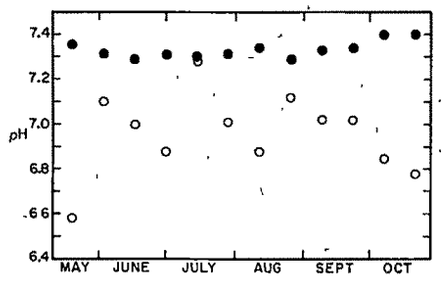


Fig. 1 Comparison of pH measured by electrodes (⊙) with pH calculated from DIC and P_{CO_2} data using equation (2) (●) for ELA lake 239 during the summer of 1982. DIC can be determined by acidifying a small volume of water to pH < 4 and then stripping the CO_2 with helium gas. The gas phase is analysed for CO_2 on a gas chromatograph or infrared analyser to a precision of better than 1%[11]. P_{CO_2} is determined by equilibrating an aliquot of water with a small volume of air and then an aliquot of the air is returned to the laboratory for GC or infrared analysis. Replicate equilibrations agree to within 2%. Equilibrations should be performed in the field because of the extreme sensitivity of the P_{CO_2} to biological processes. The hydrogen-ion content can thus be calculated with a precision of ±4% at pH 7 and ±10% at pH 5.6, which is substantially better than can be obtained by electrode measurements (±20%). The analytical precision of the pH electrode is a moot point because of the systematic errors of up to 0.5 pH units (a factor of three in H^+ activity) in the pH range 5.6–7. The measurements of DIC and P_{CO_2} are no more difficult or expensive than quality pH determination.

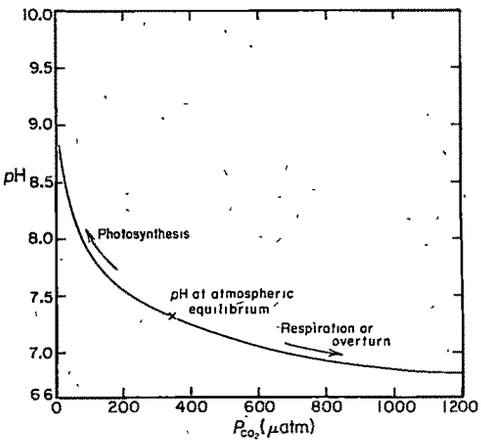


Fig. 2 Calculated dependence of pH on P_{CO_2} for lake 239 surface water. ($[HCO_3^-] = 115$ μmol l^{-1} at 20 °C.) The range corresponds to that which may be caused by algal blooms or overturn events. An upper P_{CO_2} limit of ~1,000 μatm can be achieved for surface water during fall overturn when hypolimnetic water (P_{CO_2} = 3,400 μatm; A.H., unpublished data) is brought to the surface at a faster rate than gas exchange can remove it to the atmosphere. Although we did not observe a P_{CO_2} depression in lake 239 during the summer, the response of eutrophied lakes to phytoplankton blooms[7–9] suggests that the P_{CO_2} of those lakes must be well below 10 μatm.

An intercomparison among 17 laboratories using standard electrode techniques was conducted, in which each was given an aliquot of a sample known to have a pH of 6.18 (ref. 6). They reported a mean value of 5.54 with a standard deviation of 0.74 pH units. At pH values below the accuracy and agreement were significantly better and it may be that the biases that plague electrode measurements become smaller as the hydrogen-ion activity increases.

As well as the abovementioned analytical problems, pH is sensitive to changes in P_{CO_2} (ref. 7). Photosynthesis, respiration and entrainment of hypolimnetic water, by temporarily raising or lowering P_{CO_2}, create a perturbation of the longer-term pH trend. For example, lakes that normally have a pH ≈ 7 when at equilibrium with atmospheric P_{CO_2} may have their pH driven as high as 10 during phytoplankton blooms because of the photosynthetic consumption of CO_2 (refs. 8–10). Although equilibrating water samples with standard atmospheric P_{CO_2} (340 μatm) potentially provides a means of circumventing this difficulty, this approach is subject to the electrode errors described above.

Highly accurate and reproducible hydrogen-ion concentrations can be calculated from measurements of DIC and P_{CO_2} (ref. 4) from the equilibrium relationship

$$pH = -\log \frac{[H_2CO_3^*]K_1}{([DIC]-[H_2CO_3^*])} \quad (1)$$

where $[HCO_3^-] = [DIC] - [H_2CO_3^*]$ at $pH < 8$ and $K_1 = [HCO_3^-][H^+]/[H_2CO_3^*]$, and $[H_2CO_3^*] = [H_2CO_3] + [CO_2]$.

The analysis of P_{CO_2} and DIC allows pH to be normalized to that which would prevail for equilibrium with the ambient P_{CO_2} in the atmosphere above the lake. The normalization is carried out as follows with the assumption that alkalinity is conservative:

$$pH^0 = -\log \left[\frac{P^0_{CO_2} K^0_H K^0_1 [H^+]_c}{[H_2CO_3^*]K_1} \right] \quad (2)$$

where the superscript refers to the value of a given parameter at mean atmospheric P_{CO_2} and 20 °C, K_H is the solubility of CO_2 and $[H^+]_c$ is calculated from DIC and P_{CO_2} measurements (equation (1)).

A comparison of pH obtained by direct measurement using a glass electrode with that calculated from DIC/P_{CO_2} measurements is shown in Fig. 1. Lake 239 at the Experimental Lakes Area (ELA), northwestern Ontario, is a Canadian Shield lake that was routinely monitored for pH, DIC and P_{CO_2} during the summer of 1982. The dissolved CO_2 content ($H_2CO_3^*$) of surface water averaged 13.5 μmol l^{-1} (normalized to 20 °C) during this period with a standard deviation of 0.3 μmol l^{-1} and was always close to equilibrium with the P_{CO_2} of the atmosphere. The normalized pH results calculated from the DIC/P_{CO_2} data show only a very small scatter around a mean of 7.33. In contrast, the electrode-measured pH data scatter over a range of 0.7 pH units with a mean of 6.93. The pH measurements were made using standard laboratory techniques at the ELA chemistry laboratory[11]. The comparison illustrates both the poor precision and inaccuracy of the pH electrode.

The calculated response of lake 239 surface water to changes in P_{CO_2} is shown in Fig. 2. Extremely low or high P_{CO_2} that can occur during phytoplankton blooms or overturn events, respectively, could produce variations in pH as large as 2 pH units (100-fold H^+ activity). Therefore, even if the *in situ* pH could be electrometrically measured accurately, time trends in acidity would be hard to detect if the P_{CO_2} fluctuations were not taken into account. However, when the P_{CO_2}/DIC method is used, the calculated pH (equation (1)) can be normalized as in equation (2), thus providing both the *in situ* and equilibrium values from a single set of measurements.

The perturbation of pH resulting from changes in the amount of organic acid anions may be important at lower bicarbonate ion concentrations. If during a given season there is a net production of consumption of 'organic' alkalinity, then the concentration of bicarbonate ion must change in the opposite sense to maintain the charge balance. If the P_{CO_2} remains constant through equilibration with the atmosphere, a change in the $HCO_3^-/H_2CO_3^*$ ratio produces a change in pH in accord with equation (1).

Total alkalinity (ΣaAlk) has been suggested as the best measure of acidification trends in lakes and streams. Because it can be measured with a precision of better than 1%, a reliable value for the free acidity of water at equilibrium with the atmosphere could presumably be calculated using equations (1) and (2). However, organic acid anions may comprise a substantial portion of the total alkalinity[4,12–16]. Therefore, attempts to calculate pH from measured ΣAlk without accounting for this component may be in error, especially at low $[HCO_3^-]$ ($pH \simeq 6$).

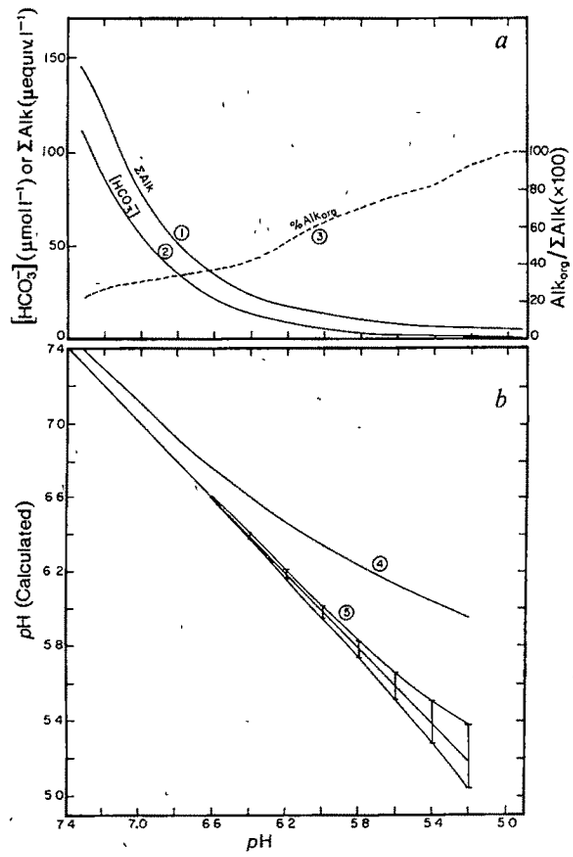


Fig. 3 *a*, The response of ELA lake 239 to acidification is shown by a simple open-system acid titration. Curves 1 and 2 represent the concentrations of ΣAlk and HCO_3^-, respectively, as a function of *p*H. Curve 3 indicates the fraction of ΣAlk made up of organic acid anions. $P_{CO_2} = 340$ μatm at 20 °C. *b*, The *p*H calculated for lake 239 by assuming that $\Sigma Alk = [HCO_3^-]$ is given by curve 4. These calculated *p*Hs deviate from the true values (curve 5) by an increasing amount as the acidification progresses. Error bars on curve 5 represent the analytical uncertainty of the calculated *p*H values using the P_{CO_2}/DIC method.

Consider again ELA lake 239, with an average ΣAlk = 146 μequiv. l^{-1}, $[HCO_3^-] = 113$ μmol l^{-1} and, by difference, $Alk_{org} = 33$ μequiv. l^{-1}. If this lake were to undergo acidification, the components of alkalinity might vary with *p*H as shown in Fig. 3*a*. Because the pKs for organic acids are generally much lower than pK_1 for $H_2CO_3^*$ (refs 12–16), most of $[HCO_3^-]$ will be consumed before there is a significant depletion in organic anions. At *p*H values near 6, ΣAlk is much greater than $[HCO_3^-]$, hence any attempt to calculate *p*H using K_1 will lead to serious error as is shown in Fig. 3*b* by comparing curves 4 (which assumes that $\Sigma Alk = [HCO_3^-]$) and 5 (which represents the *p*H calculated by the P_{CO_2}/DIC method).

The method for monitoring the acidity of lakes using DIC and P_{CO_2} measurements avoids both electrode and organic alkalinity problems and minimizes perturbations caused by fluctuations in P_{CO_2}. Given the variability of our calculated *p*H values for lake 239 (Fig. 1), we could reliably detect a change of >0.1 *p*H units in the equilibrium-acid status of the lake by making a single set of midsummer P_{CO_2}/DIC measurements at any time in the future. Collecting a few samples during a summer would reduce this detection limit by at least a factor of two.

The monitoring strategy described here is not intended for lakes that are already acidified. It is only applicable to waters with *p*H >5.5, below which the difference between DIC (that is, $[HCO_3^-]+[CO_2]$) and $[CO_2]$ becomes too small to permit accurate determinations of $[HCO_3^-]$. For lakes now on the brink of serious acidification effects (that is, in the *p*H range 5.5–7.0), our method provides a far more reliable and sensitive indicator of acidification trends than can be obtained by any other method.

Our proposal requires only that the determination of P_{CO_2} and DIC be included in the existing monitoring programmes.

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Secular climate change in old-growth tree-line vegetation of northern Quebec

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The climate of the Northern Hemisphere changed during recent centuries, as shown by the Little Ice Age episode[1] and the warming trend of the past 100 years[2,3]. The ecological impacts of these changes have yet to be evaluated in several terrestrial ecosystems, incorporating direct evidence such as detailed botanical field observations[4]. We report here results of the analysis of a ~600-year response of a lichen–spruce woodland to this long-term trend, which are thought to be the first extensive illustration of these impacts in the Subarctic. It suggests that tree-line vegetation is in a dynamic equilibrium with climate in the absence of other external disturbances; this is emphasized by spruce reaction through phenotypic adaptation—a shift from stunted individuals (krummholz) to normal trees (forest)—and differential regeneration. This study produces evidence that marginal northern forests can persist through time and that successional processes, in the absence of fire, perpetuate the original lichen–spruce facies. The longest tree-ring chronology (AD 1398–1982) yet available in eastern North America was constructed from living and dead spruces found in the lichen woodland of our study.

It has been shown recently[5] that the extensive treeless areas within the northern Québec Forest–Tundra zone are the result of late Holocene fire disturbance and climatic change. Exceptions to this deforestation trend are found around the tree line near Hudson Bay, where old-growth coniferous vegetation persisted without any fire for several centuries. One of the best examples is a lichen–black-spruce (*Picea mariana* (Mill.) BSP.) woodland, 3–4 km long, about 10 km south of the present tree line in the Bush Lake area (57°47'N, 75°45'W). This stand occupies a well-drained site that burned for the last time around 900–850 yr BP, in the immediate vicinity of the sampling area, as indicated by two radiocarbon-dated charcoals found below the organic layer (UQ-163: 890 ± 55 yr BP; UQ-158: 850 ± 85 yr BP), that is, around dendroyears AD 1075 ± 57 and 1160 ± 86 using the radiocarbon-dated calibration curve of Stuiver[6].

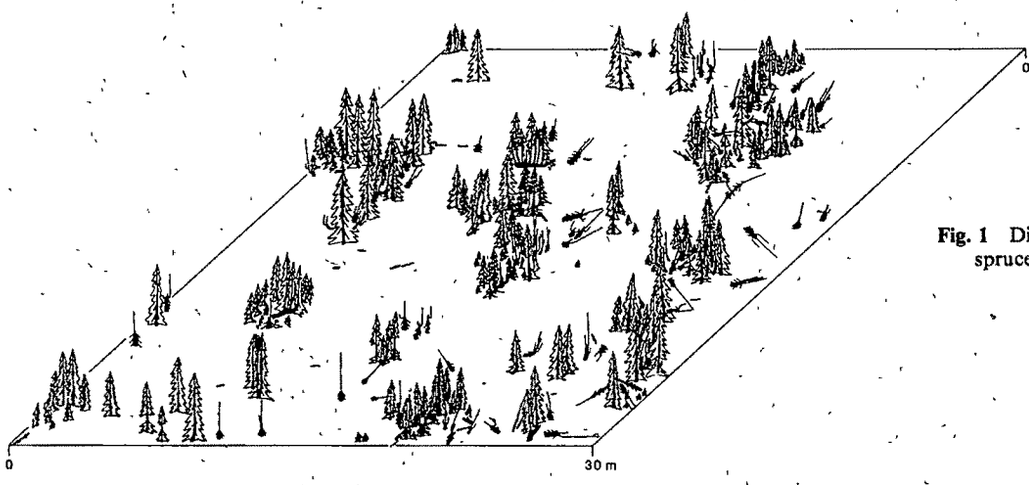


Fig. 1 Distribution of the living and dead spruces within the 900 m² quadrat.

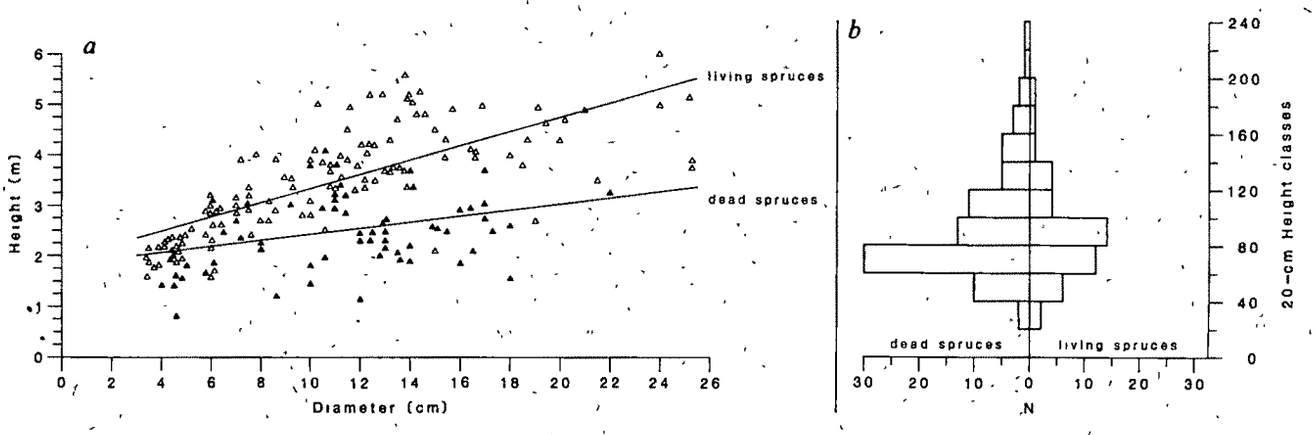


Fig. 2 *a*, Relationship between basal stem diameter and total height in living (△, $y = 1.9621 + 0.1391x$, $r = 0.74$) and dead (▲, $y = 1.8203 + 0.0604x$, $r = 0.35$) spruces. Differences in the regression slopes are statistically significant at the 0.001 level. *b*, Height (20-cm class) above ground of multiple-stem levels and branch leaders. The bulk of multiple-stem level distribution in dead and living spruces is respectively around 60–80 and 80–100-cm classes, which suggests an overal difference of 20 cm in depth of snow cover; the dead spruces were probably in an environment with less snow compared with living spruces.

The woodland consists of dominant living low spruce trees along with shrubby or subarboreal stunted leaning dead spruces (Fig. 1). We have postulated that the two contrasting growth forms developed under different climatic regimes; the abundance of dead spruces in such an environment made us suspect that the woodland was growing old in the absence of external disturbance—a unique situation for evaluating the stability of marginal northern forests.

A 900 m² (30m×30m) quadrat was used to determine the spatio-temporal development of the coniferous vegetation (Fig. 1). All the living and dead spruces were mapped and their growth forms, such as arboreal, subarboreal and shrubby, described (Fig. 1). Diameter at stem base, total height and winter damage expressed by morphological traits such as above-ground multiple-stem and branch leaders, along with bare (leafless and branchless) stems above snow cover, were measured on all individuals (Fig. 2). The origin, by layering or by seed, was determined from stem morphology, stem distribution and below-ground branch connections[7]. Samples of living spruces were taken at stem base and at the multi-stem level that is thought to mark the mean winter snow position. The dead stems were sampled immediately above the level of decomposition (stems rotted near the ground), generally 30–100 cm above ground level, and also at the snow-air interface and stem apex; although the real age, that is, the age of the stem at the ground level, could not be determined on these samples, the data were used to estimate the date of stem death.

Tree-ring measurements were performed on fine-sanded cross-sections with a standard micrometer (0.01 mm precision). The dating of dead stems was made possible because of the very good condition of preservation of these samples and the presence of such diagnostic light rings of very few latewood cells as those dated to AD 1593, 1816 and 1817 (L.F., S.P. and L.G., unpublished results). The tree-ring chronology was extended to AD 1398 by cross-dating. The oldest black spruce was 504 yr old, which extends the known longevity for that species[8] by at least 200 yr. The chronology was built from 39 radii belonging to 26 stems. Reaction wood[9] was common in leaning stems, but measurements made on the different sets of tree-rings, with or without compression wood, showed the same growth; samples with a changing direction of compression wood were rejected. Missing or incomplete rings were frequent, especially for the period 1600–1610, and annual rings were particularly narrow between 1398 and 1880, virtually at the lower limit of spruce growth. Because of these anatomical and morphological ring characteristics, the chronology has been based on the arithmetical mean of the 39 series. In addition, an overall-growth curve was produced from multiple polynomial fitting of the 39 series.

The 233 stems recorded consist of 136 living and 97 dead specimens (Fig. 1). Striking size differences were found between the two groups, especially with increasing size of basal stem diameter (Fig. 2*a*); large dead stems reached about 3.5 m in height, whereas large living spruces were 1–1.5 m higher. Living spruce boles are predominantly erect, radially symmetrical and, occasionally, eroded at the snow–air interface level, whereas dead spruces showed oblique and anisomorphic boles together with multiple-stem leaders located at a former snow–air interface

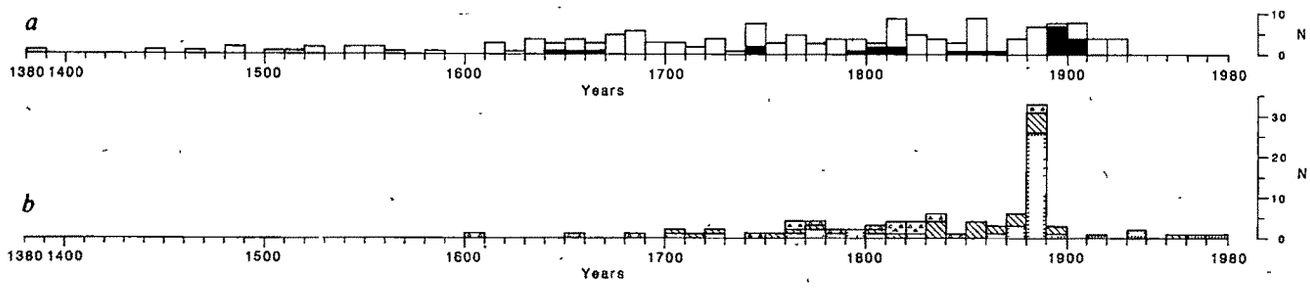


Fig. 3 *a*, Age structure of living spruces according to origin (white: layering; black: seed). Age distribution among 10-yr classes is thought to cover the difference between real stem age and stem age at the sampling level. Because the age structure is of the present-day (sampling year in 1983) living individuals, the data cannot reliably be extrapolated back in time. Absence of seedling establishment in the climatically favourable period of the 1930s and 1940s appears to be related to quadrat sampling size; areally small clustered cohorts from that period were found a few metres away, but are not incorporated here in the age structure histogram. We think that the naturally low seedling regeneration rate of this lichen woodland during the past centuries could be estimated more properly with larger sampling units. *b*, Mortality dates of spruces (triangle, death of stem apex; hatched, death of stem at the snow-air interface; dot, death of basal stem, that is, below the snow-air interface). 10-yr class. Mortality dates were estimated only for well-preserved outer stem sections, which give the overall mortality trend of established stems during the past two centuries, but not for previous centuries because of an unknown proportion of decomposed stems.

level (Fig. 1). Frequency distribution of postulated snow-air levels between living and dead spruces suggests that the former were growing in an environment with less snow (Fig. 2*b*), under colder conditions probably associated with frost desiccation and deep-freezing of exposed leaves[10,11].

The all-aged structure of the living spruce population (Fig. 3*a*) indicates continuous development during the past centuries, mainly through layering. Only 16% were identified as seed-origin individuals and 50% of these germinated between 1890 and 1910; the remaining seed population established episodically between 1640 and 1870. Layering occurred continuously throughout the past 600 yr (young layers were not sampled within the quadrat). Mortality was also an important component of the spruce population dynamics, particularly between 1700 and 1980 (Fig. 3*b*). Tree-ring analysis of dead stems revealed differential mortality according to the position of the living tissues relative to snow cover. Above-snow stems often died 100 yr earlier than stem sections located at the snow-air interface; death of the trunk followed within a few decades. An important feature here is the rather sudden mass mortality of spruces during a 10-yr period, between 1880 and 1890, when 57% of the subfossil population died. At that time, 70% of the dying spruces were older than 200 yr and 30% were older than 250 yr. Senescence appears to be one factor explaining the mass mortality; considerable reduction of the photosynthetic structure is a possible factor, as suggested by the very low growth during many decades before death. The cost of reproduction[12] may have also some influence here on the overall vigour of the old-aged and moribund spruces at a time when cone production was stimulated. In fact, this period of mass mortality coincides with a short interval of seedling establishment (Fig. 3*a*, *b*). Other factors such as pathogen attacks (for example, fungus, insects) remain uncertain.

Tree-ring responses to the long-term developmental process of spruce vegetation are clearly expressed in the 600-yr chronology (Fig. 4). Two periods of different duration are shown, a slow-growth period between 1398 and 1890 and a faster growth period afterwards. Very narrow rings were formed during the first period, especially around 1420–1435, 1480–1495, 1600–1610, 1690–1700, 1825–1830, 1835–1840 and 1855–1860. Most of the period corresponds to the Little Ice Age, particularly between 1550 and 1850 (ref. 1). Yearly variations in ring width were not important except before 1520; but, more data are needed to evaluate thoroughly the period before 1550 where short intervals of larger tree rings and presence of tree-like subfossils suggest less severe climates than during the Little Ice Age. Low stature and limited photosynthetic mass during that period could have caused variability in tree-ring responses, although the overall ring pattern is one of climate suppression on tree growth. Large tree-ring widths during the second period are associated with milder climates well documented by instrumental records[2]. Yearly variability is higher here and tree growth climaxed in the 1930s and early 1940s, during maximum warmth[2]. A reversal in the growth trend occurred afterwards and corresponds to the post-1940s cooler conditions registered in the Northern Hemisphere, except for a brief interval between 1950 and 1960 (ref. 14).

The Bush Lake lichen woodland represents an outstanding example of vegetation response to climate change in the absence of other known external disturbances. The discovery of older dead spruces may extend the chronology to 800–900 yr. The ecological impacts of the Little Ice Age climate were significant in terms of spruce morphology and regeneration, because the population formed a krummholz (stunted spruces) of predominant long-lived layers. However, episodic seedling establishment occurred during short intervals, such as 1640–70, 1740–50, 1800–20 and 1840–60. An important conclusion here is that krummholz may regenerate sexually during short milder periods, as suggested by the tree-ring chronology. Another is that shifting from krummholz to forest expresses a major change towards milder conditions culminating during this century. This shifting in growth habit appears to be a large-scale phenomenon observed throughout the northern Québec Forest–Tundra. It is also important to consider that the time lag in growth-form shifting, attributable to change in climate, is determined in part by the time when the critical position of apical buds and needles relative to the winter snow pack is reached. This suggests that winter climate defines the climate threshold where apices of growing spruces, or exposed buds, needles and twigs, located at the snow-air interface, experience harsh conditions that eventually discriminate the future growth forms among long-lived individuals. Seedlings established during the past two centuries that have developed into tree form, probably reached the snow-air interface when the winters became somewhat milder. Therefore, we may conclude that the time lag between a change in

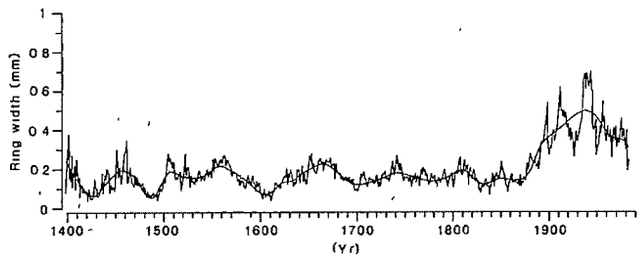


Fig. 4 Tree-ring chronology, AD 1398–1982, built from 26 stems of living and dead spruces (mean ring width in mm from 39 radii). Also included is the long-term growth curve derived from polynomial fitting[13].

climate and phenotypical spruce response is many years, sometimes a few decades, a situation controlled in growing spruces by apical-bud residence time into the critical snow–air interface. The transition from the Little Ice Age to the present regime was also characterized by mass mortality in old-aged spruces that were not able to take advantage of the new favourable climates and by the replacement of these spruces by seedlings that later formed normal trees. Although we know of no other similar example of mass mortality in northern forests that is not caused apparently by insect outbreaks, we believe that our results suggest a possible link with the changing ecological conditions that occurred at the end of the nineteenth century, when old spruces with limited photosynthetic mass were already engaged in a lengthy process of dying. A closer look at the biological effects of energy expenditure in senescent or moribund spruces during a period of climate recovery, could lead to a better understanding of this intriguing phenomenon. Finally, we conclude that the overall developmental sequence described here indicates that lichen woodlands can regenerate sexually in the absence of fire disturbance and do not degrade into tundra vegetation as suggested elsewhere[15]. It illustrates the importance of vegetation inertia, in a fire-free ambience, for persistence of communities in cold environments characterized by episodic sexual regeneration. From a climatological viewpoint, the conditions expressed by this old-growth vegetation were probably associated during the Little Ice Age with very cold windy winters with thin snow cover and cool moist summers and, during the recent warming, with milder and snowier winters (a postulated 20-cm mean increase in snow depth based on growth forms, Fig. 2*b*) and mild summers. The 600-yr chronology exhibits the marked differences observed among dead and living spruces and it may be considered as a typical example of secular climatic change in the eastern part of the North American Subarctic.

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Isotope assessment of Holocene human diets in the southwestern Cape, South Africa

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Models of seasonal mobility to exploit seasonally abundant food sources have been proposed for prehistoric hunter-gatherers in many parts of the world[1–3]. Some such hypotheses involve fundamental and insufficiently tested assumptions about the nature of both hunter-gatherer societies and the archaeological evidence that they leave. The present study is an independent test of such a hypothesis proposed for the southwestern Cape of South Africa. In this strongly ecologically differentiated area there are four distinct ecological zones that would have offered four different sets of resources to prehistoric people. Obvious modern seasonal fluctuations in these resources, plus a considerable amount of archaeological evidence, led to the suggestion that prehistoric hunter-gatherers moved in a regular seasonal cycle across the zones[4,5]; this would have allowed them to make maximum use of temporarily plentiful plant and animal foods in some areas, while avoiding lean periods in others. However, as reported here, direct measurements of food intake, as reflected in the stable carbon isotope ratios of archaeological human skeletons, reveal that this was not the case. The implications of this study extend beyond the relevance to local archaeology to more general questioning of the ways in which archaeological data should be used to generate hypotheses.

Figure 1 shows the four north–south ecological zones of the southwestern Cape: the Atlantic shoreline, the temperate coastal plain, the Fold Mountain Belt and the semi-desert Karoo. Each zone has a different geology, rainfall and vegetation. Holocene archaeological sites in the area contain quantities of plant and animal food waste, much of it identifiable as having been collected in the summer (in the mountains) or in the winter (on the coast).

Inland sites are rich in the remains of edible plants: corm casings from starchy Iridaceae corms (at their most palatable

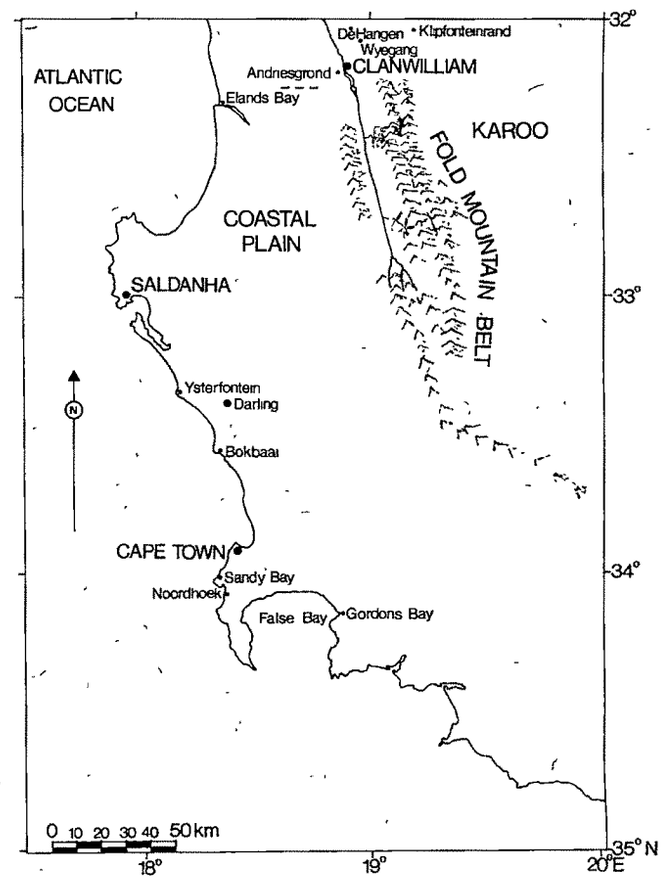


Fig. 1 Map showing geographical features of the southwestern Cape and localities of the skeletons listed in Table 1.

in late summer) and seeds from the fruits of summer-fruiting plants. The faunal assemblages are dominated by tortoise bone (tortoises are most active, and hence easiest to collect, in summer). The second most common animal is the rock hyrax (*Procavia capensis*). Examination of the stage of tooth eruption in juvenile hyrax mandibles reveals that almost half of those from

Table 1 $\delta^{13}C$ values of dated archaeological human skeletons

Archaeometry lab. no.	Skeletal register no.	Locality	Date (yr BP)	$\delta^{13}C$ (‰)	
Coastal					
UCT 1050	SAM-AP 1153	False Bay	Associated pottery (?)	−15.1	<2,000 BP
UCT 622	UCT 158	Sandy Bay	Associated pottery (?)	−15.0	
UCT 623	UCT 159	Sandy Bay	Associated pottery (?)	−13.6	
UCT 625	UCT 248	Noordhoek	Associated pottery (?)	−12.9	
UCT 459	SAM-AP 5083	Ysterfontein	Pta-926 1,490±55	−14.5	
UCT 1048	UCT 60	Saldanha	Pta-2,005 955±50	−14.6	
UCT 451	SAM-AP 4813	Bokbaai	Associated pottery (?)	−15.1	
UCT 1049	SAM-AP 1443	Gordon's Bay	Pta-2,309 2,050±50	−11.8	2,000–4,000 yr BP
UCT 1052	UCT 162	Ysterfontein	Pta-929 2,880±50	−11.5	
UCT 1092	-	Eland's Bay	Pta-1,754 3,835±50	−14.7	
UCT 1051	UCT 112	Darling Coast	Pta-2,003 4,445±50	−11.2	4,000–8,000 yr BP
UCT 1091	-	Eland's Bay	Pta-1,829 8,000±95	−14.6	>8,000 BP
UCT 206	-	Eland's Bay	Pta-3,086 9,750±100	−12.3	
UCT 202	-	Eland's Bay	OxA-478 10,860±180	−12.5	
Inland					
UCT 457	SAM-AP 1449	Clanwilliam	OxA-453 2,230±100	−17.3	2,000–4,000 yr BP
UCT 203	UCT 334	Andriesgrond	OxA-457 3,850±80	−20.2	
UCT 1093	-	Klipfonteinrand	Pta-1,642 3,540±60	−19.6	
UCT 1053	UCT 331	Wyegang	Pta-1,649 6,900±70	−18.4	4,000–8,000 yr BP

All pottery so far found in the area post-dates 2,000 yr BP; its association with skeletons therefore constitutes an approximate guide to their ages. Some of these skeletons (those from Saldanha South) lie outside the area for which the seasonal mobility hypothesis was originally formulated. They are included mainly to demonstrate the consistently positive character of coastal isotope values. Pta, radiocarbon dates from the Pretoria laboratory; OxA, dates from the Oxford radiocarbon accelerator.

the cave of De Hangen (the site with the largest faunal assemblage) were probably killed between October and May. Finally, historical accounts of meetings between European travellers and 'bushman' hunter-gatherers in the area all refer to summer encounters.

The coastal segment of the seasonal round is represented at sites such as Elands Bay Cave: like most coastal sites, this has few plant food remains but large quantities of limpet and mussel shells. If shellfish were a common, reliable resource taking the place of plant foods as a coastal staple[4–6], Parkington[4] suggested that it would have been safer to exploit them in winter. Winter outbreaks of the poisonous dinoflagellate *Gonyaulax catenella* are less common than summer ones; these diatoms render filter-feeding shellfish (such as mussels) toxic to man. The most common marine mammal from the Elands Bay deposits is the Cape Fur Seal (*Arctocephalus pusillus*). Young seals are born in November and nearly all the excavated mandibles are those of yearlings that died between July and October; seasonal rings on a small sample of canines from adult male seals obtained from the same site apparently also reflect this short time period[7]. The bones of several species of birds that are winter visitors to the nearby Verlore Vlei estuary (for example, flamingos) are found throughout the Holocene levels of the cave deposits. Winter is also the time when the Vlei, swollen by winter rains, used to break through into the sea, carrying with it organic material that would have attracted fish such as the white steenbras (*Lithognathus lithognathus*), common in the cave deposits.

Thus, there is a considerable amount of archaeological evidence for summer occupation at De Hangen and winter occupation at Elands Bay Cave. Inland and coastal sites have been seen as different segments of one integrated subsistence economy. People supposedly moved between the various resource zones in such a way as to exploit peaks in animal and, particularly, plant resources, while avoiding troughs. Recently, Parkington[5] has fitted his seasonal mobility hypothesis more securely into a chronological framework. He now expects that it will be found to apply, in the form outlined above, mainly in the period between 4,000 and 2,000 yr BP; before this, from 8,000 to 4,000 yr BP, there was a hiatus in the occupation of Elands Bay Cave and of at least one other site nearby, while after 2,000 yr BP the system was probably seriously disrupted by the emergence of pastoralists with domesticated animals on the coastal plain.

The seasonal mobility hypothesis is directly testable by measuring the $^{13}C/^{12}C$ ratio. The use of $\delta^{13}C$ measurements ($\delta^{13}C_{sample} = [^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{standard}] - 1 \times 1{,}000‰$) as a source of dietary information is now an established archaeological technique[8,9]. Most previous studies incorporating such measurements have, however, been limited by having to use global averages for the isotope ratios of the dietary base. In the study reported here, we began by analysing samples of all the foods that we know were important in the diet of prehistoric humans in the southwestern Cape. We also included a cross-section of other edible organisms that would leave no trace in the archaeological record.

The results of the analyses form very clear patterns, which are presented in full elsewhere[10]. In summary, the mean $\delta^{13}C$ value of the marine-based foods consumed by people living on the coast (shellfish, crayfish, fish, seal and seabird meat) is $-15.6 \pm 1.6‰$ (range = −12.3 to −19.4‰, $n = 29$). The edible parts of terrestrial plants have a mean $\delta^{13}C$ of $-25.4 \pm 1.8‰$ (range = −22.3 to −29.2‰, $n = 26$) whereas terrestrial animal meat has a mean of $-23.6 \pm 1.3‰$ (range = −20.9 to −25.8‰, $n = 15$). Therefore, a marine-based diet has an isotope 'signature' substantially different from a terrestrially based one; this difference would be reflected in the bones of people consuming these foods (the absolute values for bones being some 5‰ more positive as isotope discrimination proceeds stepwise along trophic levels).

Next, we analysed several archaeological human skeletons, some of which were found at the coast and some in the mountains. If they represent a seasonally transhumant population they should all have broadly similar $\delta^{13}C$ values intermediate

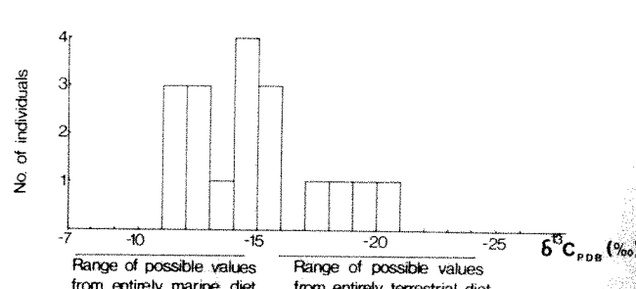


Fig. 2 Histogram of the results given in Table 1.

between the values for purely marine and purely terrestrial diets; it is obvious from Table 1 that this is not the case. Instead, the coastal skeletons reflect a predominantly marine diet and the inland skeletons an almost entirely terrestrial diet. Clearly, whatever seasonal round either group might have engaged in, they do not represent segments of the same subsistence economy.

The coastal skeletons show $\delta^{13}C$ values that range from almost entirely marine-derived (−11‰) to values which could reflect up to 50% terrestrial food composition (−15‰). We consider that the sample is as yet too small to detect geographical or chronological patterning, though this will be one of the principal aims of further work. The most interesting point is not that some coastal people consumed terrestrial foods (available, in any case, near the shore) but that, in direct contrast to nearly all models of prehistoric coastal subsistence[4–6,11], a significant proportion of the coastal population consumed an entirely marine diet and so must have spent all its time on the shore. Seventeenth-century European settlers described 'strandlopers' doing this[12]; from the isotope data it seems to be a pattern of life extending back at least as far as the beginning of the Holocene.

This result may seem surprising in view of the extremely plausible arguments for seasonal mobility summarized above. Closer examination of these arguments, however, reveals that although the evidence is consistent with such a hypothesis, it is also extremely inconclusive[10]. Although people certainly were living in the mountains in the summer and on the coast in winter, the hypothesis rests on the unproven assumption that they were the same people and that they were not living in the mountains in winter, or on the coast in summer—this would be much more difficult to demonstrate, but it is essential to do so to postulate population movement between the two areas. We suggest that comparable hypotheses formulated for other parts of the world might benefit from similar critical re-examination.

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Evidence for echolocation in the oldest known bats

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The earliest-known bats are represented by excellent fossil material, including virtually complete skeletons of *Icaronycteris index* from the early Eocene (50 Myr BP) of western Wyoming[1,2] and *Palaeochiropteryx tupaiodon* from the middle Eocene (45 Myr BP) 'Grube Messel' of western Germany[3,4]. These taxa have been closely allied[1,5] with Recent Microchiroptera, a suborder of diverse bats noted for their powers of ultrasonic echolocation[6]. A problem with this relationship is the alleged absence in the Eocene forms of specializations in the auditory region[7] and other aspects of the skeletal system[1,2]. It has been proposed, therefore, that the oldest bats are members of a group more primitive and possibly ancestral to the Microchiroptera and the visually oriented Megachiroptera[8]. Previously undescribed specimens now show, however, that *Icaronycteris* (Fig. 1) and *Palaeochiropteryx* (Fig. 2) share special basicranial features with microchiropterans which suggest comparable refinement of ultrasonic echolocation. These results support the theory that a sophisticated sonar system was present in the earliest records of microchiropteran history.

The ultrasonic echolocation of living microchiropterans has been related to the structure of the middle-ear ossicles[9–15], laryngeal-hyoid apparatus[16,17] and the cochlea[9,14,18–20]. The ear ossicles are not well preserved in *Palaeochiropteryx*, but in *Icaronycteris* there is a partial malleus with a large orbicular apophysis (Fig. 1). This rounded process is well developed in microchiropterans, but is absent or weak in non-echolocating megachiropterans[9–13]. In microchiropterans the ossicles are tightly articulated for optimal tuning at ultra-high frequencies[12–14]. Mechanical analysis[13] suggests that the orbicular apophysis shifts the centre of mass of the malleus away from the rotational axis of the ossicle chain, increases the moment

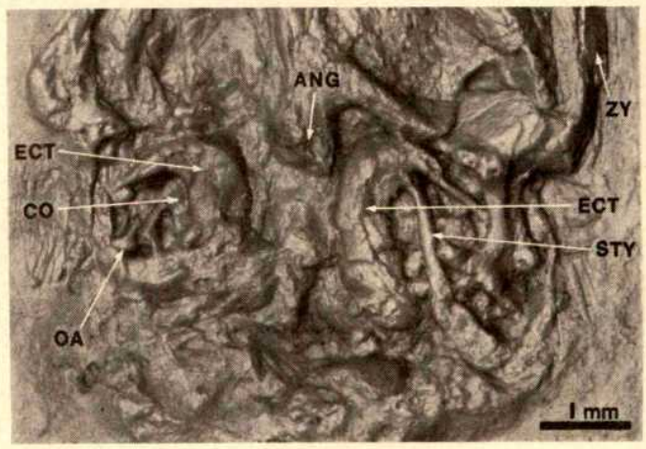


Fig. 1 Ventral aspect of cast of posterior skull of *Icaronycteris index*, University of Wyoming (UW) 2244. ANG, angular process of lower jaw; ECT, ectotympanic ring; CO, cochlea; OA, orbicular apophysis of malleus; STY, stylohyal; ZY, zygomatic arch. In this specimen the stylohyal lies lateral to its normal position ventral to the ectotympanic.

of inertia and lowers the natural frequency of the ossicle system. The apophysis may therefore contribute to the attenuation of natural frequencies that might otherwise be too high, even for echolocating bats[13].

Icaronycteris (Fig. 1) and *Palaeochiropteryx* (Fig. 2) both have a well-developed stylohyal element. In megachiropterans, as in most mammals, the stylohyal is usually a weak filamentous structure easily lost in macerated skulls[21]. The stylohyal in microchiropterans is the site of well-developed muscles that constrict the pharynx and brace the hyoid apparatus[16,17]. The hyoid stabilizers allow the extrinsic laryngeal muscles to pull from the hyoid and move the larynx forward[17], an action that precedes the emission of a series of high-frequency pulses[22]. The stylohyal, then, is an important component of a laryngeal-hyoid apparatus, whose variable design influences the charac-

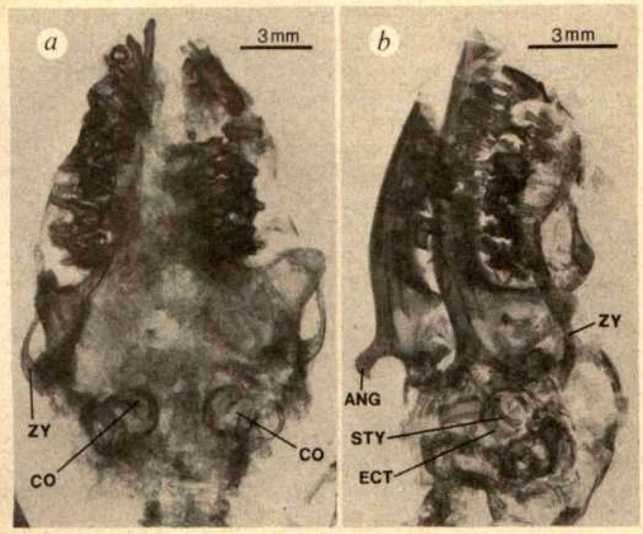


Fig. 2 *Palaeochiropteryx tupaiodon.* Positive prints of radiographs of a dorso-ventral view of Senckenberg Naturmuseum und Forschungsinstitut SMF-ME 788-B (*a*) and a lateral view of SMF-ME 1127 (*b*). Abbreviations as in Fig. 1.

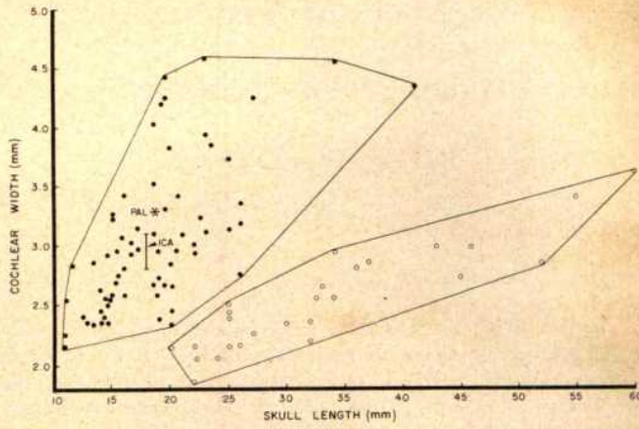


Fig. 3 Plot of maximum width of cochlea (ordinate) against skull length (abscissa) for 96 species of Microchiroptera (●), Megachiroptera (○), *Palaeochiropteryx* (*) and *Icaronycteris* (vertical line represents range of error in estimate of maximum cochlear width). Points represent 17 of 19 Recent bat families (two monotypic families of microchiropterans, the Myzopodidae and Craseonycteridae, are omitted). Convex polygons define the outer boundaries of values for each of the two suborders. Cochlear width has a weaker correlation and more positive allometry with skull length in microchiropterans ($r = 0.64$, slope = 0.80) than in megachiropterans ($r = 0.92$, slope = 0.30).

teristics of high-frequency vocalizations in different bat species[17].

The most striking resemblance between these Eocene bats and living microchiropterans is in the large relative size of the cochlea. The dimensions of this structure are easily determined in *Palaeochiropteryx* (Fig. 2). In *Icaronycteris*, the cochlea is less well preserved, but an estimate of its size can be made from measurements between auditory structures adjacent to the cochlea (Fig. 3). The maximum width of the cochlea is a meaningful functional parameter because it is equal to the maximum width of the basal turn, that region of the cochlea especially receptive to high-frequency vibrations in adult mammals[14,19,20,23–25]. A plot of maximum cochlear width against skull length for 96 species of bats shows no overlap between Megachiroptera and Microchiroptera, and supports the observation[14,15,18] that the cochlea in microchiropterans is unusually large relative to the size of the skull. The great expansion and increased torsion of the cochlea in these bats is accommodated by a unique pattern of arrested ossification of the petrosal and adjacent elements during ontogeny[26]. *Palaeochiropteryx* and *Icaronycteris* fall well within the range of relative cochlear size for Microchiroptera and exceed values for several living species within this suborder (Fig. 3). Thus, the implication is strong that these early bats share with living echolocating bats a cochlea of comparable ontogeny and function.

The basicranial evidence reviewed here supports the contention[1,5] that the oldest bats share several derived features with microchiropterans and are members of that group. Nevertheless, the fossil bats, particularly *Icaronycteris*, retain a number of very primitive features in the sternum and distal appendages[1,2,8]. This mosaic of traits suggests that the development of a sophisticated auditory and laryngeal system preceded the acquisition of certain other structures. Ultrasonic echolocation and its correlative morphology have been demonstrated in all living microchiropterans so far studied[14,15,26–28]. Thus, evidence from living taxa and the fossil record are consistent with the argument that ultrasonic echolocation is a primitive sensory mode for microchiropterans.

Echolocation also occurs in the megachiropteran genus *Rousettus* and in certain cetaceans, seals, insectivorans, rodents, birds and even some blind humans[28]. Microchiropterans are unique, however, in producing echolocation calls that are structured in frequency changes over time, a mode particularly effective in hunting volant, nocturnal prey. Other echolocators, including *Rousettus*, use transient, broad-band clicks[28]. Moreover, no other mammalian group shows an auditory architecture and ontogeny closely similar to that of microchiropterans[26]. It seems unlikely, then, that ultrasonic, time-structured echolocation was an early or primitive condition for all bats, only to be secondarily lost in the visually oriented megachiropterans. A more plausible notion is that ultrasonic echolocation was derived within bats, possibly from a condition where low-intensity, broad-band echolocation was used primarily for communication and gathering of habitat information, while vision, olfaction and direct audition (listening to sounds of prey) were used in the search for food[28]. This transition, according to the evidence reported here, occurred earlier than 50 Myr ago, the time of first appearance of bats in the fossil record.

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Course correction circuitry translates feature detection into behavioural action in locusts

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Like other flying animals, locusts must maintain course stability despite turbulence and motor errors. Accordingly, they must detect course deviations and then correct them. The compound eyes, the ocelli and the cephalic wind hairs all detect different sensory consequences of flight instability[1–3], and descending interneurones bring this information to the thorax[4–9]. One such interneurone of this population (the tritocerebral commissure giant neurone) is known to elicit correctional steering[10]. Here we characterize three additional pairs of descending deviation detector neurones and show how their information is translated into altered drive to the flight motoneurones. The central pattern generator for flight[11–13], modulated by proprioceptive feedback[14], gates the signal of the detector neurones in thoracic premotor interneurones[9], ensuring that the flight motoneurones are affected only during flight. Further, the gating process transforms the phase-independent information of the deviation detectors into a phase-dependent signal modulated at wing-beat frequency, and transfers it to those flight motoneurones active at the time and appropriate to the corrective action required.

We recorded intracellularly, with dye-filled microelectrodes, from the three pairs of descending interneurones and their identified postsynaptic cells in the thoracic ganglia of locusts (*Locusta migratoria* (L. 1758)) during flight motor activity[12], to correlate their morphological and functional characteristics. Course deviations were simulated by an artificial horizon, provided with a central wind jet (Fig. 1*b*). In some experiments the ocelli were stimulated independently via light pipes that excluded light from other sources[9]. These procedures established the role of the three interneurones as highly specific absolute deviation detectors, which integrate sensory information from the compound eyes, the ocelli and the wind hairs. Table 1 summarizes the excitatory input characteristics of the three cells.

We have named these interneurones DNI, DNM and DNC, so called because they receive input from the ipsilateral, medial and contralateral ocelli, respectively (Fig. 1*a*). All have been in part described previously, but because of confusion in the literature (Table 2), we find it necessary to rename them. Input from the compound eye confers on these cells sensitivity to large field movement and to direction of movement. Figure 1*c* shows an example of the specific nature of compound eye input. A simulated roll deviation presented to the compound eyes results in excitation of the left DNC only if the roll is clockwise and away from the horizontal position. For a roll of this specific type of given amplitude, the response of the cell increases with increasing divergence of the artificial horizon from the horizontal. The cell does not respond to a clockwise roll towards the horizontal, nor does it respond to an anticlockwise roll towards or away from the horizontal. This cell also responds with similar specificity to deviations in the pitch and yaw axes (Table 1). The optimal combination of compound eye stimulation for this cell is what the animal would experience during a diving, banked turn to the side contralateral to the axon.

Excitatory input from the wind hairs or ocelli complements the compound eye input in an aerodynamically compatible and directionally specific manner. For example, a cell which is excited by a visually simulated yaw to the left is also excited by wind directed towards the animal's head from the right which would in nature accompany this yaw. If compound eye input causes the cell to be excited by a simulated clockwise roll, it

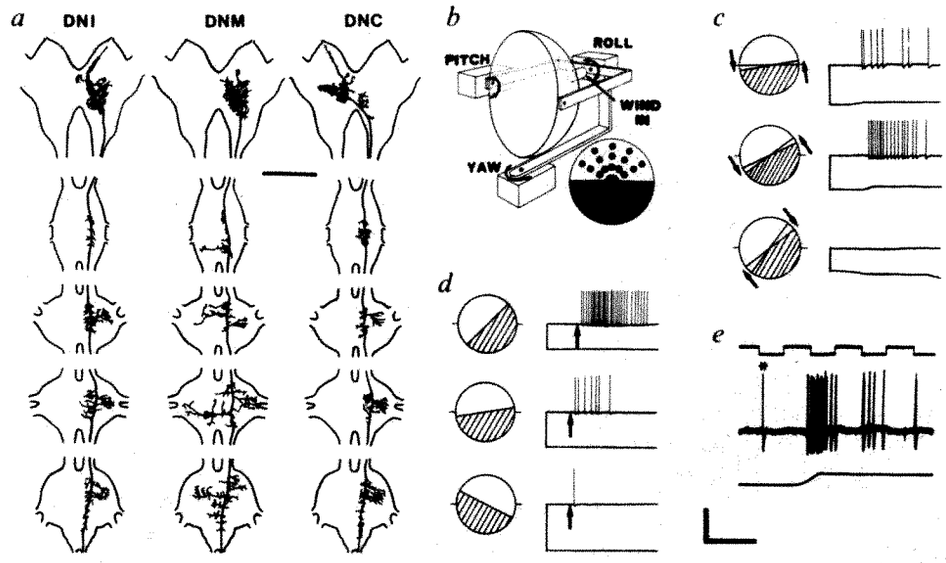


Fig. 1 The descending deviation detector neurones DNI, DNM and DNC. *a*, Morphology and nomenclature. The units are so named because they receive input from the ipsilateral, medial and contralateral ocelli, respectively, where ipsi- and contralateral are defined as the side on which the axon of the interneurone runs. This is clearly reflected in the location of the input arborizations in the brain. In the remaining ganglia, DNM is distinguished by its bilateral projection, while DNI and DNC project only unilaterally and are very similar in structure. All three units are paired. We distinguish individual units as left and right with reference to the connectives in which the axon descends to the thorax. Drawings are based on 20–30 successful intracellular fills (either Lucifer yellow[35] or hexamine cobalt chloride with subsequent intensification[36]) of each unit. Scale bar, 800 μm. *b*, Stimulus apparatus producing simulated course deviations. The animal's head was placed at the centre of a remotely steerable translucent hemisphere, on which was painted the opaque pattern shown, representing an artificial horizon and a structured visual field; the whole was illuminated diffusely with daylight from above and behind. The hemisphere, which was also equipped with a central air tube for simulation of wind on the head in flight, could be rotated around all three orthogonal axes and thus simulate pitch, roll and yaw deviations. *c*, Response of the left DNC to simulated clockwise and anticlockwise rolls away from (upper two records) and towards (lower record) the normal flying position. The sketches show the initial and final positions of the horizon, and the arrows the direction of its rotation. Only clockwise rolls away from the normal position evoke graded responses. Upper traces: intracellular recording, scale bars 50 mV, 200 ms; lower traces: roll monitor, vertical scale bar 75°. *d*, Response of same unit as in *c* to three identical frontal wind stimuli (onset at arrows) during maintained simulated visual rolls to various positions, as shown in the sketches. Calibrations as in *c*. Non-preferred orientations of the visual world inhibit the response to wind. *e*, Response of a DNI (centre trace, intracellular recording, scale bars 5 mv, 200 ms) to a visually simulated roll (lower trace, vertical scale bar 75°) and to simultaneous ON/OFF stimulation of the ipsilateral ocellus by means of a separate light pipe (upper trace, light at 565 nm modulated by 1 $\log_{10}$ unit, upward going = ON, duty cycle 200 ms). Ocellar OFF stimuli evoke only single spikes when presented alone (asterisk). These summate with the response induced by visually signalled roll. Ocellar ON stimuli are functionally incompatible with the visual information, as they imply a roll in the opposite direction; they strongly inhibit the visual response, which otherwise would consist of a long phasic-tonic burst, as in the middle record of *c*.

Table 1 Excitatory input characteristics of deviation detector neurones

	DNI	DNM	DNC
Ocellus producing reliable* excitation with OFF stimulation	Ipsilateral	Medial	Contralateral
Most effective visually simulated deviation (that is, via compound eyes)	Roll ipsilateral Pitch down Yaw ipsilateral	Pitch down	Roll contralateral (Pitch down)† Yaw contralateral
Most effective wind direction corresponds to	Yaw ipsilateral	Pitch down	Yaw contralateral

Data were derived from 60–100 experiments on each cell.
* DNI received additionally weak input from the medial ocellus, and DNM from both ipsi- and contralateral ocelli. In a fraction of the population, these weak inputs can evoke spikes.
† Weak response.

will also be excited by selective darkening of the right ocellus, which would in nature be darkened by such a roll[15].

The response properties of a deviation-detecting neurone are not, however, shaped simply by summation of excitation from three sensory sources. Mutual inhibition between the different sensory inputs is also important, and prevents a response if there is a conflict in information content. For example, Fig. 1*d* shows that the position of the visual world can alter the response of the cell to a constant wind stimulus. If the horizon is rolled to a 'visually preferred' position (Fig. 1*d*, top records), indicative of a specific deviation, and held there, the response to a frontal wind stimulus is strong (here 34 action potentials). If the horizon is held in a near-horizontal position (Fig. 1*d*, middle records), the same wind stimulus produces only a modest excitation (7 action potentials). Finally, if the horizon is rolled to a 'visually antipreferred' position, one which does not represent a deviation of interest to this cell, its response to the identical wind stimulus is virtually eliminated (1 action potential). Similarly, Fig. 1*e* shows that the ocellar input can inhibit the response to compound eye input. Darkening of the left ocellus produces an action potential in the left DNI, which responds visually to an anticlockwise roll. However, selective illumination of the same ocellus via a light pipe strongly inhibits the response of the neurone to an otherwise effective visual roll stimulus. Inhibition of the wind input by ocellar input[8] and inhibition of the compound eye input by wind input can be demonstrated in similar ways. These inhibitory relations reflect the relative probability of error for a conflict occurring in natural conditions (for example, turbulence could affect the wind hairs without producing a course deviation; the eyes would experience no change in stimulus, and the two inputs to the interneurone would convey conflicting information were it not for the inhibition). Taken together, these interactions ensure that the firing of any of these neurones is a reliable indication of a specific and well-defined deviation from course. Each neurone responds optimally to that specific combination of sensory stimuli which would impinge on the compound eyes, ocelli and wind hairs during a specific deviation. The units are formally multimodal, but we feel that this is not a useful description; the feature detected by these neurones, their 'emergent modality', is course deviation.

The cells central to the transformation of the firing of these deviation detectors into appropriate steering manoeuvres are a population of thoracic interneurones[9] that have three important characteristics[16] (see Fig. 2*e*): (1) they are postsynaptic to specific descending deviation detectors, (2) they are powerfully presynaptic to specific flight motoneurones and (3) they receive

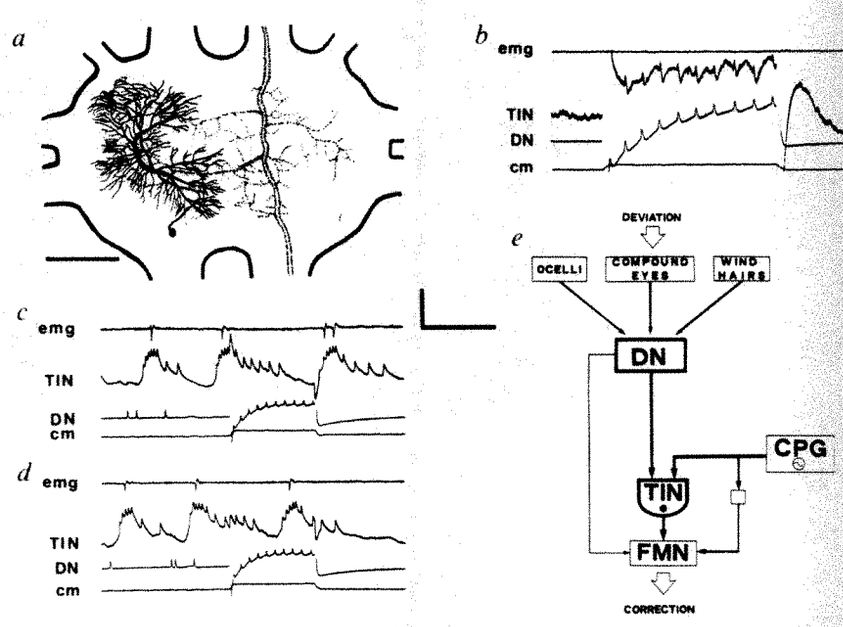


Fig. 2 Flow of information from the deviation detector neurones (DN) to the flight motoneurones (FMN) is gated in the thoracic interneurones (TIN) by summation with a phasic signal derived from the central pattern generator for flight (CPG). *a*, Presynaptic DNM (dotted) and representative postsynaptic TIN (solid) in mesothoracic ganglion (scale bar, 300 μm). *b*, Simultaneous intracellular recordings in a non-flying locust (scale bars 2 mV and 50 mV, respectively, 50 ms) from the TIN and DN shown in *a*, and an extracellular recording from the dorsal longitudinal depressor muscle ('emg') which serves as a monitor of flight activity. A depolarizing current of 10 nA ('cm') injected into DN evokes 10 action potentials, which in turn evoke small 1 : 1 excitatory postsynaptic potentials (e.p.s.ps) in the TIN. (The small high-frequency deflections on the TIN trace are artefacts caused by capacitative coupling between the two electrodes. The input bridges of both recording amplifiers are balanced, but the high current necessary to evoke a brisk burst of action potentials from an axon penetration results in a sizeable stimulus artefact.) *c*, *d*, Same experiment as *b*, but during flight motor output, indicated on the emg trace by periodic depressor muscle potentials. Scale bars 20 mV (TIN), 100 mV (DN) and 100 ms. The TIN receives strong synaptic drive from the CPG, depolarizing in the depressor phase. Note that in *c* and *d* the current pulses come at different points in the wing-beat cycle. During the depressor phase, e.p.s.ps evoked by spikes in the DN increase the number of action potentials in the depolarized TIN by ~50%, which in turn influences the postsynaptic FMN (not shown). Visual stimuli can cause a spike rate in the DN 50% greater than that induced here by current injection, so that this figure underplays the potential effect. During the elevator phase, the same DN activity does not evoke spikes in the TIN, and thus has no effect on the FMN postsynaptic to that cell. *e*, Summary diagram indicating the main relationships in the circuitry discussed here. Thickness of arrows is roughly proportional to the relative strength of the interaction. The TIN population is symbolized as an AND-gate, reflecting its role. CPG drive reaches the FMNs not only via the TINs, but also by other interneurones which do not receive DN input.

Table 2 Synonomy of the deviation detector neurones

Interneurone			Ref.
DNI	DNC	DNM	This work
O_3	O_2	—	28-30
ip 1	cl 1	—	31
O_2	O_1	—	32
i.o.	c.o.	—	33
VIP 1	VCP 1	—	6
O_3	—	O_3	8
O_4	—	—	34
Fast ipsilateral, Fast ipsilateral (medial)	Fast contralateral	Fast medial	9

Deviation detector neurones have been mentioned under different names by previous authors; all used *Schistocerca* rather than *Locusta*, but we can detect no differences. Their descriptions were based on partial dye fills (for example, thoracic ganglia but not the brain, or vice versa) and either incomplete physiological data or none at all. The only complete structure previously given[8] is actually derived from two separate units, one filled in the brain and one in the thoracic ganglia. Normally, the oldest nomenclature would be adopted (in this case the $O_{1...n}$ system[28]). This is no longer practical, as is evident from the table. Abbreviations: $O_{2,3,...}$, ocellar interneurone 2, 3, . . .; ip 1, ipsilateral 3rd-order ocellar neurone; cl 1, contralateral 3rd-order ocellar neurone; i.o., ipsilateral ocellar neurone; c.o., contralateral ocellar neurone; VIP, ventral nerve cord unit, ipsilateral to its soma, protocerebral soma; VCP, ventral nerve cord unit, contralateral to its soma, protocerebral soma.

rhythmically alternating excitatory and inhibitory drive from elements of the flight central pattern generator[13], at phase angles characteristic for each interneurone. One such thoracic interneurone is shown in Fig. 2*a*. In a non-flying animal, the firing of the presynaptic DNM evokes only subthreshold synaptic potentials in this thoracic interneurone (Fig. 2*b*). Postsynaptic flight motoneurones are consequently not affected and no behaviour results. However, in a flying animal the flight central pattern generator is operative and delivers rhythmic drive to the thoracic interneurones. If the deviation detector is now fired (Fig. 2*c*, *d*), the synaptic input it evokes in the thoracic interneurone summates with central pattern generator drive, causing the interneurone to fire an increased number of action potentials. This augmentation occurs only when the drive is depolarizing (Fig. 2*c*, *d*). Because of the rhythmic nature of the depolarizing and hyperpolarizing potentials evoked by the flight central pattern generator in the thoracic interneurone, the tonic deviation signal is transformed into phasic activity of the premotor interneurones. Sensory information is gated at the level of the thoracic interneurone. The appropriate postsynaptic flight motoneurones will consequently receive additional drive, phase-coupled with the wing-beat cycle, for the duration of the descending deviation signal. This additional phase-coupled motor drive can either alter the phase of firing of one of a pair of homologous bilateral motoneurones, or alter the number of action potentials in the discharge of affected motoneurones, or even recruit an otherwise silent motoneurone. These comprise the mechanisms which have been shown to operate during steering behaviour by intact locusts[17-20]. All the flight motoneurones and the premotor interneurones are spiking cells with all-or-none threshold characteristics, clearly a prerequisite for this mechanism. We describe elsewhere[16] circuitry in which the CPG-evoked depolarizations in the premotor interneurones summate with inhibitory postsynaptic potentials derived from the deviation detector neurones, rather than with their excitatory postsynaptic potentials, but which has similar effects on the firing pattern of the motoneurones. That summation of inputs to spiking premotor interneurones could lead to gating of the signal to motoneurones has been postulated previously[21], but not demonstrated.

The deviation detector neurones also make direct connections with the flight motoneurones[8,9]. We have shown elsewhere[16] that these connections produce much smaller postsynaptic potentials than those mediated by premotor interneurones. This is understandable in view of the need to avoid flight muscle contraction when the animal is not flying; the direct input to the flight motoneurone must be small enough to avoid firing it, even though the motoneurone receives an almost continuous barrage of sensory excitatory postsynaptic potentials from other sources. In flight, however, these direct postsynaptic potentials are also subject to a similar gating process by the flight central pattern generator, which drives the motoneurones directly[13] as well as the premotor interneurones.

Thus, at the thoracic level, a two-stage gating process ensures that the intrinsically phase-independent information from the deviation detectors: (1) elicits a response in the flight motoneurones only when the flight central pattern generator is operative, that is, in the behavioural context of flight, (2) is phase coupled, that is, is transformed into a phasic signal modulated at wing-beat frequency and (3) is automatically applied to the appropriate group of flight motoneurones which are active at the time of arrival of the signal, obviating the need for any further decision-making in the brief interval between stimulus and response.

The complete steering behaviour of the animal involves not only the modulation of wing beat treated here, but also movements of head, abdomen and hind legs[11,22-24]. It remains unclear whether the circuitry described here participates in these elements. However, we have evidence that the information brought to the thoracic ganglia by the other deviation-sensitive interneurones is processed in the same way as described above. Furthermore, the circuit principles documented here seem to be generally applicable and significant. In vertebrates, for example, locomotor control involves descending pathways from projection areas in the brain to segmental motor centres in the spinal cord and thence to spinal motoneurones[25]. Moreover, a gating process has been postulated and ascribed to the level of the segmental interneurones[26,27]. This scheme is comparable in detail to the circuitry documented here for the locust (see Fig. 2*e*). Indeed, we expect that similar principles of neuronal organization will be found in all animals that need to integrate a rhythmic motor output with non-phase-locked exteroceptive information, a need that occurs in most forms of locomotion.

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Neuronal growth, atrophy and death in a sexually dimorphic song nucleus in the zebra finch brain

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The song control nuclei of the zebra finch brain contain more neurones of larger diameter in the male than in the female[1]. This sexual dimorphism is thought to result from differential growth of neurones in the two sexes[2–4]. Using neurohistological techniques and radioactive tracers, we have studied the development of several forebrain nuclei involved in the control of song and find that the dimorphism arises from neuronal atrophy and death in the female brain as well as from an increase in cell-body size and afferent terminals from other forebrain nuclei in the male. Although the timing of these events varies from nucleus to nucleus, the sequence is essentially similar in all of them except area X. Here we describe the events in one of these nuclei, the robust nucleus of archistriatum (RA), as an example.

Zebra finches breed well in captivity and provide chicks of known ages all year round. Neurone counts were made on sections stained with cresyl violet and the neuronal connections from HVc (ventral nucleus of hyperstriatum caudal division) to RA were traced by conventional autoradiographical methods. The distinct boundaries of RA are convenient for the measurement of various cytoarchitectonic and cellular properties at all stages of development. For the measurement of RA volume, the boundaries of the nucleus in every third section were drawn with a Zeiss camera lucida onto a sheet of paper and the area within the boundaries was calculated by a computer-aided planimetric method. Because of the error involved in the sampling method, the average between the minimum and maximum estimates of the volume was chosen as the best estimate. The same planimetric method was used for the calculation of soma area from perikaryal outline tracings. For the purpose of this report, the neurone density and number were determined only for a few selected age groups and are not shown in graphical form. Sections having the largest cross-sectional areas of RA were photographed *in toto* and neurones were counted on enlarged prints. The number of neurones counted in each section divided by the volume of the section gave an estimate of the neurone density, which did not differ by more than 10% from that obtained by counting the number of neurones in random sample grids under a microscope. The total number of neurones in RA was estimated by multiplying the neurone density by the RA volume.

The cytoarchitecture of RA reveals few differences between the sexes soon after hatching but later begins to diverge towards growth in the male and atrophy in the female (Figs 1, 2). The growth in RA volume in the male is mainly the result of changes in the size and density of neuronal cell bodies; the somata become larger and less densely packed. The estimate of neurone number at day 15 ranges from 9,100 to 14,900 (median = 11,300) and from 9,200 to 14,900 (median = 9,600), respectively, for the male and the female. These numbers should be compared with those of adult birds which range from 9,300 to 14,300 (median = 13,600) for the male and from 2,400 to 3,700 (median = 2,900) for the female. Thus, the female RA loses about two-thirds of its neurones by the adult stage, whereas the male RA retains essentially the same range of neurone numbers. The sharp decline of RA volume in the female after day 30 results from both a decrease in the neurone number and size on one hand and an increase in the neurone density on the other; the cells are fewer, smaller and more densely packed. These small cells were identified as neurones by retrograde labelling from the target areas of RA neurones[3].

During their development RA neurones are mainly innervated by axons from HVc and to a lesser extent by axons from MAN

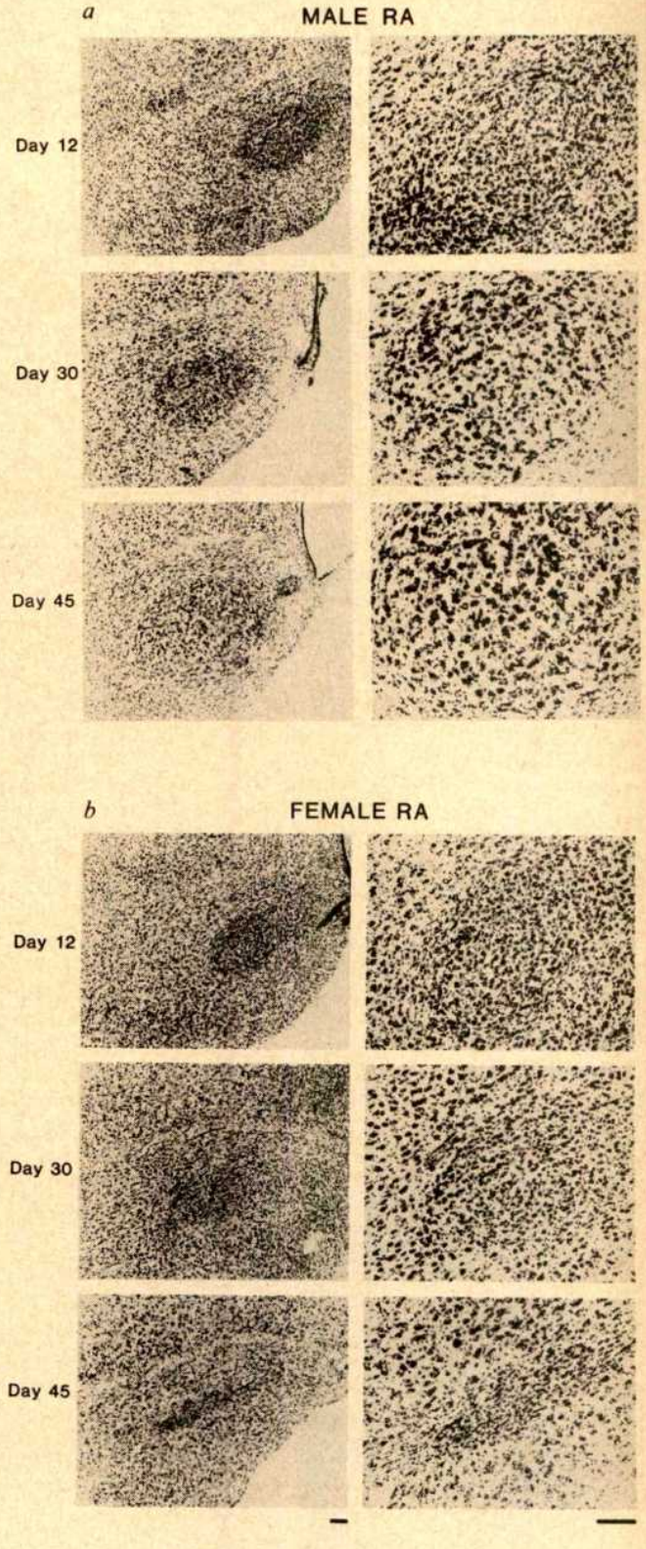


Fig. 1 Contrasting developmental stages of RA in the two sexes. At day 12 after hatching, the male (*a*) and female (*b*) RAs are similar in size; at day 30, the cell bodies of neurones are smaller in the female RA than in the male RA. By day 45 the female RA has shrunk, whereas the male RA has grown both in volume and soma size. For histological examination of brains, birds were anaesthetized with Equithesin, perfused with saline and then 10% formalin. After overnight immersion in 20% sucrose solution, brains were cut in 30 μm thickness with a freezing microtome, and these sections were stained with cresyl violet. Each photomicrograph shows the largest cross-sectional area of RA for a given age in a parasagittal section. Low- and high-magnification pictures of the same section are shown side by side. Scale bars, 100 μm.

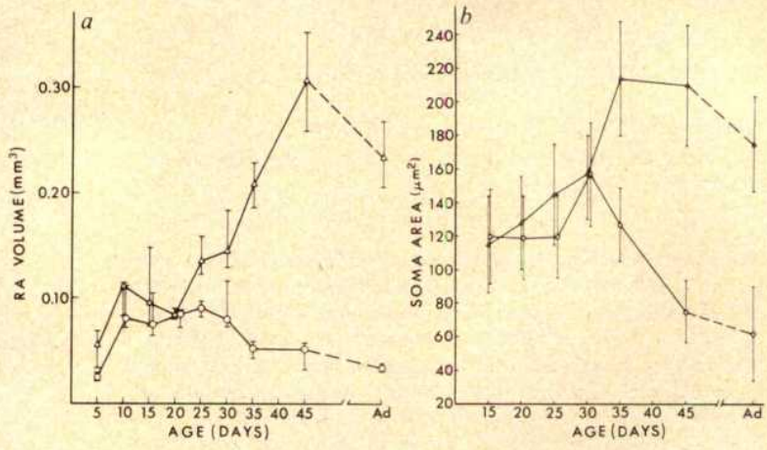


Fig. 2 Quantitative comparison of architectonic and neuronal changes (male, △; female, ○). Dashed lines indicate unsampled age intervals. *a*, Changes in RA volume. For each age and sex group, the median and range of the RA volume of five birds are plotted as a function of age. From days 5 to 20 after hatching, RA grows to about an equal volume in both sexes. Thereafter, the male RA continues to grow with a steep increase in growth rate at about day 30, although there is a temporary dip in the growth curve at about day 20. The volume of the female RA, on the other hand, remains almost constant from day 10 to day 30 when a sharp decline begins. *b*, Changes in neurone size. Each of five birds used for each age and sex group provided a sample of 50 neurones for the computation of a mean and standard deviation. The graph plots the means of the five means and standard deviations as a function of age. In the male, the mean cross-sectional area of RA neurones increases steadily, reaching a plateau at about day 35. In the female, the mean soma area remains more or less constant until about day 30 when it begins to decrease rapidly.

(magnocellular nucleus of anterior neostriatum). The time course and pattern of innervation also show differences between the sexes. An injection of tritiated proline in HVc and its immediate vicinity in 15–25-day-old chicks of either sex results in an accumulation of radioactivity (grains) around RA (Fig. 3), while control injections outside HVc and its adjoining areas do not produce this pattern of grain distribution. Furthermore, when radioactivity accumulates within the boundaries of RA as in males older than 35 days, the ring of grain density around the nucleus disappears (Fig. 3). Whatever the identity of the ring may be, labelling within the boundaries of RA indicates innervation of its neurones. Thus, the male RA appears to remain uninnervated until about day 30 when it suddenly becomes innervated. This stage corresponds to the beginning of the steep increase in RA volume. The soma density begins to decrease rapidly at about this age, presumably because the accommodation of the incoming afferent fibres results in an increase in the distance between RA neurones. The female RA appears to remain largely uninnervated, although sparse connections between HVc and RA may present in the adult[3].

The above findings indicate that cell atrophy and death constitute a normal process of sexual differentiation in the brain. In theory, differences in neurone number and size in sexually dimorphic areas of the brain may result from differential proliferation, growth, atrophy, death or migration[5,6]. However, we did not consider cell atrophy and death in our earlier work on the zebra finch. Consequently, we did not consider that early oestrogen may masculinize the female RA by preventing cell death[2–4]. As to cell migration, it is very unlikely that large neurones emigrate from the female RA and small neurones migrate into it. Neurogenesis is reported to occur in the adult zebra finch brain[7]. Because neurogenesis in the adult is generally not restricted either to the song-control system or to one sex, it is perhaps not a major factor in the sexual differentiation of the song-control system. The presence in the male and the absence in the female of neuronal connections between HVc and RA suggest a causal relationship between innervation and neuronal growth: the afferent innervation of RA may be essential for the growth of its neurones; alternately, the growth of RA neurones may induce innervation by HVc fibres. How oestrogen mediates one of these processes is not known.

The origin of brain sex differences by neuronal growth in the male and cell death in the female is not unique to the zebra finch, for all well-documented cases of brain sex differences appear to involve cell death in the female. Recent evidence indicates that differences in neurone populations between the sexes in the preoptic area and the bulbocavernosus nucleus of mammals involve growth of neurones in the male and cell death in the female[8–11]. Cell death is a common phenomenon in the development of the nervous system; however, the role of cell death in development varies from system to system[12]. For sexually dimorphic systems the significance of cell death is clear; neurones disappear in the female system because females do not need to exhibit the behaviour controlled by the relevant neurones. Why are the neurones that are destined to die produced in the first place? It could be that a genetic programme uses the same set of genes for a homologous neural circuit in both sexes and an epigenetic process determines the fate of the circuit in the two sexes.

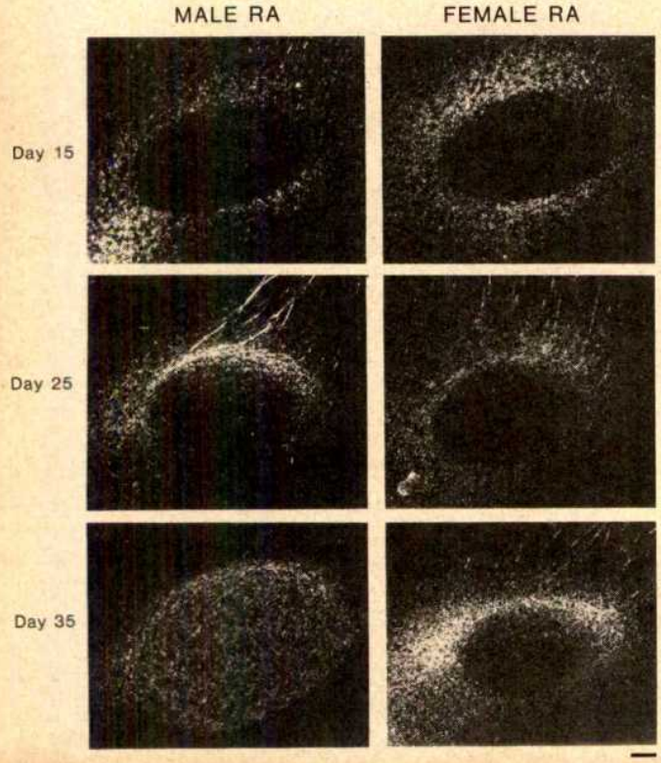


Fig. 3 Sex differences in innervation of RA. A glass pipette, 15 μm in tip diameter, was used for the iontophoretic injection of tritiated proline into HVc. The survival time after the tracer injection was 3 days. Brain sections for autoradiography were exposed for 30 days at 4 °C. Dark-field autoradiographs of RA show the distribution of silver grains for three different ages. These patterns of grain distribution appear when tritiated proline is injected in HVc and its immediate vicinity. The unlabelled oval area is RA. At day 15 both male and female RA fail to accumulate radioactivity no matter how large the injection area may be. At day 25 the male RA shows some labelling within its boundaries and is completely labelled by day 35. Note the absence of labelling outside the boundaries of RA. In contrast, the female RA remains mostly unlabelled throughout its development including the adult stage. These changes in labelling indicate when and to what extent RA becomes differently innervated in the two sexes. Scale bar, 100 μm.

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Changes of free calcium levels with stages of the cell division cycle

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Although the regulation of events in the cell division cycle by calcium or other cations has been the subject of much interest and speculation[1-10], experimental studies have been hampered by the difficulty of measuring submicromolar intracellular free calcium concentrations ($[Ca^{2+}]_i$) over an entire cell cycle. We now describe experiments using a new fluorescent calcium chelator, fura-2 (see Fig. 1c for structure), for continuous measurement of $[Ca^{2+}]_i$ from fertilization through the first cleavage of individual eggs of the sea urchin *Lytechinus pictus*. We also show for comparison the results of parthenogenetic activation by ammonia. In addition to the known transient rise of $[Ca^{2+}]_i$ at fertilization, further peaks are now revealed during pronuclear migration, nuclear envelope breakdown, the metaphase/anaphase transition and cleavage. Parthenogenetic activation by ammonia also elicits a sustained rise starting at nuclear envelope breakdown.

Fura-2 is a fluorescent tetracarboxylate chelator that exhibits a spectral shift in excitation maxima on binding calcium[11]. Increasing free Ca^{2+} increases the fluorescence excited by 350 nm light and decreases the fluorescence from 385 nm excitation. The ratio of fluorescence excited by 350 nm to that excited by 385 nm is a measure of $[Ca^{2+}]_i$ in which the uncertainties in dye concentration, cell thickness and absolute optical efficiency of the instrument are cancelled. We measured 350/385-nm ratios from single eggs, injected with fura-2, on a fluorescence microscope that had rapidly alternating excitation wavelengths[12]. Ratios were calibrated in terms of $[Ca^{2+}]_i$ by comparison with dye samples of known $[Ca^{2+}]_i$. Figure 1*a* shows the fluorescence signal during fertilization, recorded simultaneously at 350 nm and 385 nm excitation wavelengths. Shortly after addition of sperm, the fluorescence from 385 nm excitation fell dramatically whereas the fluorescence from 350 nm increased. Changes in the 350/385-nm fluorescence ratio are shown in Fig. 1*b*, while Fig. 1*c* shows the actual $[Ca^{2+}]_i$ after dye calibration. From these records we calculated that resting $[Ca^{2+}]_i$ averages 144 ± 10.3 nM (mean ± s.e.m., $n = 11$), with extremes between 70 and 250 nM (data not shown). The fertilization transient takes ~12 s from beginning to peak and rises to an average of 1.95 ± 0.16 μM ($n = 11$). Levels as high as 3.5 μM were observed, although such eggs were usually polyspermic. These values are close to those estimated previously from fertilization experiments with aequorin-loaded eggs[13]. The fertilization transient lasts ~5 min, during which time the sperm is incorporated into the egg and the sperm nucleus is transformed into the male pronucleus[14,15].

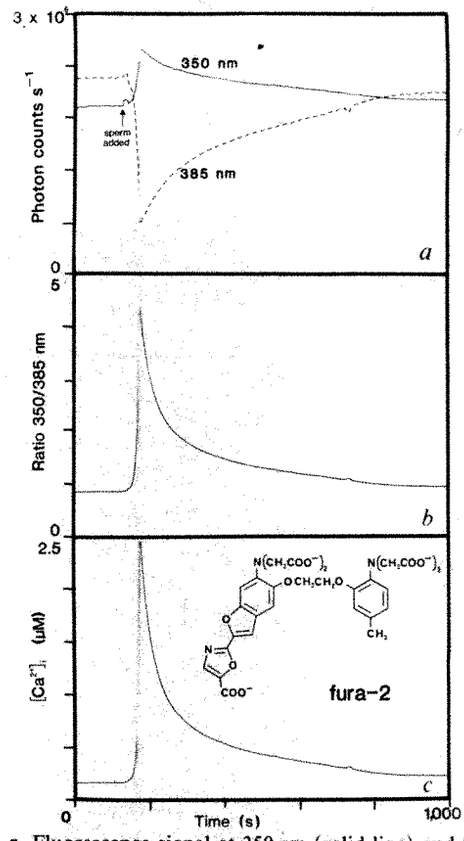


Fig. 1 *a*, Fluorescence signal at 350 nm (solid line) and 385 nm (dotted line) excitation during fertilization, ending just before the onset of a second (pronuclear migration) peak. The addition of sperm (at the arrow) caused a small artefact which can be seen to cancel out when ratios are taken (see *b*). Shortly after the addition of sperm, the fluorescence at 385 nm decreases dramatically (broken line) whereas the signal increases at 350 nM (solid line). *b*, Fluorescence ratio (R), obtained by dividing the fluorescence values at 350 nm by those at 385 nm. This ratio changes in the same direction as $[Ca^{2+}]_i$ and can be used to calculate $[Ca^{2+}]_i$ from the equation[11] $K(R-R_0)/(R_s-R) = [Ca]$, where R_0 is the ratio at 0 calcium and R_s is the ratio at saturating calcium. K represents $K_d(F_0/F_s)$, where K_d is the effective dissociation constant for fura-2 in the appropriate conditions, F_0 is the fluorescence at 385 nm in 0 Ca and F_s is the fluorescence at 385 nm in saturating calcium solutions. R_0 and R_s were obtained from droplets of fura-2 reference standards containing 50 μM fura-2, 155 mM KCl, 25 mM NaCl, 100 mM MOPS neutralized with KOH to *p*H 7.02 and either 10 mM K_2H_2-EGTA or 1 mM $CaCl_2$. The effective dissociation constant for fura-2 was determined to be 7.74×10^{-7} M by cuvette titration of 5 μM fura-2 in the above KCl/NaCl/MOPS medium with 10 mM EGTA buffers. The necessary dissociation constants for Ca-EGTA buffers were measured at 18°C in 225 mM KCl and 25 mM NaCl (ref. 11). *c*, Actual $[Ca^{2+}]_i$ values derived from the ratio in *b* after applying the calculation shown above. The inset shows the structure of fura-2.

Methods. *Lytechinus pictus* eggs were microinjected with a solution of 40 mM fura-2, 5 mM $CaCl_2$ and 0.66 M potassium gluconate (the injection solution was prepared by hydrolysing the fura-2 pentaethyl ester in 1 M KOH and neutralizing the hydrolysate with D-gluconic acid lactone). Typical dye loadings were 50-200 μM based on comparison with 100-μm droplets of dye standards. Excitation light was provided by two Spex Industries monochromators preset at 350 nm and 385 nm and selected in rapid alternation (15 Hz) by a rotating chopper mirror. These wavelengths were displaced from the true excitation peaks to avoid the transmission cutoff of glass optics at lower wavelengths. Emitted light was collected through a 500-530-nm interference filter and photon-counted in synchrony with the chopper so that the signals from the two excitation wavelengths were stored in separate memories of a Spex Datamate microcomputer. Monochromator slits of 0.25 mm were used, giving a 0.9-nm bandpass, and an attenuator screen further reduced the excitation intensity fivefold. Dye bleaching was usually undetectable, yet the photometer count rate was $1\text{-}2 \times 10^6$ photon counts per s; uninjected eggs gave $0.5\text{-}1.5 \times 10^4$ counts per s. All experiments were at 18 °C.

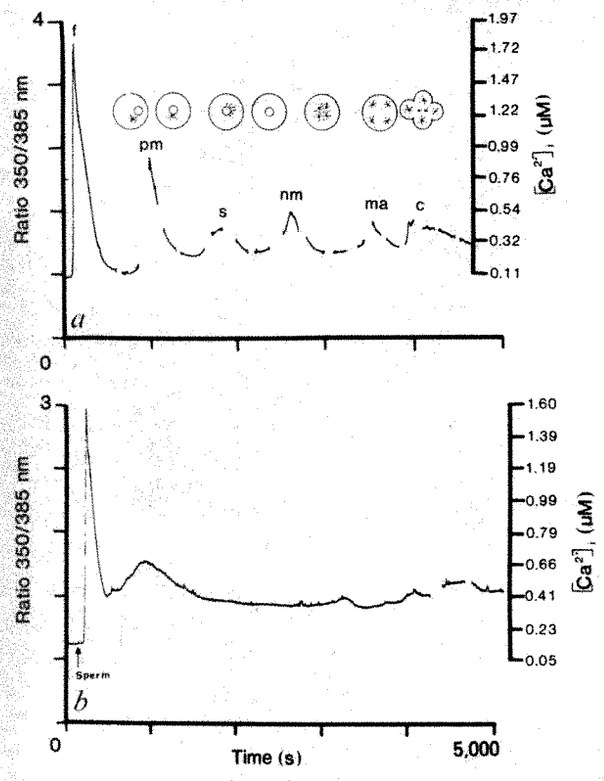


Fig. 2 Two complete records from fertilization to the first cell division. ***a*** Shows clearly the pattern of calcium fluctuations during the cell cycle seen in our records. Peaks in this record are especially prominent, as the post-fertilization baseline $[Ca^{2+}]_i$ is unusually low. However, the peak calcium levels still attain normal values, similar to those of the corresponding periods of *b*. The breaks in the trace were periods in which the trace was interrupted in order to take transmitted light photographs (not shown) to record any corresponding morphological events: f, fertilization peak; pm, pronuclear migration peak; s, streak stage peak; nm, nuclear membrane breakdown peak; ma, metaphase/anaphase peak; c, cleavage peak. This example happened to be polyspermic, and the first division resulted in four cells. ***b*** Is a typical trace where the post-fertilization baseline is higher. This egg underwent normal cleavage between the first and second breaks at the end of the record.

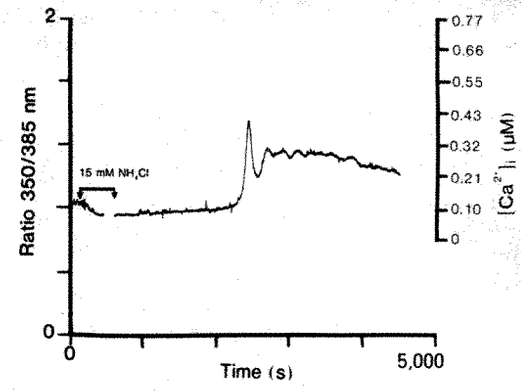


Fig. 3 A typical example of an egg activated by a 10-min pulse (arrows) of sea water containing 15 mM ammonium chloride, *p*H 8.0. From previous measurements[21], intracellular *p*H would be expected to rise from 6.9 towards 7.4–7.6 during ammonia exposure and to remain permanently above 7.3 even after ammonia withdrawal. Calcium levels can be seen to fall slightly with the addition of ammonia, but to rise sharply at 40 min after the addition. These results are typical of three similar experiments. Further experiments (not shown) give similar results even when ammonia is left in continuously or when external calcium is reduced to 10^{-7} M.

The low illumination intensities required with fura-2 and its apparent low toxicity allowed continuous measurement of calcium throughout the cell cycle. Embryos routinely cleaved at least three times (see Fig. 1 legend for details). Figure 2 shows two examples of continuous records obtained from fertilization to first cleavage. Cyclical changes in $[Ca^{2+}]_i$ coincide with specific events of embryo development and the cell division cycle. Figure 2*a* shows particularly well-defined $[Ca^{2+}]_i$ peaks corresponding to different stages of the cell cycle. In the most typical records (see Fig. 2*b*), peaks reach values similar to those in Fig. 2*a* but appear less striking because they rise from a permanently elevated plateau level of $[Ca^{2+}]_i$. Both records reveal that at 10–12 min after fertilization, a second, surprisingly large, increase in $[Ca^{2+}]_i$ occurs. Photographs taken during the breaks in Fig. 2*a*, as well as many observations during other experiments, indicate that this peak occurs during pronuclear migration. The average peak $[Ca^{2+}]_i$, 665 ± 51 nM ($n = 8$), is well above the mean post-fertilization baseline of 277 ± 28 nM ($n = 8$). EGTA-induced reduction of the external calcium concentration to 10^{-7} M did not affect this peak or subsequent peaks, suggesting that the increases in calcium result from release from internal stores (data not shown).

In 10–20% of the embryos (including that of Fig. 2*a*), we observed another calcium peak associated with the streak stage (the period of pole separation and DNA synthesis)[16]. This transient was seen in both polyspermic and monospermic embryos.

Another elevation of $[Ca^{2+}]_i$ occurred 50–60 min after fertilization, with an average peak value of 404 ± 20 nM ($n = 8$). Photographs indicate that the increase in $[Ca^{2+}]_i$ occurs close to or during nuclear envelope breakdown. This peak in free calcium may explain earlier findings of Bennet and Mazia[17] in which unfertilized eggs that had been fused with fertilized embryos underwent cortical granule breakdown in the unfertilized half when the fertilized half began mitosis. The trigger for Ca^{2+} at nuclear envelope breakdown may have triggered the release of Ca^{2+} stores in the unfertilized half.

A fourth peak was observed ~70 min after fertilization, with a mean value of 410 ± 10 nM ($n = 6$). Photographs and visual checks suggest that this peak occurs during the metaphase/anaphase transition, although precise timing is difficult because we cannot resolve the actual chromosome movements. However, in Fig. 2*a*, the spindle was newly formed before the peak, and spindle elongation, corresponding to late anaphase, did not occur until after the peak, thus indicating that the metaphase/anaphase transition occurred during the period of the peak. Although other investigators have suggested that calcium may be involved in the metaphase/anaphase transition, until now there has been no evidence that an increase in $[Ca^{2+}]_i$ actually takes place. Izant has reported that injections of calcium buffered at 1 μM or 50 nM free calcium, respectively, shorten or lengthen the duration of metaphase[4]. Wolniak *et al.* reported a decrease in chlortetracycline staining during metaphase, suggesting that a release of calcium stores occurs at the onset of anaphase[7].

Finally, we observed an increase in $[Ca^{2+}]_i$ associated with cleavage. We found that calcium rises to a mean value of 412 ± 34 nM ($n = 7$); this increase slightly precedes and occurs in conjunction with the developing cleavage furrow. The notion that calcium may regulate the activity of the contractile ring has been suggested by several investigators using injection of calcium buffers or ionophore A23187 (refs 1, 2, 6), although attempts to measure a change with aequorin have been equivocal[1,18]. On the other hand, $[Ca^{2+}]_i$ measurement in *Xenopus* embryos using ion-selective microelectrodes consistently failed to show evidence of cell-cycle-related fluctuations in calcium[19]; this was perhaps a result of species differences or localization of calcium rises that always missed the electrode tip.

The general elevation of baseline $[Ca^{2+}]_i$ and upward fluctuations during the cell cycle are not the only ionic events that follow fertilization of sea urchin eggs. A rise in internal *p*H, normally dependent on external Na^+, is known to be necessary for development[20,21]. The addition of ammonia to sea water, which artificially elevates the internal *p*H, induces many of the

events of the cell cycle, such as DNA synthesis, chromosome condensation and nuclear envelope breakdown[22]. We hypothesized that if calcium is pertinent to these cell-cycle events, ammonia might affect $[Ca^{2+}]_i$; however, at first sight this seems unlikely as ammonia does not cause a cortical reaction or any obvious change in NADP/NAD ratios, both of which are calcium-dependent consequences of fertilization[23,24]. We measured $[Ca^{2+}]_i$ in ammonia-treated eggs (Fig. 3) and found that during the first 40 min after addition of ammonia, $[Ca^{2+}]_i$ either decreased slightly or did not change. A decrease in $[Ca^{2+}]_i$ would be interesting in light of the finding that the activity of the calcium-sequestering system is enhanced by elevated pH[25]. However, at ~40–55 min, $[Ca^{2+}]_i$ rose to levels characteristic of fertilized eggs entering mitosis (350–400 nM) and remained elevated thereafter. Inspection revealed that the nuclear envelope broke down at 60–70 min, shortly after the increase in $[Ca^{2+}]_i$. Similar results were obtained even if external calcium was lowered to 10^{-7} M, indicating that ammonia can eventually reset the intracellular calcium levels even in the absence of external calcium. This finding requires a revision of the conclusions reached by Zucker *et al.* on the action of ammonia on the nuclear envelope[26]. Using aequorin, they were unable to detect these low calcium concentrations during the ammonia treatment and so concluded that ammonia exerted its effect through alkalinization alone. Although ammonia does raise $[Ca^{2+}]_i$ after 40–55 min, earlier events, such as the acceleration of protein synthesis which starts 25 min after ammonia addition, must be pH responses not mediated by calcium[27].

The observed correlation between mitotic events and changes in $[Ca^{2+}]_i$ does not prove that these changes are required for the observed events. The causal relationship between ionic and mitotic events will be clarified when a means becomes available for controlling $[Ca^{2+}]_i$ at given times.

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Transformation of mononuclear phagocytes *in vivo* and malignant histiocytosis caused by a novel murine spleen focus-forming virus

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The study of retrovirus-induced leukaemias in mice is a powerful tool for the elucidation of the normal regulation of the haematopoietic system. The acute murine spleen focus-forming viruses (SFFV) can be classified according to the haematopoietic lineage on which they exert their effects in the adult mouse. Here we report a new SFFV isolate, the AF-1 virus, with the novel ability to transform cells of the mononuclear phagocyte lineage. The virus was isolated from sarcomas that were induced on passage of a cloned Friend helper virus (F-MuLV, 643/22F)[1] in newborn BALB/c mice. We have cloned the transforming defective subunit of the AF-1 viral complex in NRK cells and isolated several subclones. Analysis of the proviral genome in two non-producer cell clones reveals that AF-1 virus contains Harvey v-*ras*-specific sequences (Fig. 1). Thus, AF-1 virus is closely related to Harvey murine sarcoma virus (Ha-MSV)[2], and is, at present, the only tool by which permanent cell lines can be obtained from mononuclear phagocytes in the mouse.

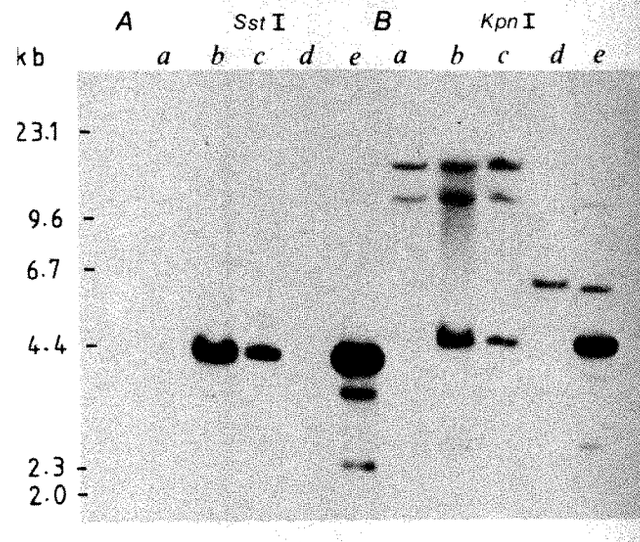


Fig. 1 Southern blots showing Ha-*ras* sequences. *Sst*I(*A*) and *Kpn*I(*B*)-digested high-relative molecular mass DNA was electrophoresed in horizontal 0.8% agarose gels and transferred to nitrocellulose filters[17]. The filters were hybridized in stringent conditions[18] to 10^6 c.p.m. ml^{-1} of ^{32}P-labelled clone BS9 of v-Ha-*ras* (specific activity 2×10^8 c.p.m. μg^{-1})[19]. Lanes *a*, NRK; *b*, AF124T; *c*, AF124TC1$_3$; *d*, 3T3; *e*, HT3. *Hin*dIII-digested *c*1857 DNA served as relative molecular mass markers: kb, kilobases. AF124T is an NRK non-producer cell clone of AF-1 virus (see text). AF124Tc13 is a subclone of AF124T. HT3 is a Ha-MSV non-producer cell clone[20].

Intravenous injection of AF-1 virus rapidly caused splenomegaly and anaemia in adult DDD (*Fv-2*^{s}) (Table 1) and B6.S mice (data not shown). DDD mice infected with AF-1 virus died within the first 25 days of the disease. C57BL/6 mice of *Fv-2*^{2} or *Fv-2*^{r} genotype were more resistant than DDD mice. C57BL/6 *Fv-2*^{r} mice survived for up to 50 days after infection. The relative resistance of C57BL/6 mice was also shown using spleen focus formation as the parameter of viral host range (T.F. *et al.*, unpublished observations).

The histological examination of AF-1 virus-infected DDD (*Fv-2*^{s}) mice revealed that the rapidly progressing spleen enlargement results from the growth of a tumour of the monocyte-macrophage/histiocyte lineage (Fig. 2). Transformed histiocytic cells were first detected in the marginal zone of the spleen 6 days after infection and then invaded the adjacent red and white pulp. At later stages these tumour cells were also found in bone marrow, lymph nodes, Peyer's patches and many parenchymal organs, but not the thymus or the brain. During the course of the disease, the cellularity of the bone marrow decreased 10-fold and at later stages the tissue recovered from the femoral cavity consisted mainly of histiocytic tumour cells. The disseminated and invasive growth of the malignant histiocytic neoplasm and the pronounced pancytopenia in AF-1 virus-infected mice were strikingly reminiscent of human malignant histiocytosis (also known as malignant medullary reticulosis), which is a systemic and fatal histiocytic tumour of both children and adults[3–6].

We also studied the effects of AF-1 virus on haematopoietic colony-forming cells (Table 1): 18 days after infection of DDD mice with AF-1 virus, no early erythroid precursor cells (burst-forming units—erythroid, BFU-E) could be detected in the bone marrow. Late erythroid precursor cells (colony-forming units—erythroid, CFU-E) were markedly decreased in the bone marrow and the spleen. The disappearance of erythroid colony-forming cells in the haematopoietic tissues of AF-1 virus-infected mice correlated well with the progressive decrease of erythroid precursor cells in the histological specimens.

The myeloid/monocytic precursor cells (CFU-C), however, were increased in the spleen of AF-1 virus-infected mice and most of them proliferated in the absence of stimulating factors. At other time points after infection with AF-1 virus, DDD (*Fv-2*^{s}) mice also showed factor-independent CFU-C in the bone marrow. In our culture system this factor-independent CFU-C formation from spleen cells of AF-1 virus-infected mice was linear, with plating densities ranging from 10^4 to 10^5 cells per dish, indicating that the factor independence is not related to the presence of factor-producing accessory cells. We obtained further evidence from studies using secondary transfer of granulocyte–macrophage and macrophage colony-forming units (CFU-GM and CFU-M) of AF-1 virus-infected mice (ref. 7 and T.F., unpublished observations). Thus, all of 17 factor-independent CFU-C from bone marrow and all of 6 factor-independent CFU-M from the spleen of DDD mice 5 days after infection with AF-1 virus continued to proliferate after transfer to liquid culture without stimulating factor for 4 months, whereas the proliferation of CFU-M transferred from uninfected controls to cultures without stimulating factors stopped after 4–6 weeks.

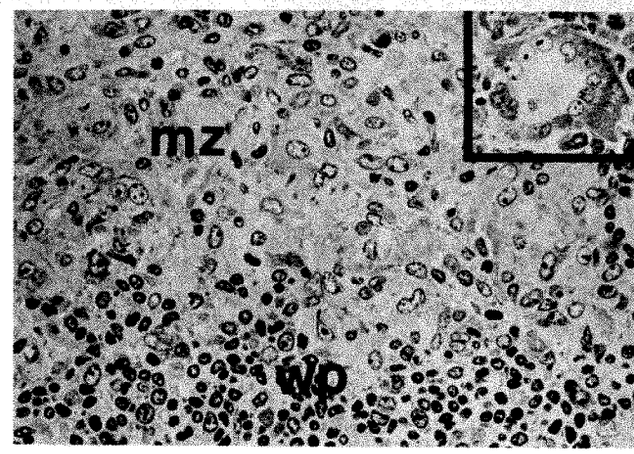


Fig. 2 Spleen of DDD mouse 10 days after infection with AF-1 virus. The normal cellular composition of the marginal zone (mz) of the white pulp (wp) is replaced by cohesively growing histiocyte-like tumour cells spreading into the red and white pulp. The tumour cells are characterized by irregular rounded, elongated, reniform or lobulated nuclei of variable size with finely dispersed chromatin and one or several prominent nucleoli. They possess abundant pale eosinophilic cytoplasm with irregular blurred cell borders. In poorly differentiated tumour cells, strong basophilic staining of the cytoplasm is seen. Bi- and multinucleated cells (inset) and mitotic figures are present. Glycol-methacrylate embedding, Giemsa stain, ×240.

Table 1 Effect of AF-1 virus on haematopoietic colony-forming cells in adult DDD mice

			Uninfected	AF-1 virus-infected
Spleen weight (mg) (mean of three measurements)			100	1,787 ± 180
Haematocrit (%)			49	34
CFU-E*	Bone marrow	None	0	0
		+Ep	180 ± 17	4 ± 1.0
	Spleen	None	0	0
		+Ep	25 ± 2.2	15 ± 1.2
BFU-E	Bone marrow	None	0	0
		+Ep	0	0
		+Wehi	0	0
		+Ep + Wehi	22 ± 3.1	0
	Spleen	None	0	0
		+Ep	0	0
		+Wehi	0	0
		+Ep + Wehi	3 ± 0.1	0.3 ± 0.2
CFU-C	Bone marrow	None	0	0
		+Wehi	104 ± 12	37 ± 4.2
	Spleen	None	0	20 ± 1.5
		+Wehi	6 ± 1.2	22 ± 0.8

Three-month-old female inbred DDD mice were injected intravenously with 6×10^3 spleen focus-forming units (SFFU) of AF-1 virus and used for the experiment 17 days after infection. AF-1 virus was rescued from 19TC13 non-producer cells with the F-MuLV clone 643/22N. This helper virus alone does not induce spleen focus formation or splenomegaly in adult mice. The technique for the assay of haematopoietic colony-forming cells has been described elsewhere[15]. Cells obtained from spleen cell suspensions or flushed out of the femoral cavity were plated at a density of 1×10^5 cells per ml in Iscove's modified Dulbecco's medium (Boehringer, Mannheim) containing 10% fetal calf serum (Flow), 0.8% methylcellulose (Fluka AG, Buchs, Switzerland), 180 μg iron-saturated human transferrin (Behringwerke, FRG) in 35-mm Petri dishes (Greiner). Where indicated, 0.5 U ml^{-1} erythropoietin (Ep) partially purified from the urine of patients with Fanconi's anaemia[15], was added. A 10% concentration of Wehi-3 conditioned medium (Wehi) was used as a source of factors (multi-CSF) that stimulate BFU-E and CFU-C formation *in vitro*[16]. Erythroid colonies of at least 20 cells were counted as CFU-E 2 days after the establishment of the culture. BFU-E form large erythroid colonies which were counted on day 9 of culture. CFU-C were recorded on the same day as BFU-E and represent the sum of macrophage, granulocytic and macrophage/granulocytic colonies.

* Data are the number of colonies per 10^5 cells plated and were obtained from three independent experiments using three mice.

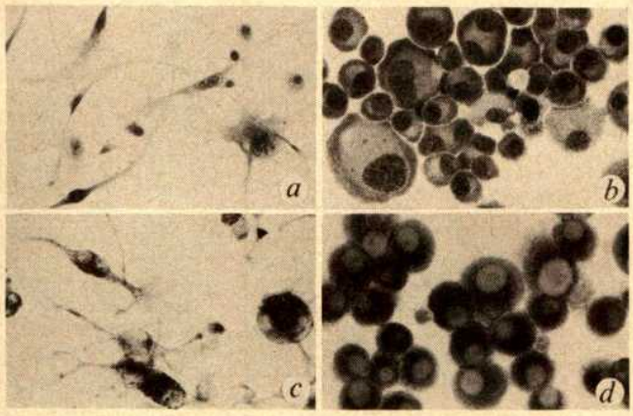


Fig. 3 HA15/A adherent (*a*, *c*) and HA15/S non-adherent (*b*, *d*) permanent cell lines obtained from spleens of DDD mice after infection with AF-1 virus. *a*, Cell line HA15/A is characterized by a pleomorphic cell type resembling macrophages. The cells obviously differ in size, cellular configuration and nuclear morphology. The cells are round or bi- and multipolar with round, oval or lobulated nuclei. The pale cytoplasm is heavily vacuolated and possesses long plump or delicately branched processes which occasionally terminate in irregular pseudopods. Multinucleated cells are present (*c*). *b*, Cell line HA15/S consists of monoblast-like cells of different size with round, chromatin-dense nuclei, strongly basophilic cytoplasm and prominent paranuclear Golgi bodies. Mono- or multinuclear polyploid cells with atypical nuclear configuration can be recognized. Both cell lines shown strong reactions with acid phosphatase (*c*, HA15/A cells) and nonspecific esterase (*d*, HA15/S cells). *a*, Giemsa, ×127; *b*, Giemsa, ×200. *c*, Acid phosphatase using naphthol AS-B1 phosphate as a substrate[6], no nuclear stain ×127. *d*, Nonspecific esterase using α-naphthyl butyrate as a substrate[5], no nuclear stain, ×200.

We found that 25% of the factor-independent AF-1 virus-infected CFU-M could be further propagated and maintained as permanent cell lines.

Cell lines were also easily established from spleen cell suspensions of AF-1 virus-infected DDD mice (cell line HA15) as early as 5 days after infection, which suggested early transformation of the target cell by AF-1 virus *in vivo*. The transformed cells proliferated readily in culture requiring neither prior passage of infected tissue *in vivo* nor a long period of tissue culture, unlike, for example, Friend leukaemia cells[8].

Shortly after establishment, the AF-1 virus-transformed cell lines mainly consisted of adherent slow-growing cells. Forced passage in culture resulted in the isolation of two sublines composed of either mainly adherent cells (HA15/A) or suspended growing cells (HA15/S). Cytologically, HA15/S cells exhibited morphological features of monoblasts and HA15/A cells resembled macrophages (Fig. 3*a*, *b*). Both sublines showed activity of nonspecific esterase and acid phosphatase (Fig. 3*c*, *d*) but no expression of Mac-1 antigen[9–11]. The lack of Mac-1 antigen expression may either be a consequence of the viral transformation or indicate that the AF-1 virus target cell is among the subpopulation of mononuclear phagocytes that does not express this antigen, for example, the histiocytes of the spleen[12]. HA15/A and HA15/S cells expressed Fc receptors, whereas only HA15/A cells could phagocytose immunoglobulin-coated polystyrene particles[13]. HA15 cells released virus that transformed fibroblasts and caused spleen focus formation and splenomegaly. The pathogenic property of this virus was not different from the AF-1 virus rescued from the non-producer cells.

Attempts to establish permanent cell lines from spleen cell cultures or CFU-C of uninfected or Ha-MSV-infected mice have failed; the cell lines ceased to proliferate after 2–3 weeks of culture. As both Ha-MSV and the AF-1 virus contain the *ras* oncogene (Fig. 1), it will be of interest to determine whether the immortalization of a subpopulation of mononuclear phagocytes is a property of either the *ras* oncogene or the viral long terminal repeat of AF-1 virus in combination with the *ras* oncogene. So far, restriction mapping of the genomes of Ha-MSV and AF-1 virus has shown subtle differences between the two in the long terminal repeat region of the genome but not in the *ras* oncogene. Recently, AF-1 virus has been molecularly cloned to pinpoint the genetic elements that make its leukaemogenic properties so unique.

In summary, AF-1 virus is a new murine spleen focus-forming virus carrying a *ras*-related oncogene that possesses the unique property of transforming cells of the mononuclear phagocyte lineage *in vivo*. The AF-1 virus-infected DDD mouse is uniquely appropriate as a model for studying the pathogenesis of human malignant histiocytosis, for which a viral etiology has been proposed[14].

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An HTLV-III peptide produced by recombinant DNA is immunoreactive with sera from patients with AIDS

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Human T-cell lymphotropic retrovirus type III (HTLV-III), also called lymphadenopathy-associated virus (LAV), has been identified as the aetiological agent of acquired immune deficiency syndrome (AIDS)[1–9]. The sera of most patients with AIDS or AIDS-related complexes, and of asymptomatic individuals infected with HTLV-III, contain antibodies against antigens of HTLV-III[4,10–12]. The characterization of these antibodies and their corresponding viral antigens is important not only for understanding immunity against HTLV-III and the pathology of AIDS, but also for the development of diagnostic methods and preventive vaccine for AIDS. Following the successful establishment of a long-term T-cell line permissive for HTLV-III replication[1], large quantities of virus have been produced, facilitating the purification of viral proteins and the development of mouse monoclonal antibodies

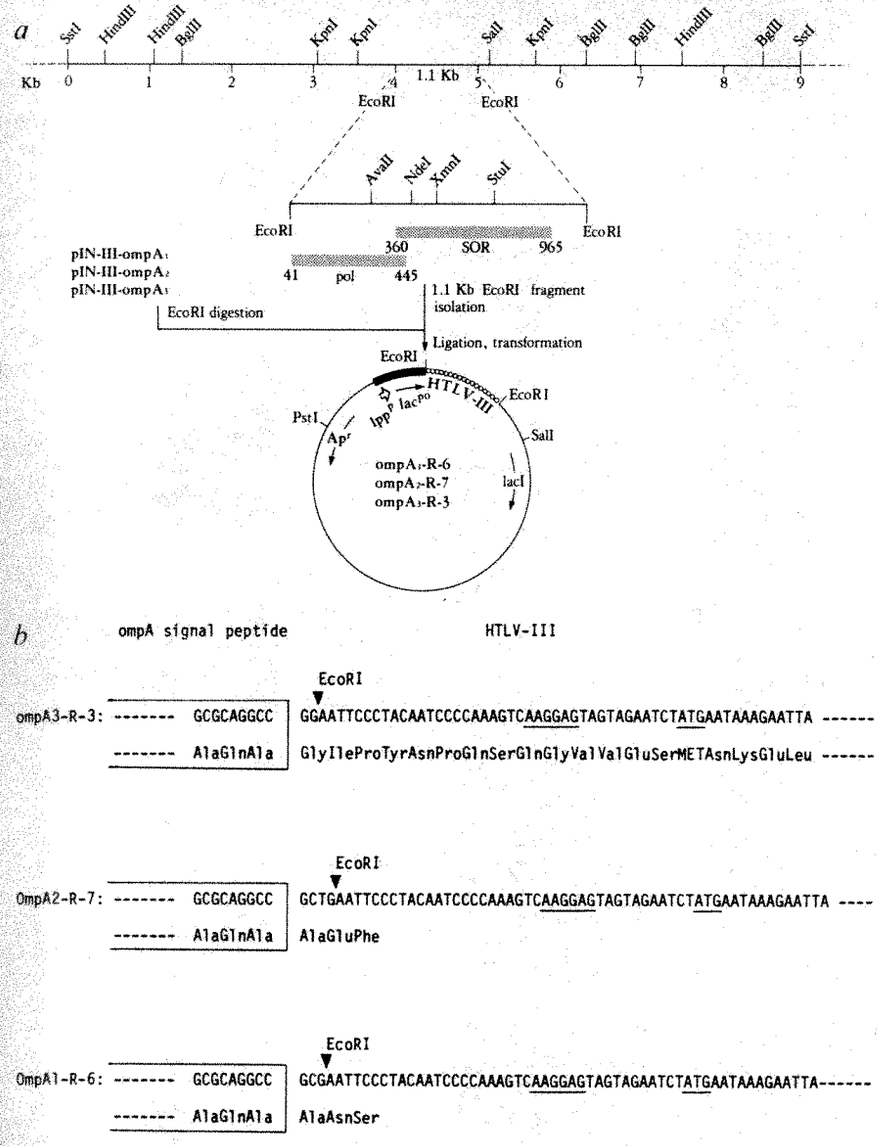


Fig. 1 ***a***, Construction of recombinant plasmids ompA1-R-6 (01R6), ompA2-R-7 (02R7) and ompA3-R-3 (03R3) carrying HTLV-III inserts. The 1.1-kb *Eco*RI fragment was excised from clone λBH10, purified by gel electrophoresis and ligated to plasmid vectors pIN-III-ompA1, -ompA2 and -ompA3 which had been cut previously with *Eco*RI. The ligation mixture was used to transform *E. coli* K-12 strain JA221 (ref. 17) and the resultant ampicillin-resistant colonies were isolated for further characterization. The 15,000-M_r peptide is presumed to start at nucleotide 41 and end at position 445 and the sequence of the short open reading frame extends from nucleotides 360 to 965 (see text). The top line shows the restriction map of HTLV-III insert of clone λBH10 (refs 13, 16). The dotted line indicates the left-hand and right-hand arms of λ vector λgtWESλB. The solid line indicates the HTLV-III DNA. The two shaded regions indicate the two open reading frames included in the 1.1 kb *Eco*RI fragment, the 3′ end of the *pol* gene and the SOR. ***b***, The nucleotide sequence of the ompA signal peptide and the pertinent region of recombinant plasmids 01R6, 02R7 and 03R3. The sequence of the ompA signal peptide is boxed. The postulated Shine–Dalgarno sequence AAGGAG and the initiation codon ATG are underlined. Abbreviations: lpp^p, *lpp* promoter, lac^{po}, *lac* promoter-operator; AP^r, ampicillin-resistance gene.

against several viral antigens[10,24]. More recently, the structure of HTLV-III proviral DNA has been elucidated[13–16]. We now report the production, by genetic engineering methods, of a peptide encoded by a gene segment of HTLV-III. A 1.1-kilobase (kb) *Eco*RI DNA segment from an isolate of HTLV-III was inserted into a *lpp* and *lac* promoter-coupled expression vector, pIN-III-ompA. *Escherichia coli* transformants of this plasmid produced a peptide of relative molecular mass (M_r) 15,000 (15K) which was strongly immunoreactive with anti-HTLV-III antibodies present in sera from AIDS patients. Lysates of the clones expressing this 15K peptide inhibited the reactivity of the p31 virion protein with AIDS sera, suggesting that it is a fragment of the viral p31 protein. The peptide reacted with sera from all 20 AIDS patients but none of the 8 normal controls tested. These results suggest that the peptide may be useful for detecting anti-HTLV-III antibodies in blood samples.

The expression vector used, pIN-III-ompA (ompA), contains the lipoprotein (the most abundant protein in *E. coli*) gene promoter (*lpp*) and the *lacUV5* promoter-operator (Fig. 1*a*)[17]. The ompA vectors also contain the DNA segment encoding the lac repressor, which allows the expression of the inserted DNA to be regulated by *lac* operon inducers such as isopropyl-β-D-thiogalactoside (IPTG). The ompA cloning vehicles contain three unique restriction enzyme sites, *Eco*RI, *Hin*dIII and *Bam*HI, in all three reading frames, so that DNA can be inserted into any of these restriction sites.

Various restriction fragments were excised from the recombinant clone λBH10, which contains a 9-kb HTLV-III DNA insert in the *Sst*I site of the vector λgtWES.λB (ref. 13). These restriction fragments were then inserted into the ompA vectors in all three reading frames and used to transform *E. coli* JA221 cells[17]. Transformants were first screened for HTLV-III DNA by *in situ* colony hybridization using HTLV-III DNA probes[18], and the positive clones then screened for expression of HTLV-III antigenic peptides using HTLV-III-specific antibodies.

This approach identified several gene segments that encode peptides showing immunoreactivity with anti-HTLV-III antibodies, among these a 1.1-kb *Eco*RI restriction fragment. When this fragment was inserted into the ompA vectors in the three reading frames (Fig. 1*a*), IPTG induction of transformants of all three plasmid constructs, designated ompA1-R-6 (01R6), ompA2-R-7 (02R7) and ompA3-R-3 (03R3), produced a 15K peptide that was strongly reactive with anti-HTLV-III antibodies in sera from AIDS patients (Fig. 2). This reactivity was not detected with sera from normal individuals (data not shown).

DNA sequence analysis of the three recombinant constructs confirmed that, as planned, each recombinant had a different reading frame of the HTLV-III (+)-strand coupled to the coding sequence of each vector. Only in 03R3 was the reading frame of the inserted DNA in phase with that set by the signal peptide in the ompA vector; in 01R6 and 02R7 the *pol* gene segment DNA was out of phase (Fig. 1*b*). Examining the DNA sequence closely, we found that there was a 6-base pair (bp) ribosome binding site, AAGGAG (Shine–Dalgarno sequence), located at nucleotide position 24–29 and an initiation codon, ATG, located 11 bp downstream (position 41–43). We believe that the 15K

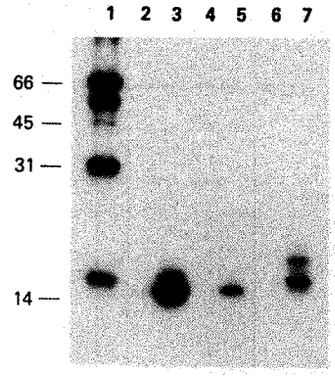


Fig. 2 Immunoreactivity with AIDS sera of HTLV-III antigens produced in bacterial cells containing plasmids 01R6, 02R7 or 03R3. Lane 1, purified HTLV-III virions; lanes 2, 3, 01R6 uninduced and induced with IPTG; lanes 4, 5, 02R7 uninduced and induced; lanes 6, 7, 03R3 uninduced and induced. Numbers on the right represent M_r standards ($\times 10^{-3}$).

Methods. Cells were grown at 37 °C in L-broth containing 100 μg ml^{-1} ampicillin to an A_{600} of 0.2. The cell cultures were then divided into two aliquots; IPTG was added to one aliquot to a final concentration of 2 mM (induced) and the other aliquot received no IPTG (uninduced). After 2 h incubation at 37 °C, the cells were collected and lysed in gel loading buffer[22]. Total cell lysates from the equivalent of 0.2 ml cell culture or purified NP40-disrupted HTLV-III virions were electrophoresed on 12.5% SDS-polyacrylamide gels by the method of Laemmli[22]. The proteins on the gels were then electrophoretically blotted onto a 0.45-μm nitrocellulose filter[23]. The blots were first incubated with a blocking buffer (5% non-fat dry milk, 0.1% Antifoam A, 0.1% Na-azide in phosphate-buffered saline) for 2 h at 37 °C and then with fresh blocking buffer containing 5% goat serum for 2 h at room temperature. AIDS patients' or normal donor sera were then added to a final dilution of 1:100 and the blots were incubated at 4 °C overnight. After incubation the filters were washed three times with washing buffer (0.5% deoxycholic acid, 0.1 M NaCl, 0.5% Triton X-100, 10 mM phosphate *p*H 7.5, 0.1 mM phenylmethylsulphonyl fluoride) for 30 min each and then incubated with fresh blocking buffer containing 5% normal goat serum for 1 h before adding ^{125}I-labelled goat anti-human IgG (10 μCi μg^{-1}) to a final dilution of 1×10^6 c.p.m. ml^{-1}. After 30 min, the iodinated probe was removed and the nitrocellulose blots were washed with washing buffer three times for 20 min each, dried and exposed to Kodak XAR-5 X-ray film.

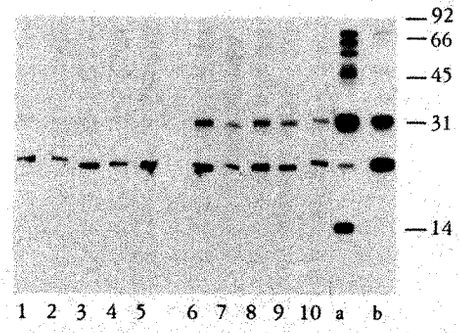


Fig. 3 Inhibition of reaction between HTLV-III antigens and an AIDS serum by 01R6 lysate. Lanes 1–5, 01R6 cell lysate of 50, 75, 100, 200 and 400 μl, respectively; lanes 6–10, control cell lysate of 50, 75, 100, 200 and 400 μl, respectively; lane *a*, no cell lysate (with serum from AIDS patient A60); lane *b*, no cell lysate (with serum from AIDS patient E.Y.).

Methods. Clone 01R6 or control cells in 10-ml cultures were induced with IPTG at an A_{600} of 0.2 and the cells were collected after 2 h, resuspended in 1 ml 10 mM Tris-HCl *p*H 8.0, and sonicated. NP40-treated HTLV-III was fractionated on a 12.5% SDS-polyacrylamide gel and electro-transblotted onto a nitrocellulose filter paper. The paper was cut into ~30 strips which were then incubated with a well-characterized AIDS serum (from patient E.Y., which had elevated anti-p31 antibodies) in the presence of increasing amounts of lysate of clone 01R6 (lanes 1–5) or a negative control (lanes 6–10). The lysates were then used in the competition inhibition immunoassay. The further immunoblotting procedure was performed as described for Fig. 2.

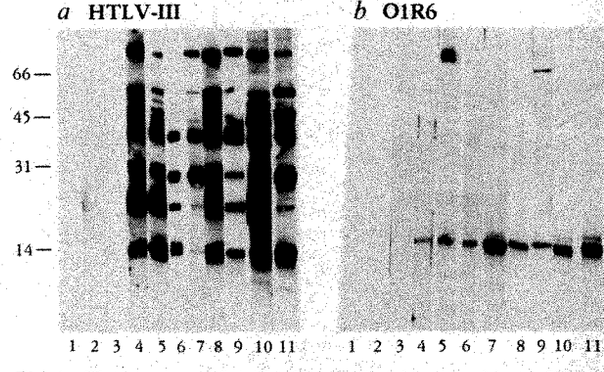


Fig. 4 The presence of antibodies against the recombinant HTLV-III-encoded 15K peptide in sera from AIDS patients and healthy individuals. Purified HTLV-III virus (*a*) or total cell lysate of bacterial clone 01R6 (*b*) were electrophoresed and electroblotted onto nitrocellulose filters, and protein strips were reacted with serum samples as described for Fig. 3. Lanes 1–3, healthy donors; lanes 4–11, AIDS patients.

peptide synthesized by all three recombinants is translated from the transcripts using this internal initiation codon. If this is true, the peptide would start from the ATG located at position 41–43 and end at the stop codon at position 446–448, producing a peptide of 135 amino-acid residues encoded by the 3'-end segment of the *pol* gene of HTLV-III. As further evidence that the 15K peptide is indeed derived from the *pol* gene, clones containing only the 5'-end *Eco*RI/*Nde*I fragment (Fig. 1*a*) still produced the same 15K peptide (data not shown).

In addition to the 15K peptide, the 03R3 construct, in which the reading frame of the HTLV-III DNA *pol* gene is in phase with that set by the vector, produced another two peptides ~19K and 16.5K in size (Fig. 2). It is possible that the 19K peptide contains an additional 35 amino-acid residues, 21 of which are from the signal peptide encoded by the ompA3 vector, with 14 encoded by the inserted HTLV-III DNA itself. The 16.5K peptide may be the processed 19K peptide in which the signal peptide is cleaved.

The 01R6 and 02R7 constructs also produced another peptide of ~17.5 K (Fig. 2) which was weakly reactive with sera from AIDS patients, but whose origin is unclear. Note that the 1.1-kb *Eco*RI fragment contains a second potential coding region, designated as the short open reading frame (SOR), extending from nucleotides 360 to 965 (Fig. 1*a*)[16]. Four of the five AUG methionine codons in this region are near the 5' end of this open reading frame. However, there is no clearly recognizable ribosome binding site at the 5' end of this open reading frame. Furthermore, several recombinant clones containing DNA fragments of the SOR, when inserted into the open reading frame vector pMR100, produced λCI-HTLV-III-β-galactosidase tripartite fusion proteins which showed little immunoreactivity with anti-HTLV-III antibodies in sera from AIDS patients[19].

Because the RNA-dependent DNA polymerase of retroviruses is a minor component of the virions, we were surprised to detect immunoreactivity against the 15K peptide in sera from AIDS patients. To identify this immunoreactive peptide with respect to the banding pattern of HTLV-III virion antigen in SDS-polyacrylamide gel electrophoresis, we used a competition inhibition immunoassay. Identical filter strips containing electrophoresed disrupted HTLV-III virions were incubated with a well-characterized serum from an AIDS patient in the presence or absence of lysates of 01R6, or control bacterial clones. Lysates of 01R6 blocked the immunoreactivity of the viral p31 protein with the AIDS serum, but lysates of control cells did not (Fig. 3). This result suggests that the recombinant 15K peptide encoded by the 3' end of the viral *pol* gene is also a part of another virion protein, p31, in contrast to a previous proposal

that p31 is a cellular protein which co-purifies with HTLV-III virions[10].

We next evaluated the prevalence in AIDS patient sera of antibodies against the 15K peptide. A panel of sera from AIDS patients and normal healthy individuals was tested in Western blot analyses using 01R6 lysate as the source of antigen. All of 20 AIDS sera and none of the 8 normal controls reacted with the 15K peptide (Fig. 4), indicating that most, if not all, AIDS patients produce antibodies against the viral p31 protein.

Thus, the 15K peptide described here seems to be encoded by the 3′ end of the viral *pol* gene located at the 5′ side of the 1.1-kb *Eco*RI fragment and to be related to the HTLV-III virion protein p31. The precise nature of the viral p31 protein remains unclear. However, in other retroviruses, such as avian myoblastosis virus and murine leukaemia virus, the 3′ end of the *pol* gene has been shown to encode, in addition to the reverse transcriptase, an endonuclease, RNase H, which seems to be essential for virus replication[20,21]. The p31 peptide encoded by the 3′ end of the HTLV-III *pol* gene may represent the corresponding RNase H of this virus. Work is in progress to purify and sequence the 15K peptide from bacterial clones expressing it. The peptide is also being evaluated for use as the solid-phase immunoadsorbent in immunoassays screening for anti-HTLV-III antibodies in serum samples.

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Hepatitis B virus contains pre-S gene-encoded domains

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One of the open reading frames on hepatitis B virus (HBV) DNA comprises the coding region (designated the *env* gene) for the virus envelope proteins. Studies on messenger RNA transcription suggest that this gene has the potential to code for three related proteins[1]: (1) a protein of 226 amino acids identified as a major protein constituent of the HBV envelope, termed S-protein; (2) a protein with 55 additional amino acids at the N-terminal coded for by a portion of the *env* gene upstream of the S-gene (pre-S); (3) a protein corresponding to the entire *env* gene (pre-S + S). Synthetic peptides from the N-terminals of proteins (2) and (3), and antisera to them have been used to study the occurrence and properties of pre-S sequences. The results presented here provide unambiguous evidence that all three *env* encoded proteins are present in HBV particles; synthetic peptides corresponding to the gene encoding pre-S are highly immunogenic and can be used in diagnostic tests for detection in human sera of antibodies preferentially recognizing HBV; such antibodies, specific for pre-S determinants, are elicited during hepatitis B infection and by immunization with HBV proteins (2) and (3); the hepatitis B vaccine licensed in the United States does not contain pre-S proteins; and the pre-S proteins of the HBV envelope contain domains specifically recognized by liver cells. These findings suggest that pre-S determinants are important in virus-neutralizing responses and should be present in HBV vaccines.

Table 1 Interaction of HBV (HBsAg) with human hepatoma cells

Cells used	Antibodies added	Percentage of cells attached to HBsAg-cellulose
HepG2 (human hepatoma)	—	80–95 (14 experiments)
HeLa	—	10–20
Rat hepatocytes	—	10–20
HepG2	Anti-pre-S(120–145) (15 mg IgG ml^{-1})	60
HepG2	Anti-pre-S(12–32) (15 mg IgG ml^{-1})	30
HepG2	Anti-pre-S(120–145) + anti-pre-S(12–32) (7.5 mg IgG ml^{-1} each)	20
HepG2	Anti-HBV serum devoid of anti-S-protein[5]	8
HepG2	—	0 (BSA-cellulose)

HBV (HBsAg) was attached to *N-N′-p*-phenylenedimaleimide-derivatized sulphydryl cellulose under conditions described for linking of pre-S(120–145)[2]. About 4 mg of HBV (HBsAg) was linked to 1 g of the cellulose derivative. A control cellulose derivative was prepared by linking bovine serum albumin (BSA) to the activated matrix. The cellulose derivative (40 mg) suspended in TS (0.14 M NaCl, 0.01 Tris-HCl, 0.02% NaN_3 *p*H 7.2) containing 10 mg ml^{-1} BSA (TS–BSA) was mixed with $\sim 2 \times 10^6$ washed HepG2 human hepatoma cells[15], suspended in TS–BSA and incubated for 30 min at 37 °C, followed by 1 h at 4 °C. HeLa cells and clone 9 normal rat liver cells (American Type Culture Collection) were used as controls. The cell/cellulose mixtures were layered on top of 1 ml of 33% (w/w) Hypaque and centrifuged at 3,000 r.p.m. for 2 min. The cellulose derivative with attached cells pelleted in these conditions; unattached cells recovered from the Hypaque/TS–BSA interphase were diluted five-fold in TS–BSA and pelleted by centrifugation. The relative proportion of adsorbed and unadsorbed cells was determined by measurement of lactate dehydrogenase (LDH) activity in appropriate aliquots of cell lysates obtained after exposure to the detergent Triton X-100 (5 mg ml^{-1} in H_2O). LDH activity was determined using Sigma diagnostic kit no. 500. In initial experiments, similar results were obtained with HBV-cellulose and HBsAg-cellulose. Because of the limited quantities of HBV available, HBsAg-cellulose was used in subsequent experiments.

Peptides corresponding to the N-terminals of proteins (2) and (3) were synthesized and used to raise antisera in rabbits. High levels of anti-peptide antibodies were elicited (Fig. 1*A*, *B*).

The use of anti-pre-S (120–145) in Western blots of HBV particles identified components of relative molecular mass (M_r) ~33,000 (~33K), ~36K, ~45K and ~67K (Fig. 1*C*, lane *d*). A similar experiment with anti-pre-S (12–32) identified the ~45K protein alone (data not shown), indicating that this component corresponds to protein (3), the full-length translation product of the *env* gene, while the ~33K and ~36K components correspond to protein (2)[2–4]. The ~45K component was not detectable in Western blots of 22-nm sub-viral particles lacking the nucleocapsid (hepatitis B surface antigen, HBsAg)[2]. This is consistent with previous evidence for antigenic determinants on the HBV envelope which are underrepresented or absent from

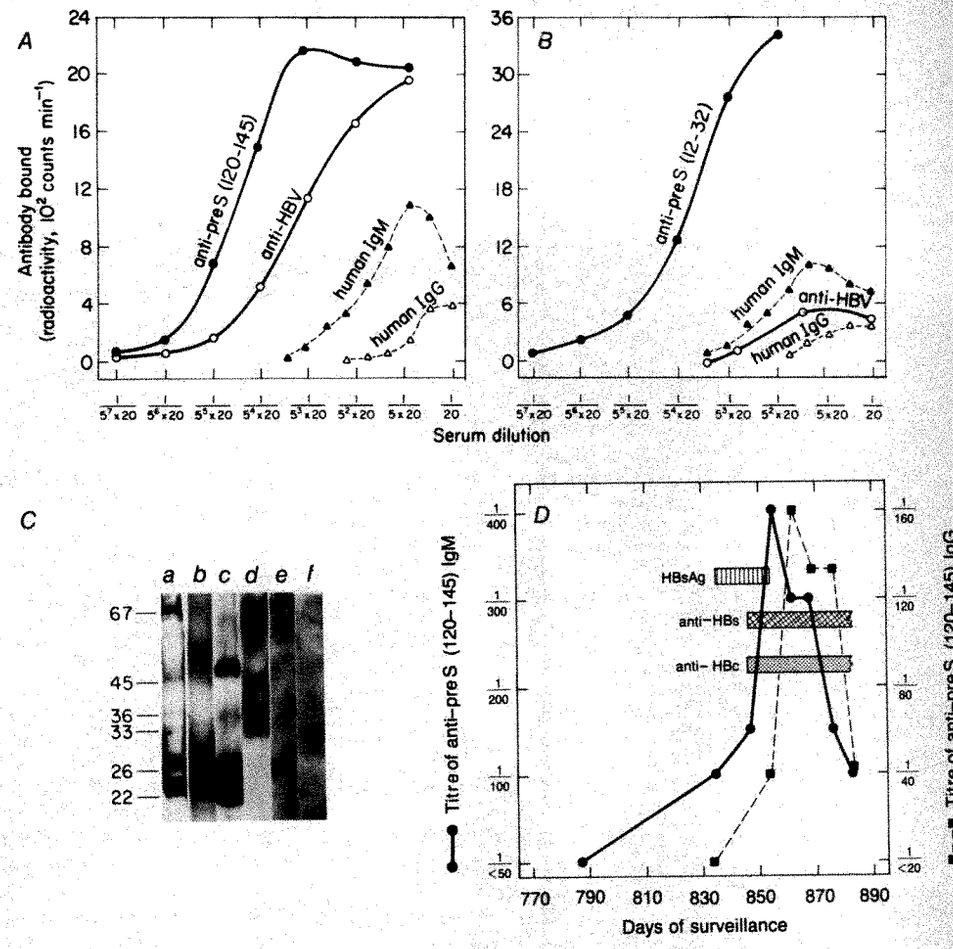


Fig. 1 ***A, B,*** Radioimmunoassays of IgG antibodies in serial dilutions of: rabbit antisera to pre-S (120–145) (*A*) or pre-S(12–32) (*B*) (●), to HBV (○) (devoid of antibodies to S-protein detectable by RIA[5]); IgG (△) and IgM (▲) in serum of a patient who had recovered from hepatitis B. The latter serum was obtained before antibodies to the S-protein were detectable. Immulon 2 Removawell strips (Dynatech Laboratories) were coated with 20 μg ml^{-1} of either free pre-S(120–145) or pre-S(12–32), then coated with gelatine (2.5 mg ml^{-1} in 0.1 M Tris, *p*H 8.8). The conditions for coating and the double-antibody RIA were described elsewhere[2,13]. The peptides corresponding to subtype adw2 (ref. 16) were synthesized as described elsewhere[2]; their sequences are MQWNSTAFHQTLQDPRYRGLYLPAGG [pre-S(120–145)] and MGTNLSVPNPLGFFPDHQLDP (pre-S(12–32)], according to the one-letter amino acid code. ***C,*** Polyacrylamide gel electrophoresis of HBV (*a, d*), serum-derived HBsAg after pepsin treatment (components of the Merck, Sharp & Dohme hepatitis B vaccine)[10] (*b, e*), and HBsAg produced in yeast[11] and lacking sequences corresponding to pre-S protein (*c, f*). Separated polypeptides were either stained with silver (*a–c*) or transferred to nitrocellulose and reacted with anti-pre-S (120–145) followed by ^{125}I-labelled protein A (*d–f*)[2]. ***D,*** Development of antibodies to the pre-S gene-encoded protein of HBV during acute hepatitis B infection. Antibodies to pre-S (120–145) were quantified; HBsAg, anti-HBs and anti-HBc were assayed using commerical test kits (Abbott Laboratories). The broken line at the end of the bars corresponding to the different markers of HBV infection indicates their presence at the end of surveillance. Antibody titres represent the highest dilution of serum at which radioactivity counts corresponding to the specimens, divided by counts corresponding to equally diluted control serum, were ≥2.1.

HBsAg[5–9]. To confirm this, we studied the reaction of an anti-HBV serum with two synthetic peptide analogues of the pre-S protein; the antiserum reacted at high dilutions with both peptides (Fig. 1*A, B*).

The synthetic peptides were also recognized by antibodies in sera of individuals who had just recovered from acute hepatitis B (Fig. 1*A, B*). Figure 1*D* shows the time course of development of antibodies recognizing pre-S (120–145) in a selected patient; similar results were obtained when antibodies recognizing pre-S (12–32) were assayed (data not shown). IgM antibodies recognizing the peptides were detected during HBsAg antigenaemia before antibodies to the S-protein (anti-HBs) or to hepatitis B core antigen (anti-HBc) were detectable. After production of the latter two antibodies, the titre of antibodies with anti-pre-S specificity declined. In some cases, antibodies recognizing the synthetic peptides were present even before HBsAg was detected in plasma, or when HBsAg never appeared in blood and the only marker for hepatitis B was anti-HBc and later anti-HBs.

HBV particles were recognized much more efficiently than HBsAg in radioimmunoassay (RIA) tests with anti-pre-S (120–145) (Fig. 2) or with antibodies to HBV particles (data not shown); this result implies that determinants specific for the preS region of protein (2) are more abundant in HBV than in HBsAg. Treatment of HBsAg with pepsin, a step used in the preparation of hepatitis B vaccine[10], resulted in a ~10^3-fold decrease in reactivity to anti-pre-S (120–145) (Fig. 2). HBsAg from vaccines derived either from infected plasma or produced in yeast[11] had ≤1/5,000 of the reactivity of intact HBsAg in these tests (data not shown; see also Fig. 1*C*). Human subjects vaccinated with either of these vaccines did not develop detectable antibodies recognizing either of the two synthetic peptides, whereas 7 out of 12 individuals who received a vaccine consisting of intact HBsAg[12] developed these antibodies.

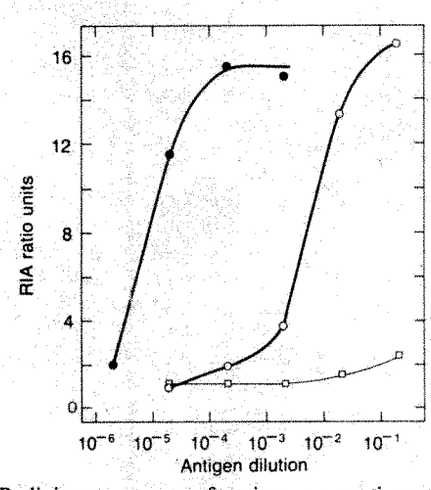


Fig. 2 Radioimmunoassays of various preparations containing HBV-specific proteins on polystyrene beads coated with anti-pre-S(120–145) IgG. The antigens tested were: HBV (●), HBsAg (○) and HBsAg treated with pepsin (1 mg ml^{-1} HBsAg, 50 μg ml^{-1} pepsin in 0.1 M glycine-NCl *p*H 2.2, 2 h at 37 °C) (□). RIA performed as described elsewhere[2]. The concentration of HBsAg S-protein was adjusted to the same level in all preparations based on RIA tests (AUSRIA, Abbott Laboratories).

Quantitative aspects of the immunological cross-reactivity between pre-S gene-encoded sequences exposed on HBV

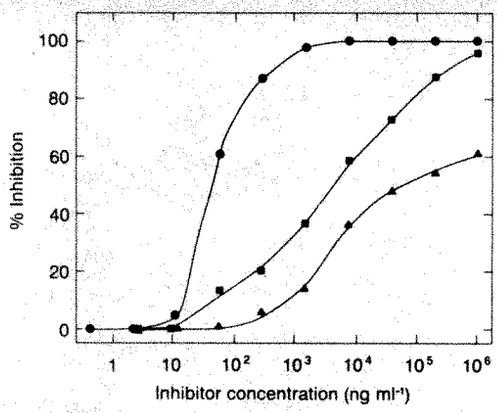


Fig. 3 Inhibition of the reaction of anti-pre-S (120–145) IgG with a pre-S(120–145)-β-galactosidase conjugate. ●, pre-S(120–145). The HBV (■) and HBsAg (▲) preparations contained the same concentration of HBsAg S-protein as determined by RIA (AUSRIA, Abbott Laboratories). The inhibition tests and the preparation of peptide-β-galactosidase conjugates were as described elsewhere[13,17]. Anti-pre-S(120–145) did not react with unconjugated β-galactosidase. The concentration of free peptide sufficient for ~50% inhibition of the reaction of pre-S(120–145)-β-galactosidase with anti-pre-S(120–145) is ~1/100 (w/w) of that for HBV. However, as the M_r of pre-S(120–145) is ~3K and that of HBV protein components reacting with anti-pre-S(120–145) (representing a minor (<20%) portion of the total HBV mass) is between ~33K and ~67K, the calculated molar concentration of the HBV pre-S protein required for 50% inhibition is approximately the same as that of the peptide analogue.

particles (or on HBsAg) and the synthetic peptide analogues were studied in more detail. The results (Fig. 3) indicate that HBV inhibited completely the reaction between anti-pre-S (120–145) and pre-S (120–145)-β-galactosidase. There is no significant population of anti-peptide antibodies which recognizes the synthetic peptide but not the native protein. Such antibody subpopulations are frequently observed in other antisera raised against synthetic peptide analogues of viral proteins[13,14]. The molar concentrations of HBV and pre-S (120–145) required for 50% inhibition were approximately the same, indicating that the antigenic determinants on the peptide analogue and on the corresponding segment of the HBV envelope protein(s) are identical or closely related. HBsAg has about one-fifth of the inhibitory activity of HBV. The inhibitory activity of pepsin-treated HBsAg was ≤1/1,000 of the activity corresponding to intact HBsAg.

To explore the involvement of pre-S gene-encoded HBV domains in attachment to cell receptors, we studied the reaction of HBsAg and HBV with liver cells. The results (summarized in Table 1) show that HBsAg (HBV) reacts with human hepatoma cells and that this reaction is inhibited by antibodies to synthetic peptides corresponding to the pre-S gene. Normal rabbit IgG, as well as antibodies to the S-protein (elicited by immunization with pepsin-treated HBsAg), had no inhibitory effects.

In conclusion, pre-S determinants clearly play an important role in hepatitis B virus infection and in the human immune response to HBV, and therefore should be included in hepatitis B vaccines.

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Intragenic recombination leads to pilus antigenic variation in *Neisseria gonorrhoeae*

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The pilus of the bacterium *Neisseria gonorrhoeae* is a fimbriate surface structure which promotes attachment of the bacterium to host epithelial cells[1–4]. Gonococcal pilus phase variation is characterized by a rapid on/off switch in which piliated (P^+) cells throw off non-piliated (P^-) variants and vice versa. Two regions of the gonococcal chromosome (*pil*E1 and *pil*E2) act as pilin expression loci[5], reminiscent of the *MAT* locus in the yeast *Saccharomyces cerevisiae*[6], while several other chromosomal regions contain silent (non-expressing) pilin sequences[5]. Biochemical and antigenic diversity is seen in pili from a wide variety of clinical isolates[7–11]. Pilins (pilus subunits) are composed of conserved N-terminal and variable C-terminal regions[12]; the conserved region of gonococcal pilin is also found in pilins produced by widely disparate bacteria[13–15]. We show here that the gonococcal pilin undergoes antigenic variation *in vitro* and *in vivo*. The protein consists of constant, semi-variable and hypervariable regions. This antigenic variation probably involves gene conversion of mini-cassettes of pilin information.

To study pilus antigenic variation in *N. gonorrhoeae*, lines of phase variants of strain MS11 were established whose members differ from each other in their piliation state (Fig. 2). Pilin transcripts from these derivatives were sequenced by the primer extension method[16]. MS11 can express at least seven different pilin genes (Figs 1*a*, 2). Within one line of derivatives, the P^- to P^+ switch resulted in the expression of a new variant (for example, 2C, E, G) or the re-expression of a former variant (for example, 3G, 10E). Thus, pilin information is not lost during phase or antigenic switching. Those derivatives which express variant genes produce pili that are antigenically different from those of the MS11 progenitor (Fig. 2).

Attempts to obtain sequences of the 3′-untranslated region of the pilin messenger RNA of variants 2C and 3I were unsuccessful (Fig. 1). These alternative sequences are probably the result of aberrant recombination events occurring between a silent locus and the expression site. Our sequence data on several expression loci in the P^- state indicate that this region is often affected by the events regulating the P^+ to P^- switch[17].

The pilin protein can be divided into three regions (Fig. 1). The first 53 amino acids are always conserved. A silent change has occurred at position 47 in variants 2C, ML3 and WG3 (data not shown), suggesting that the constant (C) region may be shorter than 53 residues. Many amino-acid substitutions are found within the semi-variable (SV) region (residues 54–114). Most changes here involve charged amino acids, but the introduction of a positive charge is often balanced by a second

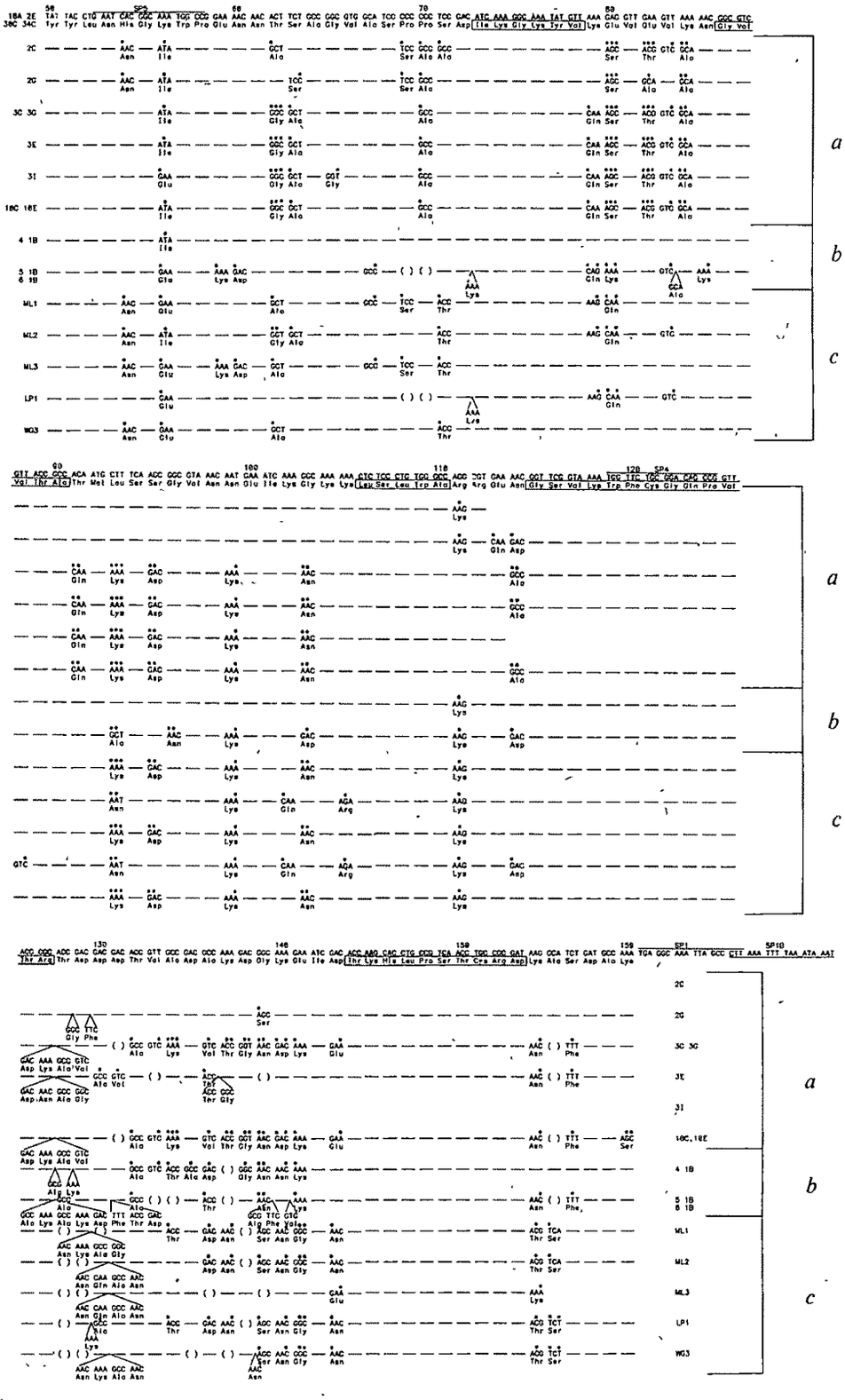


Fig. 1 Pilin gene sequences including deduced amino-acid sequences from residue 50 to the C-terminus. The MS11 pilin DNA and amino-acid sequences[5] are shown at the top. This sequence is also found in variants 2E, 10A, 30C and 34C. *a*, Sequences from the MS11-derived P^+ strains; *b*, pilin gene sequences in the expression loci of the MS11-derived P^- variants; *c*, sequences of the pilins from the clinical isolates. Strains ML1, 2 and 3 were obtained from the same individual at different times; LP1 and WG3 were isolated from two other individuals (P. F. Sparling, manuscript in preparation). Dashes indicate no sequence differences observed in these positions. Parentheses indicate codon deletions. Codon and amino-acid changes are indicated where found. If the sequence change does not alter the amino acid at that position, only the codon change is shown Dots above the bases indicate those positions within the codon which have changed. Insertions are presented below the variant sequences. Boxed amino acids in the top line represent the regions found to be conserved by comparison of all variant sequences. Primers were made by an Applied Biosystems 380A oligonucleotide synthesizer to regions which, by comparison of the different sequences, were observed to be invariant. Primer extension sequencing was done essentially as described previously[16], except that kinase-treated primers were used. The reverse complement of the sequence of each primer used is indicated by a line above or below the topmost DNA sequence. As a control for the accuracy of the technique, we have sequenced the 10A derivative and found it to be identical to that of the pilin gene from the progenitor previously determined by the Maxam-Gilbert technique[5]. A synthetic oligonucleotide complementary to a variant sequence was protected by mRNA from mung bean nuuclease digestion to the same extent as a synthetic oligonucleotide complementary to the pilin signal sequence. Thus, the pilin mRNA which was extended was also the major species expressed by the variants. Furthermore, the synthetic DNA did not prime the synthesis of any complementary DNA in RNA preparations from P^- B-series variants.

compensating substitution nearby, and vice versa. For example, a comparison of variants 10A and 10C shows that 10C has lost all charged amino acids at positions 79–84, but compensating charges in 10C can be found approximately 10 residues downstream. At the nucleotide level, these substitutions frequently involve two or three changes, both transitions and transversion, in a codon. The carboxy-terminal portion of the protein is highly variable; in addition to amino-acid substitutions, in-frame insertions and deletions of one to four codons occur. The amount of variability here would explain the low immunological cross-reactivity of pili from different strains, as this part is immunodominant over the conserved portion of the protein[11,18]. Two regions which bracket this hypervariable (HV) segment are always conserved; these centre around the cysteines at positions 121 and 151, are 13 and 10 amino acids long, respectively, and may represent epitopes common to all gonococcal pili.

In most variant genes, both the *SV* and *HV* sequences are entirely different from those of the progenitor. However, pilin variants of line 3 share the same *SV* gene segment but different *HV* ones. Thus it is possible to assemble complete genes by mixing *SV* and *HV* gene segments, a mechanism which would considerably increase the antigenic repertoire.

The absence of a good animal model for gonococcal infections has hampered the study of *in vivo* pilin antigenic variation.

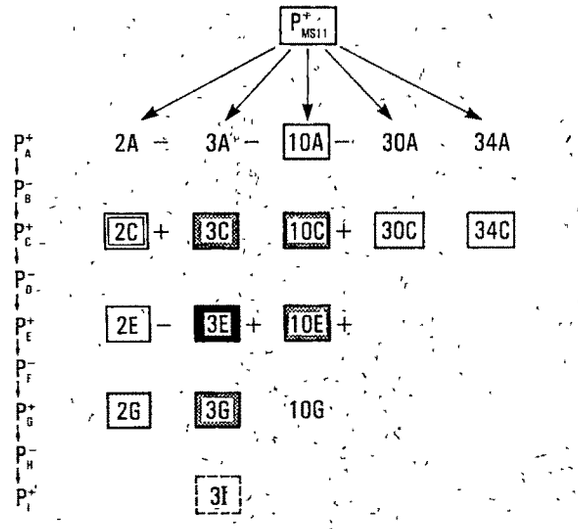


Fig. 2 Left-hand side, a schematic representation of the protocol used for deriving the lines of pilus variants of strain MS11. From the original MS11 strain, which is P^+, several independent P^+ colonies were randomly picked. (Piliation can be scored as differences in colony morphology on a plate.) Each P^+ isolate is the start of a line, each line is given a number and each constitutes the A-series, all of which are identical to the MS11 progenitor. P^- revertants are then randomly picked from each P^+ A-series. These have undergone a P^+ to P^- phase switch once and are now in the B-series. Subsequently, P^+ backswitchers can be isolated from the different lines in the B-series; they now belong to the C-series. This procedure was used to obtain lines of variants which have undergone the phase switch several times. All manipulations were done in a PII^-, or opacity$^-$, background. Piliation phenotype is given as P^+ or P^-, for piliated or non-piliated variants, respectively. The subscripts A–I denote the position within the series to which each variant belongs. The right-hand part of the figure is a schematic representation of the pilus sequence information obtained from the P^+ variants from lines 2, 3, 10, 30 and 34. Each variant sequence is denoted by the style of the box surrounding the strain number. For example, the progenitor P^+_{MS11} (at the top of the figure), 10A, 30C, 34C and 2E all express the same pilin gene variant. 2C, 3E, 2G and 3I each express a unique gene variant. Those strains which produce pilin antigenically different from that of the progenitor are indicated by +, next to the boxes, while those strains which produce pilin identical to that of the progenitor are marked by −. The absence of + or − indicates that the immunological identity of the pilin from these variants has not been determined. Immunological identity is determined by immunodiffusion[24] using crude pili preparations and antiserum directed against pili from the MS11 progenitor.

Recently, an epidemic of gonorrhoea occurred[19] whose origin could be traced to a single source and whose isolates were shown to be members of the same strain (P. F. Sparling, manuscript in preparation). We have chosen isolates from three patients for our sequence studies. One series (ML1, 2, 3) was obtained from a patient who was repeatedly infected between successful courses of antibiotic therapy. Two other isolates, LP1 and WG3, were from different patients. Our data (Fig. 1*c*) show that pilin antigenic variation did occur *in vivo* during the epidemic. It also occurred in the ML isolates obtained from a single individual at various times. However, because antibiotic therapy was instigated between each bout of infection and no sera were available from this patient, we cannot determine whether pilus antigenic variation in these cases was caused by the selective pressure exerted by the host immune response or whether these individuals were infected by partners carrying an already variant strain. However, it is clear that a single gonococcal source expressing one pilus type can, during the course of the epidemic, give rise to isolates which now produce variant pili. The C, SV and HV regions defined by the MS11 sequences are also observed in these variants. Thus, the events contributing to the antigenic variation seen *in vitro* accurately represent those which control variation *in vivo*.

The P^+ to P^- phase switch involves deletion of pilin sequences from one of the two expression loci[5]. In P^- variants 4.1B, 5.1B and 6.1B, we have observed a deletion from only one locus[5]. These variants are non-piliated, and their remaining intact expression site is not transcribed (data not shown). However, *Escherichia coli* harbouring clones of these intact expression sites make pilin transcripts, suggesting that phase variation also involves a switch-induced regulator. Examination of the pilin transcripts derived from *E. coli* shows that sequence variation in 4.1B, 5.1B and 6.1B has occurred during the P^+ to P^- switch (Fig. 1*b*). The new gene variants have all the characteristics observed for the other P^+ backswitchers and, therefore, probably encode functional pilins. These observations suggest that pilus phase and antigenic variation are closely, if not obligately, linked.

Our data strongly suggest that the pilus of *N. gonorrhoeae* can undergo extensive antigenic variation. The mixing of *SV* with *HV* gene segments predicts that the silent loci will contain truncated pilin sequences. Thus, antigenic variation would involve a multiple-step recombination event in which sections of the pilin gene are joined successfully. The generation of new pilin variants may involve those mechanisms postulated for the generation of immunoglobulin diversity (see refs 20, 21), that is, somatic mutations in the silent copies, unequal crossing-over and/or imprecise joining of *SV* and *HV* gene segments (see Fig. 1*a*, line 3). Also, the tendency of MS11 derivatives to express certain variant sequences could be explained by a mechanism similar to that governing the preferential use of certain joining sequences in pre-B-cell immunoglobulin heavy-chain switching[22]. Pilin sequences close to those involved in the initial pairing event between the silent and experession loci would be expressed preferentially.

Whether other bacterial pili undergo extensive antigenic variation is unknown. The *Moraxella bovis* chromosome contains multiple pilin gene sequences (C. Marrs, personal communication). *E. coli* K-88 pilus[23] and P-specific pilus (S. Normark, personal communication) variation involves in-frame insertions and deletions as well as single-base changes in specific regions of the structural gene. Thus, the mechanisms used by bacteria to generate immunologically variant surface proteins may be very similar, if not identical.

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The nonlinear Poisson–Boltzmann equation

SPITZER[1] has reminded us that the nonlinear Poisson-Boltzmann equation (NPBE) contains a logical inconsistency. An early incisive analysis of this inconsistency was given by Onsager[2] 50 years ago. Unhappily, more recently, a considerable confusion[1,3] has accrued concerning the nature and consequences of the inconsistency. Spitzer's[1] statement that "the NPBE violates the important electrostatic theorem of additivity of electrostatic potentials" is simply incorrect, and his recommendation that "the linearization of the NPBE is desirable, in principle" is entirely inappropriate.

First we consider the nature of the inconsistency. Onsager[2] was concerned with the microscopic NPBE as it is used to describe the potential generated by a single ion in solution that is screened by the response to it of all the other ions in the electrolyte. As Onsager makes clear, the inconsistency derives from the replacement of the potential of mean force, $w_j(r)$, in the correct Boltzmann relation (his equation (21); ref. 2), by the simpler electrostatic potential energy $e_i\psi_j(r)$ to obtain what is finally an inconsistent Boltzmann relation (his equation (13); ref. 2). The replacement is made in order to write a one-particle NPBE. Note that neither the Poisson equation nor the superposition theorem of electrostatics as such is germane to the inconsistency studied by Onsager. The confusion is compounded by a supposed connection of the linear superposition of electrostatic potentials to a linear dependence of space charge ρ on potential ψ (refs 1,3). In actuality, the linear superposition theorem of electrostatics[4] holds for an arbitrary distribution of space charge $\rho(r)$. Thus, in the analogue to the NPBE appropriate to Fermi–Dirac statistics, namely, the Thomas–Fermi equation[5], $\rho \propto \psi^{3/2}$. For space-charge-limited currents in a planar vacuum diode[6], $\rho \propto \psi^{-1/2}$; in the analogous solid-state diode[7], $\rho \propto \psi^{-1/3}$, and so on.

We next take up the consequences of the inconsistency. As noted above, the inconsistency refers directly to the microscopic NPBE. However, it is important to note that most of the literature on the NPBE since World War II, including the Spitzer letter[1], is not concerned with this equation but rather with the macroscopic NPBE. This latter equation describes the spatial dependence of the average potential generated by relatively large, charged surface(s) in contact with the electrolyte. This local average potential is what remains after all microscopic fluctuations have been averaged out. For the macroscopic NPBE a new phenomenon comes into play when linearization finally fails (that is, at high surface-charge concentrations), which is the formation of a counter-ion condensate close to the surface[8]. Fixman[9], in a theoretical study of the problem within the framework of statistical mechanics, pointed out that substantial errors deep in the condensate lead to far smaller errors outside the condensate. He aptly described this phenomenon as "the buffering action of 'the condensed phase'" (ref. 9). This very pronounced decoupling of a large surface charge from the 'distant' bulk electrolyte is also a direct mathematical consequence of the NPBE, as shown for planar geometry by Verwey and Overbeek[10]. The buffering action of the condensate, as predicted by the NPBE, is even more striking for cylindrical and spherical geometries, as shown by numerical studies of this equation by Lampert and Martinelli (to be published elsewhere). Fixman's numerical calculations led him to conclude that the NPBE is acceptable[9] up to local, condensate concentrations as high as 2–3 M, provided that the bulk salt concentration does not exceed ~0.1 M, which happens to be, approximately, that for physiological saline. It is also relevant here and perhaps not widely known that some 15 yr after his incisive criticism of the microscopic NPBE, Onsager used the macroscopic NPBE to calculate 'The effects of shape on the interaction of colloidal particles'[11].

Spitzer[1] alludes to the difficulty of testing experimentally the Gouy–Chapman–Stern (GCS) model for incorporating adsorption into double-layer theory, as constructed with the NPBE. If that is the situation in electrochemistry and colloid chemistry, then matters are improved considerably in biophysical studies of charged phospholipid bilayer membranes immersed in an aqueous solution. McLaughlin *et al.*[12] showed, by adding small concentrations of divalent cations to a host solution of monovalent ions, that the observed shift in membrane potential disagreed strongly with predictions of linearized PB theory, but agreed adequately with GCS theory. In a recent critique of the work of several groups using various experimental techniques, McLaughlin concludes[13] "that fortunately, the GCS theory appears to be a good first approximation for describing the adsorption of cations to bilayer membranes".

M.L. is on medical leave from Princeton University.

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Homologies in the avian tarsus

MCGOWAN has recently presented evidence from the avian tarsal joint for a wide taxonomic separation between ratite and carinate birds. He implies that the pretibial bone of carinates is formed by ossification of a portion of the cartilaginous precursor of the calcaneum (a "calcaneal spur"), while in ratites the comparable structure is an ascending process of the astragalus. However, the youngest specimen he described was a 12-day-old quail embryo. Examination of a large series of cleared and stained chicken embryos shows that the 7-day-old embryos have separate cartilaginous calcania, astragali and tibiae, but no ascending processes or pretibial bones. Eight-day-old chicken embryos show a separate triangular pretibial chondrification above the calcaneum which does not appear to be part of either the astragalus or the calcaneum. On day 9 the pretibial, astragalar and calcaneal cartilages fuse. The pretibial cartilage appears to fuse with both the calcaneal and astragalar cartilages. Only in the fused state could one mistake the pretibial chondrification for an extension of the calcaneum. By day 17 the pretibial bone (calcaneal spur of McGowan[1]) has begun to ossify; at this point it is the only tarsal bone undergoing ossification (Fig. 2*b–d* of ref. 1). Thus, the pretibial bone is the last carinate tarsal element to chondrify and the first to ossify.

McGowan[1] shows that the first bone to ossify in the tarsal complex of ratites is the "ascending process". We believe that this concordance between the ossification of the ascending process of ratites and the pretibial bone of carinates would be hard to explain if they are not the same bone. This is especially true when we consider that the calcaneum is the last bone to ossify in McGowan's preparations.

McGowan[1] describes the "ascending process" of ratites as an ossification with a disk-like base and separate from the astragalus. He describes an early stage in the development of the distal end of the tibiotarsus as comprising a medial ossification and a lateral cartilaginous area. The lateral cartilaginous area has a dorsal spur which he considers to be "homologous with the precursor of the carinate pretibial bone". The enlargement of the astragalus found in some ratites might be a derived character state related to neoteny. The earlier ossification of the astragular portion of the joint may increase the relative

importance of the astragalus in highly neotenic birds. Despite McGowan's statements, the pretibial bone seems to represent both a separate chondrification and ossification in carinates; we believe that this is also the case in ratites and that the difference (if it exists) between ratites and carinates is the result of varying amounts of overlap with the calcaneum and the astragalus. Because of the extreme lateral placement of the pretibial bone in some carinates, it may be confined to the region above the calcaneum (see Fig. 11 of Martin *et al.*[2]), and after the chondrification has fused with the calcaneum it may resemble a 'spur' of that bone. On the other hand, falcons have the pretibial bone very centrally situated as in the ostrich, and most, if not all, of the fusion of the distal end of the pretibial bone is with the astragalus (Fig. 179 of ref. 3). A juvenile swan, *Cygnus buccinator* [Kansas University 79260], shows a pretibial bone ossifying separately from the calcaneum and partially overlapping the astragalus; except for its slightly more lateral position, it corresponds to McGowan's description of the "astragalar process of the ostrich" which is a "well-ossified ascending process, expanding distally into a disk of bone".[1] We believe that the present evidence indicates that ratites and carinates have a homologous ossified ascending process (pretibial bone) separate from both calcaneum and astragalus, but that the position of this process may vary. We believe that, as originally suggested, the pretibial bone is a neomorph ossification providing a derived character for birds. The late chondrification of this element supports such an interpretation. The Mesozoic birds (*Archaeopteryx*, *Enaliornis*, *Baptornis* and *Hesperornis*) have the pretibial bone laterally placed and fused to the calcaneum as in carinates, but not as in theropods (Fig. 1*b* of ref. 1), and we would therefore caution against arguing that the pretibial bone of carinates is a derived character state within the Class Aves.

To view these results as in any way confirming the theropod origin of birds would be to beg the question. The only way McGowan could conclude that the ratite tarsal condition is more primitive than that of the carinates is by already assuming that theropod saurischians are the out-group most proximate to birds; this approach does not address the fact that pseudosuchians and crocodilians are also contested nearest out-groups[4-7]. McGowan's own interpretation of his material would indicate that the bulk of the known birds (the carinates) do not have a theropod-like ascending process.

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MCGOWAN REPLIES—According to Martin and Stewart, the ascending process of the carinate tarsus commences as a chondrification independent of the cartilaginous astragalus and calcaneum. This subsequently ossifies as a separate element, and a similar ontogeny is believed to occur in ratitites. The pretibial bone thus formed, a new element which is not homologous with the ascending process of theropods, provides a shared derived character for birds.

My X rays of juvenile ostriches (ref. 1, Fig. 3*b*–*c*) confirmed Huxley's[2] belief that ratites, like theropods, have an ascending process that is continuous with the bony astragalus. Whether this process develops in ratites as an integral part of the astragalus, or, as Martin and Stewart contend, as an independent chondrification, and ossification, can only be determined embryonically. The earliest ratite embryos available to me were close to hatching, at which stage there is a prominent bony ascending process expanded distally into a disk of bone embedded in cartilage. That this entire structure is the astragalus, and not an independent ossification, is confirmed by examining early ostrich and emu embryos[3]; these show that the cartilaginous astragalus is drawn up into an ascending process, as Parker[4] described for the kiwi, and this subsequently ossifies to give the bony ascending process. Ossification commences at the tip of the cartilaginous process, spreads distally and eventually extends across the entire width of the cartilaginous astragalus, as shown in my X rays of juveniles. Ossification of the corresponding process in carinates is confined to the process itself (ref. 1, fig. 2*b*–*e*).

I have examined swan embryos (*Cygnus olor*), and find that the pretibial bone develops as an ossification of a cartilaginous spur, as described for other carinates[1]. Inasmuch as the pretibial bone ossifies separately from the bony calcaneum, my observations[1] do not conflict with those of Martin and Stewart, and I believe that if they examined later stages of swan development they would find that the pretibial bone eventually fused with the calcaneum, as it does in the chicken (ref. 1 fig. 2*f*).

One area of common ground I now share with Martin and Stewart pertains to the homology of the ascending process in ratites and carinates, but our conclusions differ radically. An examination of early duck embryos (days 8–11 of incubation) revealed a dorsal cartilaginous process from the lateral aspect of the astragalus, similar to that seen in early emu embryos (15–20 days)[3]. This early association with the astragalus is soon obscured by the fusion of the cartilaginous astragalus and calcaneum, and from then on the process develops in close association with the calcaneum, as described previously[1]. Similar observations for gulls lead me to believe that the carinate pretibial bone is an astragalar derivative, supporting its homology with the ascending process of ratites.

Ratites, like theropods, have a bony ascending process that is continuous with the astragalus, but carinates have a derived condition where the process fuses with the bony calcaneum. Martin and Stewart deny that the (predominently lateral) carinate pretibial bone is a derived character within birds, noting that the ascending process is laterally placed in the earliest bird, *Archaeopteryx*, but this is the case in theropods[1]; the significant point is not its orientation but whether it is continuous with the astragalus or the calcaneum.

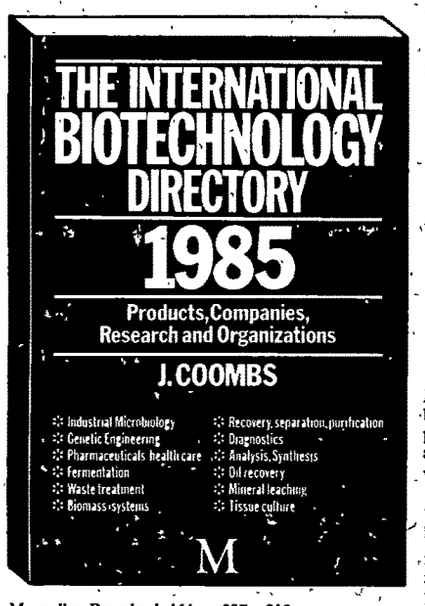
My conclusion that the ratite tarsus is more primitive than that of carinates does, of course, hinge upon theropods being phylogenetically closest to birds, a position I took at the outset with my reference to Ostrom's work[5]. Citing a review by Benton[6], Martin and Stewart point out that pseudosuchians and crocodilians have also been proposed as the nearest avian out-groups. Their own bias for the crocodilian hypothesis is contained in a paper by Martin *et al.*[7]. Note, however, that Walker[8], the leading proponent of the crocodilian hypothesis, has recently reassessed the evidence, concluding that the hypothesis for a common ancestry of crocodiles and birds (above the thecodont level) should be rejected.

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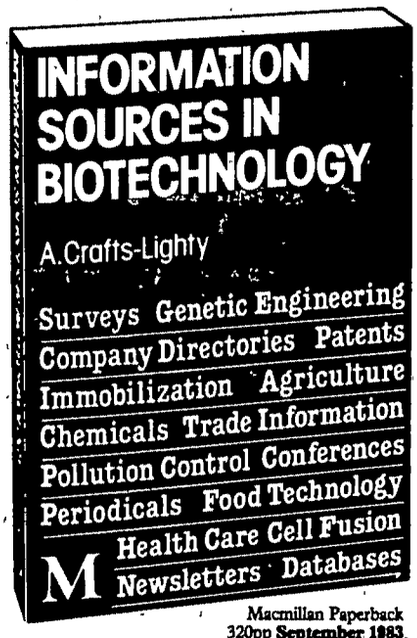
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In the aftermath

P.M. Kelly

Nuclear Winter: The Human and Environmental Consequences of Nuclear War.
By Mark A. Harwell.
Springer-Verlag: 1984. Pp.179. DM 54.

WITH a fresh spate of books on the "nuclear winter" about to reach the marketplace, it is appropriate that Mark Harwell's sober and detailed account of the biological aspects of the subject should be available first. It deals not only with the effects of a possible nuclear winter but also with the more direct, and inevitable, blast, fire and radiation damage caused by nuclear explosions. The book is based on a technical support document produced, in collaboration with other biologists and ecologists, for the conference "The World After Nuclear War".* It was at this meeting, held in Washington DC in late 1983 and the culmination of a series of exercises designed to assess the scientific validity of the nuclear winter theory, that the first detailed findings concerning the possibility of widespread cooling and concomitant damage to the biosphere were announced.

Shortly after the Washington conference, two summary papers were published in *Science* (**222**, 1,283-1,300). One dealt with the atmospheric component of the theory; the other with the biological aspects. To those aware of developments only through these papers or through brief, sometimes opinionated, accounts in the scientific press and the news media, there seemed to be a lot of fuss about a rather speculative notion. This was a mistaken impression. A great deal of carefully documented background research was available to anyone who cared to look for it, as Harwell's account of the rigorous analysis supporting the biologists' case clearly demonstrates.

But how certain is the atmospheric scientists' case? Since 1983, the principal conclusion of the initial investigators, the *possibility* that a nuclear war would cause a major climatic excursion — an abrupt, marked but short-lived change — has been confirmed by other researchers and by two review projects mounted by the US National Academy of Sciences (NAS) and the Royal Society of Canada (RSC). There are many areas of uncertainty, and the NAS and RSC reports carefully define them, but the *risk* of a nuclear winter now seems undeniable.

The NAS committee concentrated on the atmospheric aspects of the problem, but noted that the impact of the climatic excursion on those who survived the initial effects of a nuclear exchange and on the biosphere as a whole "deserves careful independent study". Progress on the physical side has been rapid since the Washington conference. Many points in the original investigations have been clarified and the computer simulations of the nuclear winter effect have been substantially improved. On the biological side, progress has been slower. In part, this has been an unfortunate by-product of the understandable reluctance of atmospheric scientists to provide the type of detailed information on climate needed for rigorous impact assessment. Computer models of the climatic system tend not to be sufficiently accurate on the spatial and time scales of concern.

One solution to this problem is to use scenarios for possible, in the sense of physically plausible, nuclear winter climates in much the same way as atmospheric scientists assessing the climatic effects use a range of strategically plausible nuclear war scenarios. This is Harwell's philosophy. While concentrating on the consequences of a nuclear exchange in the 5,000 megaton range, the book provides the data necessary to assess the implications of any scale of exchange (or nuclear winter).

Beginning with the development of a nuclear war scenario, which provides the input data for the "consequence analyses", the direct effects of blast, fire and fallout (both local and global) are considered first. This area has been addressed in depth in a number of studies, and Harwell's main contribution is to quantify the impact in terms of casualties and damage to the biosphere in the United States. This section is an excellent introduction to the literature on these aspects of the post-nuclear world. The concept of the nuclear winter is then introduced and a detailed discussion of its effects on the biosphere forms the main part of the rest of the book.

In considering the consequences of the principal assaults — temperature and sunlight reductions, radioactive fallout, and the loss of technical and social support for agriculture — Harwell assesses the net impact on the human population and on the flora and fauna, the food supply for the survivors. Using numerical models whenever available, the impact is quantified in terms of human casualties and food availability. The emphasis is, for the most part, on the northern middle latitude combat zone, and grassland, forest, freshwater and estuarine ecosystems are subject to particular scrutiny. Inevitably, some conclusions are more speculative than others, but the reader is given all the information necessary for a personal assessment of the uncertainties.

In the midst of this description of the gross distortion and near-destruction of the ecology of the combat zone, some surprising insights into possible diets emerge. Ducks, for example, through metabolic generation of heat, could survive temperatures of −40°C for a couple of weeks (in the unlikely event that enough food was available). The analysis ends with a brief section entitled "Recovery Processes". I find the use of the word "recovery" in this context somewhat inappropriate. "Coping" and "adaptation" seem more suitable terms given that many aspects of the pre-nuclear environment (and civilization) might never be recovered.

Harwell also discusses possible psychological responses to the devastation that would occur, but wisely steers clear of dogmatic statements. Similarly, the nature of the fragmented post-nuclear social structure is covered but no firm conclusions are reached. These areas have yet to be considered in any depth but, as the author observes, such factors might well be crucial in determining whether or not use could be made of what limited food supplies would be available. Of particular concern, on the individual level, is the prospect of "psychic numbing" through which unacceptable aspects of reality are shut out and which could severely handicap the ability to survive in the aftermath of nuclear war. Would any form of social organization, whether voluntary or enforced, be possible in such circumstances?

The final section, "Summary of Consequences", is a sobering account of the conclusions of the study: the state of the world after nuclear war. It left one question uppermost in my mind. What can be done to provide viable civil defence and support for those who survived the initial attacks in the face of the multiple hazards of the post-nuclear world?

Harwell's stated intention is "to provide a more comprehensive view of what the world would be like to the immediate survivors of a nuclear war" (p.xv). With the assistance of some (rather harrowing) imaginative effort on the part of the reader, a portrait of the world after nuclear war does indeed emerge from the text. But the main strength of the book lies in the clear, dispassionate accumulation of the technical information needed to assess the implications of the use of nuclear weapons. Harwell has produced an exhaustive, wide-ranging and, in many respects, pioneering treatise, which will be an invaluable reference tool for anyone concerned about nuclear issues. □

*The book resulting from the conference, *The Cold and the Dark*, has been published in paperback in Britain by Sidgwick & Jackson under the title *The Nuclear Winter: The World After Nuclear War* (price £5.95).

P.M. Kelly is a Senior Research Associate in the Climatic Research Unit, University of East Anglia

Fundamentals of the fourth state

C.N. Lashmore-Davies

Handbook of Plasma Physics, Vols I and II.
Edited by A.A. Galeev and R.N. Sudan.
North-Holland: 1983/1984. Vol. I pp.751, $180.75, Dfl.425. Vol. II pp.850, $190.50, Dfl. 495.

AT A TIME when the growth in the publication of papers on plasma physics shows no sign of slowing, the appearance of two volumes which set out the basic ideas of the subject and provide summaries of all the main lines of research is particularly welcome. These two books are worthy successors to the Leontovich reviews and the Simon and Thompson series, and have the advantage of being published together. They contain 33 articles, 17 of which were contributed by 19 authors from the United States, 15 by 12 authors from the Soviet Union and one by two Japanese authors.

Volume I deals with the principles of plasma physics, and is divided into four parts. The first covers the motion of charged particles in electromagnetic fields, atomic collision processes relevant to plasmas and the various radiation phenomena which can occur in plasmas, concluding with an illuminating discussion of the different modes of description of plasmas for low frequency phenomena and an account of the subtleties of collisional transport in magnetically confined plasmas.

The remaining parts of Vol. I deal with the collective nature of plasmas. An important feature of a plasma is the great variety of waves it can support. The nature of the linearized wave motions allowed by ideal MHD and the effect of allowing for a distribution of particle velocities are both described. Also included are treatments of fluctuations, geometrical optics and the conversion of one linear wave into another in an inhomogeneous plasma.

When there is a reservoir of free energy in a plasma some of these wave modes can become unstable resulting in a great variety of instabilities. The many aspects of plasma instabilities from ideal and resistive MHD, the kinetic instabilities driven by the pressure gradient of a confined plasma, non-thermal particle distributions in velocity space and whether a given instability behaves like an amplifier or an oscillator are all systematically considered. An inevitable consequence of instability is that a plasma moves into a state in which its motion is non-linear. Since a plasma can support so many different wave motions it has been conjectured that a state of weak turbulence might exist, and in the final part of Vol. I the concepts behind this theory are lucidly set forth.

Volume II follows on from its companion, containing Parts 5–9, and is mainly concerned with non-linear consequences. In many practical situations an enhanced level of long wavelength Langmuir waves can be generated (for example by an electron beam or a laser) which, according to weak turbulence theory, should relax to a coherent Langmuir oscillation. The resolution of this apparent paradox and the existence of three-dimensional, localized, high-frequency electric fields are beautifully described by the discoverer of the phenomenon. Also discussed are the spectra resulting from weak turbulence and the numerical modelling of strong Langmuir turbulence.

In hot plasmas the effect of binary collisions is often insufficient to explain various transport processes. The simplest of these phenomena is the dissipation of a current in an unmagnetized plasma, due to the generation of waves rather than inter-particle collisions, although even this apparently simple example is not fully understood. Other examples considered are the dissipation of intense laser light and finite amplitude waves in plasmas through the non-linear generation of waves. Similar but far more complicated situations occur in the cross-field diffusion of particles and energy in magnetically confined plasmas. This complex issue is surveyed, together with an attempt at a comprehensive statistical description of plasma turbulence. These effects are generally referred to as anomalous although in many applications they represent the normal situation rather than the exception.

Part 8 consists of two excellent reviews on the diagnostic methods upon which the subject of plasma physics rests; the first is concerned with radiative techniques and the second with the methods used in hot magnetically confined plasmas, complementing those discussed in the first article. Volume II also contains an informative account of the methods of particle simulation by computer and a thorough discussion of the equilibrium and stability of non-neutral relativistic electron beams and their interaction with plasma.

Together, the books satisfy the aims of the general editors in encompassing most of the present-day knowledge of plasma physics, albeit from a user point of view. They are not suitable for someone requiring an introduction to the subject; although they take the reader to the frontiers of current research, they do not cover the general topic of the foundations of plasma kinetic theory nor do they contain more than a very brief account of the fundamental and fascinating subject of Landau damping. But those actively involved in research on plasma physics will find these volumes invaluable, as will experienced workers in other fields wishing to acquaint themselves with a particular aspect of the subject. □

C.N. Lashmore-Davies is at the Culham Laboratory, Abingdon, Oxfordshire (Euratom/UKAEA Fusion Association).

Off the record

John Parkington

From Hunters to Farmers: The Causes and Consequences of Food Production in Africa.
Edited by J. Desmond Clark and Steven A. Brandt.
University of California Press: 1984. Pp.433. $63.25, £52.25.

AFRICA, as one of my mentors once sagely remarked, is a very big place. It encompasses environments as diverse as the sand seas of the Sahara and Namib, the thirstlands of the Kalahari and Sahel, the heath communities of the Cape and Mahgreb, the savannahs of Serengeti and the tropical forests of Zaire. To understand the social and ecological events leading to and following from the transition from food gathering to food production over such a vast and varied area is a Herculean task. Yet archaeologists are nothing if not optimists, and the goal of *From Hunters to Farmers* is nothing less than this. A collection of 30 papers, it sets out to provide a synthesis of current knowledge and is, in effect, the published proceedings of a symposium held in Los Angeles in 1978.

The volume is divided into four sections, the first consisting of three general papers which present a depressing but honest picture of how little we know about past climates, past population distributions and the circumstances surrounding the adoption of food production in Africa. Most of the contributions fall into the second section and are regional summaries of greater or lesser geographical scope. I wondered at this point what distinguishes papers that are quoted long after publication. Do they contain dazzling theoretical insights or essential primary observations? Or do they reflect the spirit of their times? Regional reconstructions based on modest but accumulating data sets are almost by definition transitory phenomena. We all write and then rewrite them.

To my mind the most interesting section is the third, in which the effects of the acquisition of domestic plants and animals by Kalahari San are documented. These three papers combine empirical observations with theoretical perspectives and will probably have longer lives than the regional accounts. Because they contribute to the realization that the San are, and have been, far from isolated from agriculturalists and pastoralists, they will have immediate im-

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pact. The only paper in the fourth section describes the difficulties involved in palaeodemographic reconstruction and provides a good overview of what we would like to know but usually find hard to document archaeologically.

As the editors undoubtedly expected, this book is more valuable in what it shows up rather than what it shows off. The contributors have demonstrated that we know very little of the climatic and environmental circumstances that preceded the appearance of domesticates, that the preservation of reasonable samples of plants and animal bones in archaeological contexts is variable but generally poor, that we have very inadequate dating of critical changes, and that models for understanding *how* and *why* people shift into agriculture or pastoralism are only now emerging. More seriously we see an archaeological record that is so residual and fragmentary that it becomes malleable and an easy victim for a predatory hypothesis. In these circumstances it is extremely tempting to "fit" the archaeological evidence onto superficially more substantial linguistic histories, climatic reconstructions or, more commonly, ethnographic models.

Several of the contributors (particularly but not only Stahl, Ambrose, Klein, Gifford-Gonzalez, and McIntosh and McIntosh) make the point that archaeological observations have to generate some internal and independent consistency. This comes only from sustained multidisciplinary research into regional sequences of events, something that few parts of Africa have so far enjoyed. What is known is largely in this book. Although I would liked to have read more discussion of theory and method, to have seen more concern with social than ecological issues and to have heard more talk of process, I recognize and recommend here a valuable summary of our current understanding of "the causes and consequences of food production in Africa". □

John Parkington is in the Department of Archaeology at the University of Cape Town.

Search for hay in a needlestack

Harry Hertz

Organic Trace Analysis.
By K. Beyermann.
Ellis Horwood/Halsted: 1984. Pp.365. £35, $74.95.

THE search for trace organic substances in complex inorganic and organic matrices is one of the most challenging problems facing chemists. It pervades all aspects of our lives: the water we drink, the food we eat, the environment in which we work and live, the quality of our manufactured goods and many aspects of our day-to-day health. Klaus Beyermann's *Organic Trace Analysis*, a generally very readable translation of the original German edition, is the first comprehensive description of this developing field of analytical chemistry.

Beyermann has aimed the treatise at a diverse audience, from the professional analyst to "science administrators, biologists, politicians [and] forensic experts". While this is an admirable goal, it has led to occasional identity crises and uneven presentation of various topics. Nevertheless, if readers are willing to pick and choose what to read they will find that the book does present an effective overview of the field.

An outstanding feature is the references, some 3,000 of them. Much of the information on previous studies is reported in tables, permitting easy access to the literature. Furthermore, all the tabular entries (as well as the text itself) are extremely well indexed in the back of the book, making this an outstanding rapid-reference volume. The order of presentation follows the logical sequence of analysis: from general aspects through sampling, sample handling, component separations and detection.

There are, however, some flaws. The book as a whole is less evaluative of the literature than one would like, the author usually choosing to cite relevant works without any commentary. Section 2.4, which deals mainly with statistics, was not contributed by Beyermann and is not as well written as the rest of the book. The level of detail in this introductory section is perhaps excessive, while the references here are more parochial than they are elsewhere; this could dissuade some readers from continuing. And the topic of sampling and storage is treated in a manner which belies the problem in this area of assuring analyses which accurately represent the native (*in situ*) concentration of the species of interest.

As with any rapidly evolving field, by the time a book has been through the publication mill new discoveries have appeared and contributed to the science being discussed. Here consideration of such techniques as fast atom bombardment in mass spectrometry and the impact of monoclonal antibodies on immunoassay is not included, and thus the book already has a somewhat dated look in parts. For all that, the publication of *Organic Trace Analysis* is undoubtedly a milestone in analytical chemistry. It is a handy reference volume for the practising analyst and a valuable introduction for the generalist who is prepared selectively to read sections of the book. □

Harry Hertz is Director of the Center for Analytical Chemistry at the US National Bureau of Standards, Gaithersburg, Maryland.

Angles on aphids

M. Llewellyn

Aphid Ecology.
By A.F.G. Dixon.
Blackie/Methuen: 1985. Pp.157. £17.95, $39.95.

APHIDS are specialist phloem-feeding insects that have received world-wide attention, both because of their importance as agricultural pests and because of the intricacy and diversity of their biology. A book on aphid ecology by a leading research worker in both fields will thus attract attention from a number of angles. Satisfying all potential readers — especially in a mere 136 pages of text — is a tall order, but Professor Dixon has succeeded remarkably well.

This book is as much about the biochemistry, physiology, genetics and evolution of aphids as about patterns and processes of ecology; it contains not only information about a specialist taxon of insects but constitutes an integrated approach to ecology which, although often talked about, is rarely attempted. Many areas are excellently reviewed. The accounts of such topics as "galling", polymorphism, genetic change, sexual morph production and host-alternation are a delight, and a large literature on the population dynamics of several species has been crystallized into a fascinating and informative chapter. A few subjects do not catch the imagination, however; the section on host plant finding and nutrition, for example, is not particularly convincing. The book is peppered with the author's own ideas and theories — on aphid size, ovariole number, reproductive strategies and the aphid fauna of the tropics — which may not find universal approval but which will stimulate the work of aphid biologists for some time to come.

There are well over 100 graphical figures, as befits an experimental, quantitative approach to ecology, but I often found them of little value because of the lack of adequate background information. The condensed treatment of some topics, in particular reproductive strategies and aspects of migration, may baffle the general reader, and it is a pity that the space taken by some figures was not used to extend consideration of difficult concepts such as these.

After reading this book few will disagree that a broad approach to ecology, drawing upon diverse areas of biology, is anything other than desirable. Here it has resulted in a challenging text which surely heralds the demise of the specialist and blinkered approach to ecology so prevalent over the past 30 years. □

M. Llewellyn is a Lecturer in the Department of Biology at Queen Elizabeth College, University of London.

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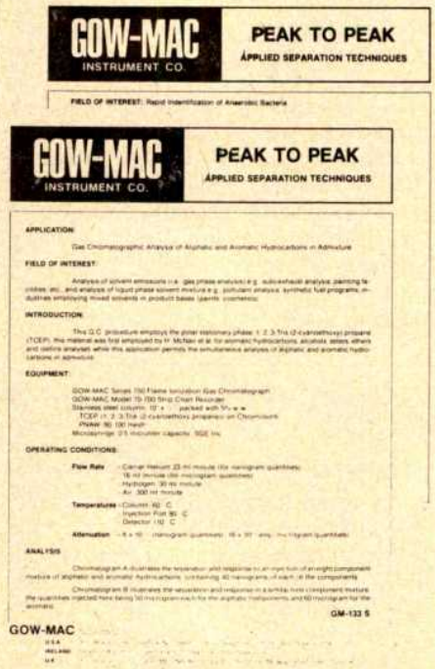
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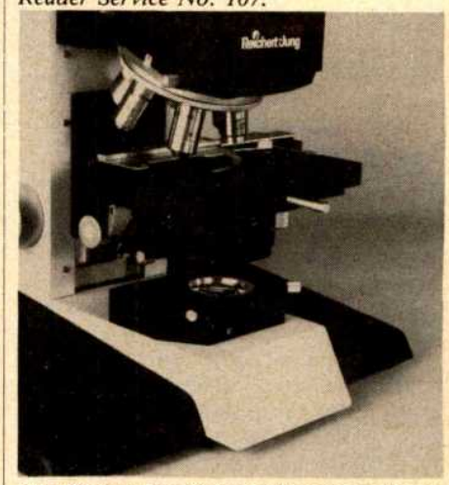
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May meetings

14–16 May 1985, **International Conference and Exhibition on Biotechnology**, Boston, USA (B. Burris, Cahners Exposition Group, 7315 Wisconsin Avenue, PO Box 70007, Washington, DC 20088, USA).
14–17 May 1985, **International Society for Neurochemistry Symposium on Exitatory Amino Acids**, Bournemouth, England (Dr P.J. Roberts, Department of Physiology and Pharmocology, University of Southampton, Southampton SO9 3TU, UK).
21–24 May 1985, **Conference on Lasers and Electro-optics**, Maryland, USA (Optical Society of America, 1816 Jefferson Place, N.W. Washington, DC 20036, USA).
22–24 May 1985, **International Congress on Tissue Integration in Oral and Maxillo-Facial Reconstruction**, Brussels, Belgium (Prof. D. van Steenberghe, Dept. of Periodontology, Faculty of Medicine, Catholic University Leuven, Kapucijnenvoer 7, B-3000 Leuven, Belgium).
24–25 May 1985, **International Meeting on Genetic Polymorphism and Fertility in Mammals**, Rome, Italy (Prof. G. Montalenti, Accademia dei Lincei).

June meetings

3–6 June 1985, **Karlsruhe International Conference on Analytical Chemistry in Nuclear Technology**, Karlsruhe, FRG (Prof. Dr Hans J. Ache, Kemforschungszentrum Karlsruhe GmbH, Institut fur Radiochemie. Postfach 3640, D-7500 Karlsruhe 1, FRG).
4–6 June 1985, **Symposium on Artificial Intelligence**, COGNITIVA 85, Paris, France (Marie-France Chicanne, CESTA, 1 rue Descartes 75005 Paris, France).
6–8 June 1985, **International Conference on Biological Reactive Intermediates**, University of Maryland, USA (Dr R. Snyder, Dept. of Pharmocology and Toxicology, College of Pharmacy, Rutgers University, Piscataway, NJ 08854, USA).
10–14 June 1985, **International Conference on Computers in Chemical Research and Education**, Oberbayern, Germany (Dr J. Brandt, Institut Für Organische Chemie, Technische Universität München, Lichtenbergstr. 4, D-8046 Garching, FRG).
14 June 1985, **International Seminar on Immunopathology of Rheumatic Diseases**, Montpellier, France (Prof. J. Sany, Service d'Immuno-Rhumatologie et Readaption Fonctionelle, Centre Gue-de-Chauliac, Hôpital Saint-Eloi, 34059 Montpelier, France)
17–20 June 1985, **International Conference on Developments in Analytical methods in the Pharmaceutical and Biomedical Sciences**, Camerino, Italy (Prof. I. Antonini, University of Camerino, 62032 Camerino, Italy).
19–21 June, **International Symposium on Molecular Aspects of Neurobiology**, Florence, Italy (Dr E. Folco, Fondazione Giovanni Lorenzi, Via Montenapoleone, 23-20121 Milan, Italy).
23–27 June 1985, **International Conference on Modern Trends in Activation Analysis**, Copenhagen, Denmark (Dr K. Heydom, Riso National Laboratory, Postbox 49, Dk-4000, Roskilde, Denmark).
25–28 June 1985, **International Conference on Surface Engineering**, Brighton, UK (The Conference Office, The Welding Institute, Abington Hall, Abington, Cambridge CB1 6AL, UK).
26–28 June 1985, **International Congress on Lasers in Medicine and Surgery**, Bologna, Italy (Medicina Viva, Viale dei Mille, 140 43100 Parma, Italy).

July meetings

30 June–5 July, **International Conference on Proteins of Iron Metabolism**, Villeneuve d'Ascq, France (Prof. G. Spik, Universite des Sciences at Techniques de Lille I, Laboratoire de Chimie Biologique, F-59665 Villenueve d'Ascq Cedex France).
1–3 July 1985, **International Symposium on Physico-Chemical Properties and their Role in Environmental Hazard Assessment**, Canterbury, UK (H.L. Bennister, 174 Beverley Drive, Edgeware, Middlesex HA8 5ND, UK).
2–5 July 1985, **International Conference on Improving University Teaching**, Utrecht, The Netherlands (T.B. Massey, The University of Maryland University College, College Park, MD 20742, USA).
7–11 July 1985, **International Symposium for Comparative Research on Leukemia and Related Diseases**, Hamburg, Germany (Prof. F. Deinhardt, Max v. Pettenkofer Institut, Pettenkoferstr. 9a, D-8000, Muenchen 2, FRG).
7–12 July 1985, **International Conference on Natural Products**, North Carolina, USA (Prof. D.G. Kingston, Dept. of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA).
7–12 July 1985, **International Conference Ion Beam Analysis**, Berlin, FRG (IBA'85, HMI Berlin, Postfach 39 01 28, D-1000 Berlin 39, FRG).
7–10 July 1985, **International Congress on Engineering and Food**, Alberta, Canada (ICEF-4 Dept. of Food Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2PF).
8–12 July 185, **International Meeting on NMR Spectroscopy**, Cambridge, UK (Dr J. Gibson, Secretary (Scientific), The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).
8–12 July 1985, **International Symposium on Controlled Release of Bioactive Materials**, Geneva, Switzerland (USA: CRS Administrative Office, 16 Nottingham Drive, Lincolnshire, IL 60015 312/945-1616. Europe: Symporg S.A. P.O. Box 76, CH-1222, Vesenaz-Geneva, Switzerland 022-524-104).

Please send 'Announcements' entries to:
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August meetings

3–8 August 1985, **International Leukocyte Culture Conference** to be held jointly with the National Meeting of the Reticuloendothelial Society, Ithaca, New York, USA Awards of up to $500 will be made for papers, on the basis of scientific merit. There will also be an award of $500 for the winning presentation (RES/LCC Conference Office, Dr Sherwood M. Reichard, Medical College of Georgia, Augusta, Georgia 30912, USA).
11–17 August 1985, **International Anatomical Congress and Exhibition**, Barbican Centre, London, UK (IAC Secretariat, 100 Park Road, London NW1 4RN, UK).
12–14 August 1985, **Symposium on the Ethics of Animal Experimentation**, Stockholm, Sweden (M. Thelestam, Dept. of Bacteriology, Karolinska Institutet, S-104 01 Stockholm, Sweden).
18–21 August 1985, **International Symposium on Order in the Amorphous State of Polymers**, Midland, Michigan, USA (S. Butler, Symposium Secretary, MMI, 1910 West St. Andrews Road, Midland, Michigan 48640, USA).
21–24 August 1985, **International Meeting on Metallothionein and other Low Molecular Weight Metal-binding Proteins**, Zurich, Switzerland (Prof. J.H.R. Kagi, Institute of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland).
25–30 August 1985, **IUPAP Nuclear Physics Conference**, Harrogate, UK (The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).
26–30 August 1985, **International Symposium on Nuclear Medicine and Related Medical Applications of Nuclear Techniques in Developing Countries**, Vienna, Austria (Int. Atomic Energy Agency, IAEA-SM-283, Vienna International Centre, PO Box 100 a 1400 Vienna, Austria).
29–31 August 1985, **20th Meeting of the European Association for the Study of the Liver**, Helsinki, Finland (EASL, The Finnish Medical Society Duodecim, Mrs Leena Antikainen, Runeberginkatu 47 A, SF-00260 Helsinki, Finland).

September meetings

2–6 September 1985, **International Symposium on Marine Natural Products**, Paris, France (Drs A. Ahond and C. Poupat, ICSN du CNRS, 91190 Gif s/Yvette France).
2–7 September 1985, **European Drosophila Research Conference**, Balatonszeplak, Hungary (J. Szabad, Institute of Genetics, Biological Research Center, H-6701 Szeged, Hungary).
3–5 September 1985, **Symposium of the**

Uranium Institute, London, UK (Symposium Secretariat, Conference Associates UIS, 27A Medway Street, London SW1P 2BD, UK).

3–6 September 1985, **Noordwijkerhout Symposium on Innovative Approaches in Drug Research**, Noordnijkerhout, The Netherlands (Dr J.F. Rodrigues de Miranda, c/o Prof. Dr H. Timmerman, Vrije Universiteit, Department of Pharmocochemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands).

3–6 September 1985, **European Nuclear Medicine Congress**, London, UK (European Nuclear Medicine Congress 1985, Institute of Nuclear Medicine, Middlesex Hospital Medical School, Mortimer Street, London W1N 8AA, UK).

8–13 September 1985, **International Congress of Pure and Applied Chemistry**, Manchester, UK (Dr J.F. Gibson 30th IUPAC Congress, Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

8–13 September 1985, **European Immunology meeting**, Jerusalem, Israel (Prof. M. Schlesinger, Secretariat of the 7th Immunology Meeting, PO Box 983, Jerusalem 91009, Israel).

9–13 September 1985, **IUPAC International Congress of Pure and Applied Chemistry**, Manchester, UK (Dr J. Gibson, 30th IUPAC Congress, Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

3 Sept–4 October 1985, **International Remote Sensing Workshop**, South Dakota, (US Geological Survey, 917 National Center, Reston, VA 22092, USA).

8–13 September 1985, **International Conference on Biothermodynamics**, Styria, Austria (Dr P. Laggner, Institut für Röntgenfeinstrukturforschung, der Österreicheischen Akademie der Wissenschaften und des Forschungszentrums, Graz, Austria).

8–13 September 1985, **Laurentian Hormone Conference**, Alberta, Canada (The Laurentian Hormone Conference, 45 Shattuck Street, Boston, MA 02115, USA).

9–10 September 1985, **International Symposium on Drug Analysis: Current Challenges**, Ottawa, Canada (Dr G.L. Mattok, Bureau of Drug Research, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2).

9–14 September 1985, **International Conference on Magnetospheric Systems**, Toulouse, France (Centre National d'Études Spatiales, Department des Affaries Universitaires, 18 avenue Édouard-Belin, 31055 Toulouse, France).

Appointments

Dr Archie Johnston (director of the Health and Safety Executive's Reseach and Laboratory Services Division) has been appointed to the Health and Safety Executive to make up the three man team. The executive is responsible for implementing the provisions of the health and Safety at Work Act 1984.

Professor John L. Jinks has been appointed to be Secretary and Deputy Chairman to the Agricultural and Research Council from 1 May 1985. He has been chairman of the council's plants and soils research committee for the past two years.

Dr Jo Eirik Asvall has been appointed Regional Director for Europe of the World Health Organization (WHO). For the past six years Dr Asvall has been Director of Programme Management at the WHO Regional Office for Europe.

Sir Francis Tombs is to become chairman of The Engineering Council on 1 May 1985 for a three-year period. Sir Francis is a past president of the Institution of Electrical Engineers.

Mr John Webb (managing director of Bayer Limited) has been appointed Chairman of the Chemical Industry Safety, Health and Environment Council (CISHEC), the body responsible for the UK chemical industry's position on health, safety and the environment.

Dr Ronald S. Paul has been elected Chief Executive Officer of the Batelle Memorial Institute having been president and Chief Operating Officer for the past three years.

Awards

Dr D.A. Henderson, who led the global campaign that successfully eradicated smallpox, has been awarded the Albert Schweitzer International Prize for medicine by the Burroughs Wellcome Fund (supported by the Burroughs Wellcome Company). Dr Henderson is dean of The John Hopkins School of Public Health, Baltimore, Maryland.

Loughborough University is to confer the honorary degree of Doctor of Technology on Olympic gold medallist and world record breaking athlete **Sebastian Coe**. Coe has a BSc degree in Economics from Loughborough, where he was an undergraduate, research student and research assistant. It is hoped that the ceremony will take place in July this year.

750 Overseas Research Students (ORS) Awards will be offered on a competitive basis in 1985 to overseas postgraduate students. The scheme is administered by the Committee of Vice-chancellors and Principals of the universities of the United Kingdom. Each award will cover the difference between the tuition fee for a home postgraduate student and the "full-cost" fee chargeable to an overseas postgraduate student. Candidates must be overseas graduate students who, in session 1985-86, are commencing full time study as registered research students for a higher degree at one of the academic institutions participating, or are already on such a course and do not have an ORS award. Awards may be held in any field of study by students of outstanding merit and research potential. Further details and application forms from the registrar of any of the academic institutions taking part in the scheme, including Aberdeen, Leeds and Sussex Universities.

The Simon Fraser University, Canada, announces the R.H. Wright award in Olfactory Research of $25,000. The award will be made to an individual who has recorded outstanding achievements in research in olfaction. Names of proposed candidates and supporting information should be sent to B.F. Clayman, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada.

The European Science Foundation is offering short and long term research fellowships in toxicology. Fellowships can be awarded to applicants of any nationality working in any field of the natural and life sciences, provided that their project contributes to furthering research in toxicology within Europe. The deadline for submitting completed applications is 30 September 1985, and application forms may be obtained from: ESF Research Fellowships in Toxicology (PGT), Dr John Smith, European Space Foundation, 1 quai Lezay-Marnesia - F - 67000 Strasbourg, France.

The British Ecological Society is offering grants ranging from £50 to £500 to amateur and professional scientists undertaking ecological research and survey, usually within the United Kingdom, but grants are also given to projects abroad. Application forms are available from the British Ecological Society (S.E.P.G.), Burlington House, Piccadilly, London W1V 0LQ. Further details from Dr M.B. Usher, Department of Biology, University of York, York YO1 5DD.

The Gerontological Society of America invites nominations for its 1985 Brookdale Awards for distinguished contributions to gerontology. Three awards of a $25,000 cash prize will be given to gerontologists recognized nationally and internationally in their field, two of whom will be US citizens and one a non-US citizen. Further details from Linda Harootyan, The Gerontological Society of America, 1411K St, NW, Suite 300, Washington, DC 20005.

Miscellaneous

Columbia University chemical engineer **Helmut W. Schulz**, at 72, has won official recognition for a 44-year old idea that was long wrapped in military secrecy. The US government has awarded him $100,000 in recognition of an innovative proposal he made as a young man for an energy efficient gas centrifuge method of uranium enrichment. This is the largest sum ever granted for an invention put under military wraps and thus unavailable for use by its inventor. □

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TECHNICIAN

required for cell biology laboratory at The Institute of Cancer Research, Sutton, Surrey, to investigate the mechanisms of cell killing by cytotoxic drugs and radiation. Previous experience in tissue culture, cell fractionation and basic biochemical techniques would be an advantage. However, further training will be given.

Salary will be in the range of £5,738 — £7,159 per annum depending on qualificatons and experience + £623 London Allowance pa.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 301/B/85. (6370)A

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DEPARTMENT OF IMMUNOLOGY

LECTURER

Applicants are invited for the above post, which is situated within the Leeds General Infirmary available from 1 October 1985.

Applicants must have a PhD and at least five years post-doctoral experience, together with three years teaching experience including formal lectures to undergraduates.

Informal enquiries may be made to Professor Gowland (Tel (0532) 432799 ext 3540).

Salary on the academic scale for Lecturers (£7,520 — £14,925)(under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no. 91/3. Closing date for applications 6 June 1985. (6341)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENTS OF BIOCHEMISTRY AND SURGERY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post on a project funded by the Cancer Research Campaign to investigate biochemical and immunochemical aspects of glycoproteins produced by primary human breast cancers. The work will be carried out primarily in the Department of Biochemistry in close collaboration with Professor Blamey of the Department of Surgery. Initial salary will be at the first or second point on Range 1A for Research and Analogous Staff, depending on qualifications and experience.

Applications including a curriculum vitae and names of two referees should be submitted by May 17th to Professor F W Hemming, Department of Biochemistry, Queen's Medical Centre, Nottingham NG7 2UH (0602 700111 ext. 3619). (6356)A

MEDICAL RESEARCH COUNCIL

MRC Tuberculosis and Related Infections Unit at the Hammersmith Hospital, London W12 OHS.

POSTDOCTORAL MOLECULAR BIOLOGIST

is required to work in a team engaged in the study of structure and function of protein antigens of mycobacteria. Background experience in recombinant DNA and/or peptide technologies is desirable. The appointment is for a period of 3 years with a possibility of extension up to 5 years, starting from September 1985.

Remuneration will be based on the University non-clinical academic staff scales. Applications with curriculum vitae, list of publications, names of two referees and a brief summary of current research interests should be submitted to the Director of the Unit, Dr. J. Ivanyi (address above, phone 01-743-4594, Ext 60). (6334)A

COMPUTER PROGRAMMER/ ANALYST

The ICI Corporate Bioscience Group has an opportunity for a computer programmer/analyst to lead the development of computing facilities for the group to support research in molecular genetics, plant sciences and cellular biology.

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Candidates should have a degree (or equivalent) in computer science, physical science or related subject and at least two year's full-time computing experience in a scientific environment. Familiarity with the use of 8086-based microcomputers is desirable and a knowledge of biochemistry or chemistry would be an advantage.

The vacancy is in the laboratories of a large science community located at Runcorn Heath in Cheshire. You would be a member of a Mond Division team which provides specialist support to the various groups on the site.

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For an application form contact Sue Owen on Runcorn (0928) 513712 or write to:-
Mrs S Owen,
Personnel Department,
Imperial Chemical Industries plc,
PO Box 8, The Heath,
Runcorn, Cheshire
WA7 4QD.

(6405)A

ICI

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The Professor will be expected to give an overall lead to research and teaching, as well as contributing within his/her own subject area(s)

Further particulars can be obtained from the Registrar and Secretary, University of Bradford, West Yorkshire, BD7 1DP.

Closing date for receipt of applications is Friday, 7th June, 1985. (6337)A

DURHAM UNIVERSITY

DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for a

LECTURESHIP IN GEOPHYSICS

tenable in any area of solid-earth geophysics, but with some preference for candidates in marine geophysics or seismology.

Applicants must have previous research experience in geophysics. It is hoped to fill the post from not later than 1 October 1985. Salaries on the scale £7,520-£14,925 per annum with superannuation.

Application (3 copies) naming three referees should be sent by 31 May 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE. From whom further particulars may be obtained.

(6367)A

DIRECTOR

LUDWIG INSTITUTE FOR CANCER RESEARCH BRANCH OF MONTREAL

The Ludwig Institute for Cancer Research is seeking an outstanding scientist to direct the research program of its proposed Branch in the field of cancer genetics encompassing the latest developments in such areas as cell biology, biochemistry and molecular biology. The Ludwig Institute is creating a new Branch which will conduct its research in Montreal, Quebec, in conjunction with the Royal Victoria Hospital and in association with McGill University. University and hospital academic appointment of an appropriately qualified candidate is anticipated. Planning cells for the Branch to occupy space exceeding 12,000 sq. ft.

Candidates must have demonstrated outstanding research capabilities, both in their own personal research and in attracting and leading excellent younger investigators. A Director will be a leader in basic science (Ph.D. and/or M.D) and will have an associate staff member who will lead the clinical investigative activities. Interested candidates should submit an up-to-date curriculum vitae and bibliography, about five selected recent reprints, and names of three internationally known referees no later than July 1st, 1985 to: **Dr. Lloyd Old, Scientific Director, Ludwig Institute for Cancer Research, Stadelhofer-sdtrasse No. 22, 8001 Zurich; Switzerland.**

Priority will be given to Canadian citizens and permanent residents.

(NW1736)A

DEPARTMENT HEAD EPIDEMIOLOGY/BIOSTATISTICS

The Hospital for Sick Children, one of North America's leading paediatric centres with a strong research focus, and affiliated with the University of Toronto, is establishing a Department of Epidemiology/Biostatistics, and is presently seeking a Department Head. It is planned to establish a "core" department to which other individuals in the Hospital with substantial epidemiology/biostatistical interests will relate Cross appointments in the Department of Preventative Medicine and Biostatistics in the University of Toronto are anticipated.

The preferred candidates will have clinical training (MD) as well as epidemiological training, with sufficient experience to undertake the responsibility of developing and administering a hospital department involved in research, consultation, service and teaching. The Department will also be expected to provide expertise in consultation relating to a Quality Assurance Program, clinical epidemiological research and research in medical economics.

Interested candidates should apply, with curriculum vitae and names of three references, to The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8, Attention Kerr M. Chalmers, Secretary to the Search Committee. (NW1743)A

ΕΘΝΙΚΟ ΙΔΡΥΜΑ ΕΡΕΥΝΩΝ

1958

THE NATIONAL HELLENIC RESEARCH FOUNDATION

ANNOUNCEMENT

The Institute of Biological Research of the National Hellenic Research Foundation seeks personnel for the following positions:

- One senior scientist to head a group of Genetic engineering — Molecular Biology of eukaryotes-Microbial genetics
- Two research associates to participate in the above group
- One research associate specialized in Cell Biology-Cytogenetics
- One research associate specialized in fluorescence and NMR spectroscopy in biological systems
- One research associate specialized in enzyme technology-Biotechnology

All candidates should hold a PhD and be greek citizens.

Full c.v. with publications and addresses of at least two persons for references should be addressed to. Mrs. D. Stefanou, Secretary, Institute of Biological Research, NHRF, Vassileos Constantinou 48, Athens, Greece, not later than June 15. (W1849)A

UNIVERSITY OF EDINBURGH

Regius Chair of Geology

The Secretary of State for Scotland invites applications for the Regius Chair of Geology in the University of Edinburgh which falls vacant on 1st October, 1985 following the retirement of Professor Sir Federick Stewart.

The successful candidate will be expected to have made an internationally recognised contribution within some field of the geological sciences and will assume the position of Head of Department. He/she must have outstanding leadership and administrative qualities and the ability to interact positively with academics and industry.

Further particulars of the appointment may be obtained from the Secretary, Room 3/114, New St Andrews House, Edinburgh EH1 3SY, with whom applications (13 copies, except in the case of overseas candidates who may submit only 2 copies), giving the names of three referees, and should be submitted not later than 9 August 1985.

6119)A

Recombinant DNA Specialist post-doctoral level

If your sights are aimed at the field of genetic engineering, focus in on a laboratory where sophisticated technology and the latest ideas go hand in hand with the strength of France's n° 1 chemical and pharmaceutical group-which also happens to be among the top 10 in the world The Rhône Poulenc Group provides Génétéca with an industrial scale structure for research, application and sales, thus giving Génétéca every chance to produce broad ranging applications and all of the resulting financial benefits. Ten years ago, the field of genetic engineering didn't even exist. Our main projects performed until 1984: Génétéca has successfully cloned and expressed the gene of a pharmaceutical enzyme, the numerous genes – approximately 20 – of the biosynthetic route to the vitamine B 12, an important drug and food additive, and the gene of two medically valuable human proteins

If you would like to broaden your professional horizons, join a laboratory that really counts in this field. You will find your working environment, just thirty minutes from the heart of Paris, both pleasant and stimulating.

Please send your application (résumé) to GENETICA,
160 quai de Polangis, 94340 Joinville-le-Pont, Paris - FRANCE

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GENETICA
PARIS FRANCE

postdoctoral position polynucleotide structure

The Department of Exobiology of the University of Nijmegen conducts research in the field of chemical evolution. We are seeking a chemist with recent Ph.D. to participate in a project on primitive mechanisms of polynucleotide synthesis and replication.
Appointment will be in principle for one year, although there may be a possibility of extension.
Experience in the use of spectroscopic techniques for studying the structure and dynamics of polynucleotides is desirable, but other backgrounds will be considered.
Salary is dependent on experience (minimum Dfl. 3.502,– per month).
Candidates should submit a curriculum vitae referring to announcement nr. 85-25 and have two letters of reference sent within four weeks from the publication of this announcement to: Personnel Department of the Faculty of Science, Toernooiveld, 6525 ED Nijmegen, the Netherlands.

university of nijmegen

(W1840)A

Royal Postgraduate Medical School

(University of London)

DEPARTMENT OF HAEMATOLOGY

'NEW BLOOD' LECTURESHIP IN MOLECULAR GENETICS

Applications for the above post are invited from suitably qualified candidates with research experience in genetics and molecular biology. This Department has a strong research base with established groups in cell culture studies and in molecular genetics. The Department also hosts the MRC Leukaemia Research Unit. Our main research theme is the study of genetic and acquired blood disorders at the molecular level. The successful candidate will be expected to develop in due course his/her own autonomous research programme within this scope. The appointment will start 1 October 1985.

Salary in the range £7,520 to £14,925 plus £1,233 London Allowance a year.

Candidates, who should normally be under 35, may obtain further particulars from the Personnel Office or Professor L Luzzatto, Department of Haematology, Royal Postgraduate Medical School, Ducane Rd, London W12 0HS.

Applications, including curriculum vitae and the names of three referees, should be sent to the Senior Assistant Secretary at the Royal Postgraduate Medical School (ref: N7/8).

Closing Date: 7 June 1985 (6391)A

THE JOHNS HOPKINS UNIVERSITY

Applications are invited at the Postdoctoral Level for: (1) Pharmacologist to investigate the pharmacokinetics and biological properties of mismatched double-stranded RNA interferon inducers.

(2) Immunologist/Protein Chemist to carry out detailed studies on DNA-protein interactions using monoclonal antibodies as a model system; to quantitate DNA base modifications induced by irradiation and oxygen radicals using an immunological approach. Appointments are immediately available.

Applicants should submit a curriculum vitae, a statement of research interests, transcripts and three letters of recommendation to: Dr. Paul O. P. Ts'o, Division of Biophysics, The Johns Hopkins University, School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, Maryland 21205. (NW1735)A

F O M

Association Euratom-FOM
FOM-Institute for Plasma Physics
"Rijnhuizen"
Nieuwegein, The Netherlands

This Institute is working in experimental and theoretical plasma physics. Part of the work is done in collaboration with other European laboratories, including JET.

Applicatons are invited for the post

HEAD OF THE EXPERIMENTAL DIVISION

whose task is to direct approximately forty experimental physicists and laboratory assistants and to share responsibility for the management of the Institute with the Director and the theoretical, technical and administrative division heads.

Candidates should have made significant contributions to thermonuclear plasma physics and should have proven abilities to direct an experimental group and to participate in international collaboration.

For infomation concerning this post, please contact Prof. C.M. Braams, director of the Institute tel. 03402-31224. Applicants, as well as persons who wish to propose qualified candidates, are invited to apply within two weeks after this advertisement appears, to the Secretary of the Selection Committee: Mr. H.J. Lammers, Postbus 1207, 3430 BR Nieuwegein, The Netherlands.

RESEARCH SCIENTISTS IN ORGANIC CHEMISTRY

The Dana-Farber Cancer Institute, affiliated with Harvard Medical School, announces openings for Ph.D. scientists of organic chemistry with a background in synthesis. Some experience in biochemistry would be desirable. Appointees will join a team involved in research and development of immunotoxins as reagents for treatment of human cancers. Appointments will be at either postdoctoral or more senior levels depending on experience.

Applicants should arrange for a curriculum vitae and 3 letters of recommendation to be sent to Dr. Walter A. Blättler, Division of Tumor Immunology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

An Affirmative Action/Equal Opportunity Employer. (NW1733)A

TUMOR IMMUNOBIOLOGIST

The Department of Pediatrics, Southern Illinois University School of Medicine invites applications for an Associate Professor alternate track faculty position. Ph.D. in immunology or related field required with four years postdoctoral experience including research related experience in tumor immunology. Minimum salary $39,000, salary is negotiable based on experience. Applicants may have research experience in one or more of the following areas: Surface antigens or tumor cells, polyamines, natural resistance, bone marrow transplantation.

Send curriculum vitae and description of research abilities to: L. G. Thatcher, M.D., Chairman, Department of Pediatrics, S.I.U. School of Medicine, P.O. Box 3926, Springfield, Illinois 62708. S.I.U. School of Medicine is an equal opportunity/affirmative action employer. Applications will be accepted until June 14,

HEAD, BASIC SCIENCE DEPARTMENT

The School of Dental Medicine, University of Connecticut Health Center, is seeking to fill the position of Head, Department of Oral Biology. The position is a full-time, tenure track appointment that will be filled at the rank of Professor. The applicant must hold a PhD degree in one of the biomedical sciences or a professional doctoral degree with equivalent research training, be actively engaged in basic research, and have teaching experience at the graduate level. Some administrative experience is preferred.

The Department of Oral Biology serves the basic science needs of the School of Dental Medicine. The Department is multidisciplinary, with its ten full-time faculty representing the areas of molecular biology, cell biology, developmental biology, and the neurosciences. The Department's principal educational responsibility is the administration of a large Ph.D. program; it also provides additional teaching contributions to the undergraduate dental and medical curricula, and dental residency programs. **Resumes should be sent to: Dr. John Patterson, Chairman, Oral Biology Search Committee, Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032. Affirmative Action/Equal Opportunity Employer.** (NW1732)A

POSTDOCTORAL POSITION

Studies of the biochemistry and genetics of antibiotic production by *Streptomyces*. Characterization of mutants; plasmid isolations; in vitro DNA manipulations; gene cloning; enzyme isolation. Background in rDNA work and protein purification required with Ph.D. in microbiology, genetics, or biochemistry. Available 1 October 1985, for a period of 1 or 2 years. **Send résumé and two letters of recommendation to: Professor C.R. Hutchinson, School of Pharmacy, University of Wisconsin, Madison, Wis. 53706; telephone: 608-262-7582.** (NW1744)A

Public Service Commission of Canada / Commission de la Fonction publique du Canada

Research Scientist, Earth Structure Studies

Energy, Mines and Resources
Earth Sciences
Ottawa, Ontario

We require a scientist to conduct field investigations and research studies into the nature and structure of near-surface rock bodies and major geological features of crustal and mantle scales. Emphasis will be on the application of electrical induction techniques, which may include audio-magnetotelluric surveys, real-time magnetotelluric surveys, standard magnetotelluric surveys and controlled source induction surveys. Other geological and geophysical methods may be incorporated as necessary. You will be expected to carry out field investigations in all parts of Canada, from the east and west coasts to the Arctic Ocean Basin. The research studies will demand extensive use and development of state-of-the-art computer-based processing and interpretational methods.

You require graduation with a doctoral degree from a recognized university, or a lesser degree with evidence of research experience and productivity equivalent to that of a doctoral degree in geophysics, geology, physics, mathematics or a related field. It is essential that you possess experience in the collection, processing and analysis of geophysical data, with emphasis on the magnetotelluric method. Knowledge of either English or French is essential.

We offer a salary ranging from $28,176 to $52,409 commensurate with your qualifications and experience.

Forward your résumé and/or application form, quoting reference number S-85-31-5193-47JG-(G26), to.
Joan Girling (613) 996-8054
Public Service Commission of Canada
Ottawa, Ontario, Canada K1A 0M7

Closing date: 28 June 1985 (NW1729)A

The Public Service of Canada is an equal opportunity employer Canada

HERIOT-WATT UNIVERSITY
Department of Physics
LECTURESHIP IN PHYSICS
(Reference No. 42/85)

Applicants should be either experimental or theoretical physicists or electronic engineers. Strong preference will be given to candidates with research interests in optoelectronics, laser physics, semiconductor physics, instrumentation. A Ph.D. and subsequent research experience is expected.

Information Technology Lectureship (Reference No. 43/85)

The department has a flourishing research group working on optical bistability based devices. Candidates with experience in optical signal/image processing are sought in order to expand our work in Optical parallel Processing and Optical Computer Architecture.

Appointees to the above posts will be expected to contribute variously to teaching on the degree courses of Physics/Applied Physics/Computational Physics and the M.Sc. course in Opto-electronic and laser Devices. Industrial collaborations are encouraged both in the teaching and research activities.

Salary scale £7,520 - £14,925 per annum (under review) plus USS benefits.

For further particulars and an application form please write to the Staff Officer, Heriot-Watt University, Chambers Street, 4081A

MOLECULAR AND CELLULAR IMMUNOLOGY
UNIVERSITY OF CALIFORNIA, BERKELEY

The Department of Microbiology and Immunology at the University of California, Berkeley is seeking highly qualified applicants to fill a faculty position at the Assistant Professor level beginning July 1, 1986. Applicants should hold a Ph.D. or M.D. degree and have a strong record of scholarly achievement and outstanding research contributions. Responsibilities include development of a graduate-level course in molecular aspects of cellular immunology, participation in undergraduate instruction in immunology, and maintenance of a strong independent research program to provide training for graduate students and postdoctoral fellows. Preference will be given to candidates whose major research interest involved molecular mechanisms of cellular immunobiology.

Please send curriculum vitae, summary of research interest, and the names of three references by July 15, 1985 to:
Dr. James P. Allison
Chairman, Search Committee
Department of Microbiology and Immunology
University of California
Berkeley, CA 94720.

The University of California is an Equal Opportunity Affirmative Action Employer. (NW1742)A

RESEARCH & DEVELOPMENT SCIENTISTS OXFORD

Genetics International an American Biotechnology company involved in the development of novel biomedical assays require highly motivated individuals interested in the areas of Immunology, Biochemisty, Enzymology, Electrochemistry and Synthetic Organic Chemisty.

These positions would suit persons holding either a first degree M.Sc. or a Ph.D. with or without prior industrial experience. Applicants should be ideally in their twenties and be able to demonstrate their personality and maturity and contribute to a multidisciplinary area.

The salary and benefits package for these positions is very attractive, and will include a pension scheme and if applicable generous relocation expenses will be provided. Opportunity exists for the individuals to participate in the company Executive Stock Option Scheme.

To help us assess your suitability for these posts please send in a letter of application together with a C.V. and names of 3 referees.

Please Apply to:
Mrs Frankie Minal,
G.I. (UK) Inc.,
11 Nuffield Way,
Ashville Trading Estate,
Abingdon, Oxon. OX14 1RL.

An equal opportunity employer. (6338)A

UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

Department of Biological Chemistry

POSTDOCTORAL RESEARCH ASSOCIATE

Position is available for three years from September 1, 1985 to study molecular mechanisms of hormonal regulation of heart Ca^{2+} channels. Research involves receptor binding, protein phosphorylation, Ca^{2+} flux, and electrophysiological studies using cultured heart cells. Background in biochemistry, cell biology, or pharmacology is essential with a commitment to training in biochemical approaches. Salary negotiable.

Applications along with CV and the names of three references should be sent to: Dr. Terry B. Rogers, Dept. of Biochemistry, Univ. of Maryland, School of Medicine, 660 W. Redwood St., Baltimore, MD, 21201.

Equal Opportunity Employer. (NW1734) A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals, Houndmills, Basingstoke, Hants. RG21 2XS, UK

or

Paul Siman, Bio/Technology, 65 Bleecker St., New York, NY 10012.

THE UNIVERSITY OF SYDNEY

CHAIR OF PHYSICS (ASTROPHYSICS)

Reference no 16/05

Applications are invited for the Chair of Physics (Astrophysics) which will become vacant in the School of Physics at the end of 1985 following the retirement of Professor B Y Mills.

Salary will be at the rate of $A52,026 per annum (under review).

Applications in duplicate, quoting reference no, including curriculum vitae, list of publications and the names and addresses of three referees, by 31 July 1985, to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information is available.

The University reserves the right not to proceed with any appointment for financial or other reasons.

Equal Employment Opportunity is University Policy.

Further information is also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1844)A

THE UNIVERSITY OF MANCHESTER

THE DEPARTMENT OF GERIATRIC MEDICINE

RESEARCH ASSOCIATE

Applications are invited for the above position to investigate the effect of age on the structure of the female human urethra. Applicants should have a background in quantitative morphological techniques and immunocytochemisty. The salary will commence at £7,520 pa and the appointment is for two years only. (Superannuation). Please address all correspondence to Dr I Davies, Department of Geriatric Medicine, University Hospital of South Manchester, Nell Lane, Manchester M20 8LR. (6358)A

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY

BABRAHAM CAMBRIDGE CB2 4AT

A 3 year (MRC funded) post for a

SCIENTIFIC OFFICER

available in the Immunology Department for work with Dr Bruce Roser on the regulation of the immune response to transplantation antigens. The work will involve some minor animal surgery, cell transfer, cell separation and identification techniques (including fluorescent antibody methods and FACS 420 analysis), and in vitro techniques including monoclonal antibody production and screening. Previous experience should preferably include at least one of these techniques. Applicants should have a degree, or equivalent, in immunology, physiology, biochemistry or cell biology. Salary scale (under review): £5,909 × 6 increments to £8,153. Application forms obtainable from the Director of the Institute quoting ref BR6. Closing date: 24th May

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL POSITION IN HORMONALLY REGULATED MAMMALIAN GENE EXPRESSION

Applications are invited for this MRC funded post to study how adrenalin and cyclic AMP regulate salivary gland genes. The project involves cloning and characterising cDNA and genomic sequences for salivary proteins induced in large amounts in the mouse by adrenaliane. Experience with recombinant DNA methods is desirable but not essential. Applicant should have or expect to receive PhD.

Tenable for 3 years from 1 October 1985 on Range IA (£7,520 — £8,92 a year).

Details from Dr C J McDonald Department of Biochemistry, th University Sheffield S10 2TN. Te (0742 78555 ext 4239) to whom applications, accompanied by curriculum vitae and the names o three referees should be addresse before 3 June 1985. Quote ref R255/G. (6343)A

UNIVERSITY OF HEIDELBERG

DEPARTMENT OF NEUROBIOLOGY

POSTDOCTORAL RESEARCH

position available at the Departmer of Neurobiology for electron-micro scopist interested in the role of ce adhesion molecules during nervou system development and immuno logical techniques.

Send applications to Dr I Schachner, Im Neuenheimer Fel 504, 69 Heidelberg, Fed Rep Germany. (W1846)A

UNIVERSITY OF LEEDS

BIOTECHNOLOGY UNIT

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for the abov post for work on a collaborative pro ject between the Biotechnology Un and the Department of Biophysics i a protein engineering group engage in the purification, crystallisation an biochemical studies of a potentiall interesting industrial enzyme

Post available immediatley for fixed period of up to three years.

The applicant should have a goo honours degree in Biochemistry an a PhD or equivalent experience in pro tein chemistry/biochemistry.

Salary according to IA Range fo Research and Analogous Staff (£7,52 — £12,150)(under review) accordin to age, qualifications and experienc

Application forms and further pa ticulars may be obtained from th Registrar, The University, Leeds LS 9JT quoting reference no. 201/ Closing date for applications 30 Ma

UNIVERSITY OF LEEDS

DEPARTMENT OF PURE AND APPLIED ZOOLOGY

PREDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post, supported by the AFRC, for work on the cold hardiness and overwintering survival of aphids, in collaboration with the Entomolgy Department, Rothamsted Experimental Station.

A good honours degree in Agricultural Science, Applied Biology, Biochemistry or a related discipline are required

Post available from a date to be arranged for a fixed period of up to three years.

Salary on the IB Range for Research and Analogous Staff (£6,600 — £8,920)(under review) according to age, qualifications and experience.

Informal enquiries may be made to Dr J S Bale (Tel 0532 431751 ext 7423).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no 56/14. Closing date for applications 30 May 1985.

(6346)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

DEPARTMENT OF PHYSIOLOGY

SERC POSTDOCTORAL RESEARCH FELLOW

required for 3 years for work on the electrophysiology and pharmacology of amino-acid function in lamprey spinal cord. A background in electrophysiology would be an advantage. Initial salary up to £8753 pa.

Application form and further details from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE Closing date 31st May.

(6392)A

LEUKAEMIA RESEARCH FUND CENTRE AT THE INTSTITUTE OF CANCER RESEARCH

TECHNICIAN/RESEARCH OFFICER

required to help organise a molecular biology laboratory and actively participate in its research programme into Cellular and Molecular Biology of Human Leukaemia. Experience in recombination DNA techniques essential. Candidates should have a degree, HNC or equivalent qualification.

Salary will be in the range of £5,738 — £6,942 + £1,042 pa London Weighting allowance, depending on qualifications and experience. Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 301/B/86. (6371)A

THE UNIVERSITY OF AUCKLAND
New Zealand

LECTURESHIP/SENIOR LECTURESHIP IN MARINE BIOLOGY

MARINE BIOLOGY
(Leigh Laboratory)

Applicants should have a higher degree and teaching and research experience in either botanical or zoological aspects of Marine Science. The laboratory's current activities cover intertidal and subtidal experimental ecology of both plants and animals, phytoplankton and zooplankton studies and fish physiology and behaviour. The successful applicant will be expected to support and encourage these or related research programmes, assist in the teaching of marine biology and to contribute generally to the management of the Laboratory. Salaries will be established within the appropriate scale for Lecturers NZ$23,622-$27,929 and Senior Lecturers NZ$29,430-$37,240. Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications in accordance with the Method of Application should be forwarded as soon as possible, but not later than 31 May 1985. (W1845)A

BIRBECK COLLEGE
University of London

DEPARTMENT OF CRYSTALLOGRAPHY

LECTURESHIP IN MOLECULAR BIOLOGY: PROTEIN ENGINEERING

Applications are invited for a lectureship to be held in the Department of Crystallography established under the UGC's "New Blood" scheme and tenable from 1st October 1985.

Applicants should have a first degree in a relevant biochemical subject and practical experience in recombinant DNA techniques including site-specific mutagenesis in the study of protein structure and function. The successful candidate would be expected to work in collaboration with the structural molecular biology group of Professor T L Blundell, FRS and his colleagues.

Candidates should normally be less than 35 years of age but exceptionally well-qualified canidates just over this limit may be considered. Appointment will be at an appropriate point on the Lecturers' scale £8,753-£16,158 pa inclusive of London Allowance (under review). Further particulars may be obtained from the Assistant Secretary (Personnel), (CE), (N), Birkbeck College, Malet Street, London WC1E 7HX (Tel: 01-580 6622, ext 529), to whom applications in duplicate including full cv and names of two referees should be sent by 7th June 1985.

(6376)A

BIOGEN

is a biotechnology company with laboratories in Switzerland and the United States, developing commercial applications of molecular biology in the areas of agriculture, chemicals, pharmaceuticals and energy.

In Geneva vacancies now exist for additional

PROTEIN CHEMISTS

for work involving the purification and characterization of proteins from recombinant microoganisms. Experience with HPLC would be an advantage.

The successful candidates will possess a PhD degree with at least two years of postdoctoral experience and an appropriate record of research productivity as documented by publications.

We offer a challenging scientific career with the opportunity to interact with talented colleagues in a stimulating environment.

Please send your résumé accompanied by the names and addresses of at least three references whom we may contact on your behalf to —

BIOGEN S.A.
Personnel Manager
46, Route des Acacias
1227 Carouge — Geneva
Switzerland.

(W1837)A

PRESIDENT
THE AMERICAN MUSEUM OF NATURAL HISTORY

The trustees of The American Museum of Natural History invite applications for the position of President and Chief Executive Officer of the Museum. The President is responsible for the effective governance of this prestigious museum and related entities.

Reporting to the President are the Director, the Deputy Director for Research (9 curatorial departments), Deputy Director for Administration, Assistant Director for Financial Affairs, Department of Development and Public Affairs, Department of Library Services, Department of Exhibition and Graphics, Department of Education, *Natural History* Membership, and the Planetarium.

The annual operating budget of the Museum is $35 million, there are 800 employees, 23 buildings, a $95 million endowment, and 2.5 million visitors a year.

Candidate should have the following qualifications:

* Demonstrated imaginative leadership and an ability to work with diverse constituencies
* A strong interest in the natural sciences and appreciation of scholarly work A Ph.D. in the Natural Sciences would be highly desirable.
* Proven administrative and financial skills in the administration of large organizations.
* Experience in major fund raising.

Letters of nomination or application, coupled with resume/vitae and information on current compensation should be submitted in confidence no later than July 1, 1985 to: Box 1737, c/o Nature Classified, 65 Bleecker St, New York, NY.10012.

Resumes will neither be acknowledged nor returned.

The American Museum of Natural History is an equal opportunity employer. (NW1737)A

MELBOURNE TUMOUR BIOLOGY BRANCH
LUDWIG INSTITUTE FOR CANCER RESEARCH

CELL/MOLECULAR BIOLOGIST, REF.3/85

The Melbourne branch of the Ludwig Institute is undertaking a research programme directed towards the understanding of the cellular and molecular basis of growth regulation in normal and neoplastic tissues.

A Post-doctoral Scientist is required to work in an active programme on the retroviral transformation of murine epithelial and T-lymphocyte cells in vivo and in vitro. Experience in tissue culture and in either basic protein chemical or molecular biological techniques are required.

Initially the position will be for a period of 2 years with the possibility for extension. The salary will be equivalent to research fellow (grade 1 levels 1-4, dollars Aust 21,411 to dollars Aust. 23,284 per annum) depending on experience. The Ludwig Institute will pay the transfer expenses to Melbourne including an Apex Company airfair for the successful candidate and his/her dependents.

Applications should include above reference number, curriculum vitae and the names and addresses of 2 referees. Address to: — The Director, Ludwig Institute for Cancer Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia. (W1836)A

UNIVERSITY OF LINZ/AUSTRIA
Institute of Biophysics offers

3 POSTDOCTORAL POSITIONS

The Institute provides, under guidance of Prof. H. Schindler, most advanced techniques of membrane reconstitution, aimed at relationships between membrane structure and functions, especially of ion transport through membrane channels. The new collaborators are invited to join current studies of ion channels involved in heart function and other electrical and chemical excitation processes. Applicants should be competent in either of the three fields:

Membrane Physiology (strong background either in electophysiology including patch clamp techniques, or in physiology of cardiovascular membranes including experience in cell-culturing),

Membrane Biochemistry (Broad expert knowledge in isolation of membranes and of membrane proteins to purity),

Membrane Biophysics (physicist or physico-chemist with either practical experience with membranes or involved in theoretical approaches to membranes)

For all three posts, training in membrane reconstitution is not required and will be provided. Each appointment will be, in the first instance for one year with the possibility for prolongations and a chance of promotion to a senior position

Applications, including curriculum vitae, names and addresses of two referees, and a detailed account of previous relevant experiences should be sent before 15th May 1985 to Prof. H. Schindler, University of Linz, Institute of Biophysics, A-4040 Linz, Austria. Tel: 01043/ 732/23 13 81 Ext. 530, 531. (W1841)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MEDICINE

TECHNICIAN GRADE 3

to assist with a project concerning Radioimmunoassay of peptide and steroid hormones, measurement of catecholamines by HPLC. Assistance with hypothalamic perfusions and related work.

Candidates must possess ONC or appropriate equivalent and three years appropriate experience.

Salary within a range £5,399 — £6,325 per annum.

This post is available for one year.

Application forms may be obtained from the Registrar, the University, PO Box 147, Liverpool, L69 3BX.

Quote Ref: RV/259/N (6355)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF MICROBIOLOGY

RESEARCH TECHNICIAN GRADE 3

Applications are invited for the post of Technician Grade 3 tenable for a maximum of three years. Initial salary will be in the range of £5,399 — £5,701 per annum.

The position is funded by the Wellcome Trust and the successful applicant will be involved with the identification and biotyping of medically important yeasts. Some experience of microbiology is essential: experience in medical mycology would be an advantage.

Send applications, including a curriculum vitae and the names and addresses of two referees, to Dr F C Odds, Department of Microbiology, University of Leicester, Leicester LE1 7RH. (6339)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS Centre for Applied Microbiology & Research

Therapeutic Products Laboratory

BIOCHEMIST/ MICROBIOLOGIST

A graduate is required for research into the isolation and evaluation of methionine degrading enzymes for cancer therapy. The post is for a maximum period of 3 years and is financed by the Cancer Research Campaign. The work will be under the direction of Dr M Tully (telephone Idmiston (0980) 610391 ext 424) from whom further information may be obtained.

The successful applicant will be graded Basic Grade Microbiologist.

Salary £6,559 — £7,063

National Health Service terms and conditions will apply.

Application forms are obtainable from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Telephone Idmiston (0980) 610391.

Closing date 31 May 1985.

Please quote Reference No 41 when applying. (6348)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF GENETICS

NEW BLOOD APPOINTMENT

Applications are invited for a U versity Lectureship or Demonstrat ship (equivalent to UAL) in the fi of molecular plant genetics under new blood scheme. The pers appointed will be expected establish a research programme a to undertake teaching.

Relevant pensionable scales stipends are as follows:

UL £10,330 a year rising by annual increments to £15,930 U £7,980 rising by 4 annual increme to £9,860.

Informal enquiries concerning t post can be made to Professor J I Finchman, Department of Geneti Downing Street, Cambridge C 3EH.

Further particulars may obtained from Secretary to t Appointments Committee, Facu of Biology A, Zoology Departme Downing Street, Cambridge CB2 3 to whom applications (3 copi including Curriculum Vitae a names of three referees should be s to reach him by 31st May 19 Applications for this post should marked 'NBGEN'. (6375)A

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nature classified

NEW YORK OFFICE
Garth Walkes
65 Bleecker Street
New York, NW 10012
Tel: (212) 477-9600

TORONTO OFFICE
Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1LI
Tel: (416) 690-2423

LONDON OFFICE
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ZOOLOGY

POST DOCTORAL RESEARCH FELLOW IN DEVELOPMENTAL NEUROBIOLOGY

Applications are invited for an MRC funded post to investigate axonal and cellular interactions in vitro, using amphibian nervous tissue. Candidates should have experience in neural tissue culture and a background in cell biology or biochemistry would be helpful. The person appointed will join a small team investigating the specificity of nerve connections in development and regeneration. A well equipped laboratory and technical assistance are available.

The post is tenable for three years starting in September or October this year. The salary range (Grade 12) is £7,520 - £12,150 per annum.

Applications (three copies, including CV and the names of two referees) to Dr R M Gaze, Department of Zoology, West Mains Road, Edinburgh, EH9 3BT, by 30th May 1985.

Please quote reference No 5250. (6364)A

THE UNITED MEDICAL AND DENTAL SCHOOLS

St Thomas's Campus

RESEARCH TECHNICIAN

required from July '85 for neurophysiological laboratory. Duties involve general practical work and also fabrication/testing of laboratory equipment. Previous laboratory experience required; interest/training in histological procedures, electronics or photography useful.

Salary on Whitley Council MLSO Scale, 3 year post on MRC Grant.

Apply with cv and names and addresses of 2 referees to Staffing Officer, Dean's Office, St Thomas's Hospital Medical School, London SE1 7EH, before 23rd May, 1985. For informal enquiries, please contact Dr S McMahon, tel 01 928 9292 Ext 2241. (6373)A

UNIVERSITY OF EDINBURGH

TEMPORARY LECTURER IN ZOOLOGY

Applications are invited for a temporary Lectureship in Zoology for one year only from 1 October 1985. This post is financed by the Nuffield Foundation and the maximum salary is point 4 of the Lecturer scale. The main teaching duties are in evolution and population biology. Further particulars can be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL with whom applications by letter (2 copies) including a curriculum vitae and the names of two referees should be lodged not later than 31 May 1985. Please quote reference number 1188. (6296)A

UNIVERSITY OF YORK

DEPARTMENT OF ELECTRONICS

POSTDOCTORAL RESEARCH ASSISTANT/RESEARCH ASSISTANT

Applications are invited for a 3 year post associated with the development of a dispersive EXAFS facility for the SERC Synchrontron Radiation Source at the Daresbury Laboratory. EXAFS is an exciting x-ray investigatory technique, using a dispersive geometry which enables atomic structure to be measured on the millisecond timescale.

The person appointed will be concerned with the design and development of this facility, which will exploit solid-state devices as x-ray imagers under the control of microprocessors. The project is interdisciplinary covering aspects of applied physics, advanced electronic and mechanical system implementation and software techniques.

Work will take place at York and at Daresbury Laboratory, Cheshire.

Salary on a scale up to £8,920 per annum, with USS.

Three copies of applications, with full curriculum vitae and naming two referees, should be sent by 31 May 1985 to Registrar's Department (Appointments), University of York, Heslington, York YO1 5DD. Further particulars are available. Please quote reference number N/6200.(6398)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY & EMBRYOLOGY

RESEARCH ASSISTANT

required for project on the control of nerve and muscle differentiation using a variety of techniques. Experience of tissue culture an advantage.

The post is funded by the MRC until 31 August 1987. Salary will be at a point on the RA range IB scale £6,600 - £8,400 according to age, qualifications and experience.

Applications (cv and 2 referees) to: Dr A Warner, Dept of Anatomy, University College London, Gower Street, London WC1E 6BT. Closing date: 25 May 1985. (6350)A

UNIVERSITY OF LONDON

READERSHIP IN PLANT BIOCHEMISTRY TENABLE AT ROYAL HOLLOWAY AND BEDFORD COLLEGES

The Senate invite applications for the above Readership from 1 October 1985. Preference will be given to candidates who have considerable experience in the molecular biology of plant development and an interest in postgraduate courses. Applications (11 copies) should be submitted to the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further details should first be obtained.

Closing date for receipt of applications: 14 June 1985. (6351)A

UNIVERSITY OF ZIMBABWE

LECTURESHIPS/SENIOR LECTURESHIPS
ASSOCIATE PROFESSORSHIPS

DEPARTMENT OF BIOLOGICAL SCIENCES (3 posts)

Post A Freshwater Biology: A Lecturer specialising in some aspect of Freshwater Biology is required to teach general undergraduate courses in ecology and to contribute to an MSc programme in Hydrobiology. Any area of Freshwater Biology (except Fisheries Biology) will be considered but candidates specialising in phytoplankton/primary production studies will be of particular interest. Experience of tropical lakes or running waters is desirable. Applicants must have a first degree and a PhD and/or research experience in the appropriate area of specialisation Appointment will be for two years in the first instance.

Post B Plant Physiology: A Lecturer is required to contribute to undergraduate and postgraduate courses in Plant Physiology Specialists in any plant physiological discipline will be considered but the Department will view with particular favour applicants specialising in photosynthesis, water relations or seed physiology. Applicants must have a first degree and a PhD and/or research experience in some aspect of plant science.

Post C Nematology/Soil Zoology: Applicants should be experienced in the general area of Nematology and/or Soil Zoology. Any area of specialisation within these disciplines will be considered but preference will be given to candidates with research experience with plant-infecting nematodes. The successful candidate should have a first degree in some aspect of biological or agricultural science and a PhD and/or research experience in the appropriate area of specialisation. He/She will be required to contribute to undergraduate courses in invertebrate zoology, parasitology and soil and ecology, and to appropriate postgraduate courses.

SALARY SCALES
Lecturer Z$11,880 - $20,076; Senior Lecturer Z$20,580 - $23,400; Associate Professor Z$21,084 - $23,904 (including allowance). Appointment on the above scales according to qualifications and experience.

CONDITIONS OF SERVICE: Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on short-term contract basis with an initial period of two years. Short-term contracts may, in exceptional cases, be extended.

Six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees should be addressed to the Director, Appointments and Personnel, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe (Telex: 4-152 ZW). Applicants in the UK should also send an additional copy to the Secretary General, Association of Commonwealth Universities, (Appts), 36 Gordon Square, London WC1H 0PF. Closing date for receipt of applications is **14 June 1985.** (W1848)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF GENETICS

LECTURESHIP

(Molecular Genetics)

Applications are invited for a Lectureship in Genetics available from 1st October 1985. We are seeking to appoint a research scientist active in the field of molecular genetics who wishes to undertake a research programme involving the use of recombinant DNA techniques. Facilities are well established in the department of prokaryote gene cloning, the molecular genetics of fungi, DNA sequencing, the cloning of higher plant DNA and animal virus research.

Salary will be on the Lecturers' scale: £7,520 — £14,925 per annum, according to age, qualifications and experience.

Further particulars may be obtained from the Senior Assistant Registrar (F P), The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (3 copies) together with the names and addresses of three referees should be lodged not later than 27th May 1985. (6369)A

Southampton THE UNIVERSITY

Department of Chemistry

POSTDOCTORAL RESEARCH FELLOW IN INTERFACIAL STUDIES

Applications are invited for the above post funded by the Department of Energy, for work in the measurement of very low values of interfacial tension, under the Enhanced Oil Recovery programme. The work will involve the use of computerised video digital techniques applied to interfacial studies and the extension of these measurements to pressures up to 100 bar.

The post is currently available and the appointment will be initially for a one year period on a salary range up to £9390 per annum.

Applications naming two references should be sent to Dr D J Schiffrin, Chemistry Department, the University, Southampton, S09 5NH from whom further information can be obtained. (6381)A

UNIVERSITY OF LONDON
THE INSTITUTE OF DERMATOLOGY
Department of Electron Microscopy and Cell Pathology

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above position which will be available for 2 years in the first instance. The chief aim of the research will be to develop in-vitro techniques for studies on cell-substrate interations as part of a long term investigation on genetic and acquired blistering disorders. Experience in cell culture methods and immunohistochemistry is desirable.

Salary according to age and experience on 1A scale. Further details from Dr. R.A.J. Eady, Institute of Dermatology, Homerton Grove, London E9 6BX. (Tel: 01-985 7061, Ext: 66).
(6404)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF GENETICS

Applications are invited for the post of
RESEARCH ASSISTANT (1B)
for three years from 1st September 1985 to work on cell differentiation in the slime mould Physarum polycephalum. Starting salary £6600, rising to £7520 pa. The project, funded by SERC, is a collaboration with Sheffield University which will employ time-lapse filming to analyse cellular events of development from amoeba to plasmodium in mutant strains. No previous experience is necessary but applicants should have, or expect shortly to obtain, a degree in one of the Biological Sciences. Knowledge of aseptic techniques and/or photomicrography would be an advantage.

Applications with curriculum vitae and names of two referees should be submitted to Dr Jennifer Dee, Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, by 17th May 1985 Further details may be obtained from Dr Dee: telephone (0533) 551234 ext 393.
(6384)A

Southampton THE UNIVERSITY

INSTITUTE OF CRYOGENICS

Applications are invited for the post of Lecturer in the Institute of Cryogenics. Candidates should have a Ph.D. and research experience in applied low temperature physics or cryogenic engineering and a strong interest in teaching. The post will require development of links with industry via collaborative research and continuing education activities.

Salary within scale — £7,520 — £14,925 per annum (under review). The appointment will be for an initial five year period.

Further particulars may be obtained from Mr. D.A.S. Copland, The University, Southampton, SO9 5NH, to whom applications (7 copies from U.K. applicants) should be sent not later than 27 May 1985 quoting reference number2155. (6359)A

DURHAM UNIVERSITY
DEPARTMENT OF MATHEMATICAL SCIENCES

Applications are invited for the post of
POSTDOCTORAL SENIOR RESEARCH ASSISTANT
in Elementary Particle Theory tenable for fourteen months from 1 August 1985 or later by arrangement under the direction of Drs E F Corrigam and D B Fairlie for investigations in the Stucture of Gauge Theories. There is scope for working on a variety of different topics.

Initial salary in the range £7,520 — £9,860 per annum plus superannuation.

Application (3 copies) naming 3 referees should be sent by 31 May 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may be obtained. (6368)A

UNIVERSITY OF CAMBRIDGE
CAVENDISH LABORATORY

RESEARCH ASSOCIATE IN HIGH ENERGY PHYSICS

Applications are invited for the above SERC-funded post. Candidates should have or be shortly completing a PhD in Particle Physics or have related experience. The laboratory has an active research programme at the antiproton-proton Collider at CERN, and is involved with the construction of the OPAL detector for the study of electron-positron collisions at LEP. It is envisaged that the successful candidate would join the Collider research programme.

The appointment commences on 1 July 1985. Salary will be in the range £7,520 to £9,860 pa depending on age and experience. The appointment will be initially for two years but is renewable.

Letters of application including curriculum vitae and the names of two referees should be sent to Mr J Deakin, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE.

Further particulars can be obtained by contacting Dr J G Rushbrooke at this address. Closing date for applications is 20 May 1985.
(6363)A

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF ZOOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

A one-year post is available from 1st October 1985 to work on a SERC funded project looking into risk-sensitive and time-constrained foraging in shrews. Applicants should have a working knowledge of optimal foraging theory and experience with laboratory behavioural studies. The appointment will be within the first two points of the postdoctoral scale.

Applications with curriculum vitae to Dr C J Barnard, Department of Zoology, University of Nottingham, University Park, Nottingham, NG7 2RD. (6397)A

MAX-PLANCK-INSTITUTE FOR IMMUNOBIOLOGY
Freiburg, FRG

searches for a
POSTDOCTORAL RESEARCH FELLOW OR AN ASSISTANT RESEARCH SCIENTIST

Applications are invited for work in the fields of T-cell differentiation and cancer-related immunobiology, mainly employing molecular biological techniques. Therefore experience in molecular genetics is of advantage. The position is immediately available.

Applicants should forward their CV and letters of recommendation as soon as possible to: Dr J T Epplen, Max-Planck-Institut fur Immunbiologie, Stubeweg 51, D-7800 Freiburg i Br, FRG. (W1839)A

INSTITUTE OF CANCER RESEARCH

ELECTRON MICROSCOPE TECHNICIAN

required for the Institute of Cancer Research, Fulham Road. The work involves mostly transmission E M of fixed material with some negative staining and scanning work. The unit carries out E M studies for many departments within the Institute giving rise to a considerable variety of experimental material. Qualifications required are HNC or Degree in Biological Sciences. Previous E M experience considered essential. The post would particularly suit applicants with technical qualifications.

Salary will be in the range of £5,738 to £5,925 + £1,042 London Allowance, depending on qualifications and experience.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 301/B/84. (6366)A

THE QUEEN'S UNIVERSITY OF BELFAST
DEPARTMENT OF PHYSIOLOGY

RESEARCH ASSISTANT

The research assistant will work with Dr N G McHale on a project investigating the mechanism of lymph flow in sheep. The research is funded by the Wellcome Trust and the appointment will be for two years. Applicants should preferably have, or expect to obtain, a good honours degree in Physiology or a related discipline.

Commencing salary: £6,600 per annum.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: 31 May 1985. (Please quote Ref 85/W) (6401)A

UNIVERSITY OF OXFORD
NUFFIELD DEPARTMENT OF PATHOLOGY

RESEARCH ASSISTANT

Applications are invited for the above position to work on the origin and function of osteoclasts in normality and bone disease. Applicants should have a degree in cell biology or molecular biology. The salary is funded by the Arthritis and Rheumatism Council for three years, on a maximum starting point of £6,600.

Applications, together with curriculum vitae and the names of two referees, should be sent to: Professor J O'D McGee, Nuffield Department of Pathology, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, by 30th May, 1985, from whom further details can be obtained.
(6382)A

UNIVERSITY OF LIVERPOOL
CYTOGENETICS UNIT

SCIENTIFIC OFFICER (CYTOGENETICS)

Applications are invited from Science graduates for a basic grade post on the Whitley A scales. This Unit provides the Cytogenetics service for the Mersey Regional Health Authority. The post primarily involves work in prenatal diagnosis and experience in human cytogenetic techniques/tissue culture is desirable.

Salary, depending upon qualifications and experience, within the range £6,559 - £7,807 per annum (under review).

Application forms may be obtained from the Registrar, the University, PO Box 147, Liverpool L69 3BX. Forms should be received not later than 31 May, 1985.

Quote Ref: RV/269. (6379)A

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when replying to these advertisements

UNIVERSITY OF OXFORD

New blood lectureship in Synaptic Pharmacology

In association with Lincoln College

Applications are invited for the above university lectureship tenable from 1 October 1985. Applicants should be under 35 years of age on 1 October 1985. Stipend according to age on the scale £7,520 to £15,930 (under review). The successful candidate may be offered a stipendiary Tutorial Fellowship at Lincoln College.

University Lectureship in Pharmacology

In association with Somerville or Hertford College

Applications are invited for the above post tenable from 1 October 1985. Stipend according to age on the scale £7,520 to £15,930 (under review). The successful candidate may be offered to stipendiary Tutorial Fellowship at Somerville College, the statutes of which provide that all Fellows shall be women, or, if a man is appointed, at Hertford College.

Further details may be obtained from Professor A.D. Smith, Department of Pharmacology, South Parks Road, Oxford OX1 3QT, to whom applications (nine typed copies, or one from overseas applicants) with the names of three referees should be sent by 30 May 1985. (6388)A

MOLECULAR BIOLOGIST

The Danish Cancer Society is opening a position as research professor to build up a molecular biology research unit at the Fibiger Institute, Ndr. Frihavnsgade 70, Copenhagen, Denmark.

The institute is a multidisciplinary research institute involved in several areas of cancer research including tumor endocrinology, environmental carcinogenesis and tumor virology. The director of the Fibiger Institute is dr. Jørgen Kieler. It has a total staff of 50 employed including 5 senior investigators. It is sponsored by a core grant from the cancer society and project grants from the scientific grant committee of the Cancer Society. An international scientific committee acts as an advisory body for the institute.

The successful candidate will be responsible for establishing a research programme in molecular oncogenesis and should advise the other research units on aspects of molecular biology and its application.

The successful candidate should have extensive knowledge of recombinant DNA techniques and their application to cancer research problems. The candidate should be internationally recognised in the area of cancer research, and should have a proven record of scientific accomplishments.

The appointment is for a 5-year period. A tenure position may become vacant during the initial period.

Research facilities, research funds and staff requirements are negotiable with the Executive Committee of the Cancer Society. Salary is negotiable depending on scientific qualifications and accomplishments of the candidate

The application should be received by the chairman of the Danish Cancer Society: Professor, dr. med. Steen Olsen, Sølundvej 1, DK-2100 Copenhagen Ø., Denmark, *before 1 July, 1985.*

Application should include:
- **Curriculum vitae and bibliography**
- **Names of 3-4 referees**
- **A short summary of past and current research activities and a proposal for future research.**

The applications will be evaluated by a committee appointed by the Scientific Grant Committee of the society after consultation with the International Scientific Advisory Committee.

Additional information is available from the director or from the chairman of the Danish Cancer Society. (W1821)A

SACCHARIDE CHEMISTRY

Monsanto Company's Health Care Division has an opending for a carbohydrate chemist experienced in the chemical synthesis of monosaccharides and polysacharides. Applicants should be sufficiently experienced in this specialty to design and carry out the synthesis, purification and characterization of known and novel monosaccharides and polysaccharides with a high degree of proficiency. The position may entail directing a group of chemists in the preparation of polysaccharides of biological interest.

Please forward your resume in confidence to: **Dr. P.R. Schenkenberg, Box N-1; MONSANTO COMPANY; Mail Zone G4NG; 800 N. Lindbergh Blvd., St Louis, MO 63167.** An Equal Opportunity Employer M/F/H/V.

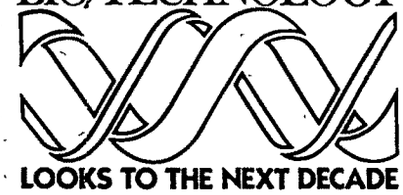
(NW1741)A

Monsanto

ATTENTION SCIENTISTS AND RESEARCHERS

Posters are now being accepted for BIO/TECHNOLOGY's premier conference

BIO/TECHNOLOGY



LOOKS TO THE NEXT DECADE

January 20-22, 1986, Clarion Hotel, New Orleans

You are invited to submit abstracts of work with commercial implications to:

Research Editor
BIO/TECHNOLOGY Conference
65 Bleecker Street, New York, NY 10012

Ideas suitable for support from corporate or venture funding are particularly welcome.

Conference Themes:

Day 1: The Molecular Biology of Plants & Animals
Day 2: Commercial Production Processes
Day 3: Innovations in Pharmaceuticals & Immunology

POSTDOCTORAL RESEARCH ASSOCIATE
Physical Chemistry

To conduct fundamental experimental research on gas-liquid interactions related to acid rain formation $19,000-$20,000/year. **Send resume and names of three references to Prof. P. Davidovits, Boston College, Dept. of Chemistry, Chestnut Hill, MA 02167.**

Boston College is an equal Opportunity/Affirmative Action Employer (NW1731)A

FELLOWSHIPS

POST M.D. AND PH.D. FELLOWSHIPS IN CANCER RESEARCH

The University of Manitoba and the Manitoba Cancer Treatment and Research Foundation have been designated a Terry Fox Training Centre making available a number of post-doctoral research positions. This co-ordinated program is designed to bring together a number of trainees within one cancer centre and to further their research and knowledge in the cancer field Research areas include Biochemistry, Cell Biology, Immunology, Medical Physics and Bioengineering, Epidemilogy and General Oncology. **Enquiries should be addressed to: Dr. L.G. Israels, Manitoba Cancer Treatment and Research Foundation, 100 Olivia Street, Winnipeg, Manitoba R3E OV9 Canada. Telephone (204) 787-2241.** (NW1738)A

continued on page 16

GRANTS and SCHOLARSHIPS

UNIVERSITY OF WALES COLLEGE OF MEDICINE
DEPARTMENT OF CHILD HEALTH
Applications are invited for a
POST-GRADUATE SCHOLARSHIP

to investigate androgen action and metabolism in disorders of sexual differentiation in humans, using tissue culture and cell fractionation techniques.

Applicants should have, or expect to gain, a first or upper second class honours degree in Biochemistry or a related science discipline.

The scholarship will commence in September 1985 and is tenable for a 2 year period (subject to a probationary year), with possible extension for a further year thereafter. The results are expected to be submitted for a PhD degree.

Further particulars and application forms (quoting Ref No C10/61) may be obtained from the Registrar and Secretary, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN (Tel No 0222-755944 Ext 2296) as soon as possible Closing date for application: 31st May, 1985. (6402)H

ASSISTANTSHIPS

FACULTY OF SCIENCE

RESEARCH ASSISTANTSHIPS
Marine Biology/ Chemistry/ Human Geography

Salary £5910 - £6657

(pay award pending)

Posts are available for the following projects -

1 Ecology of the velvet swimming crab, Liocarcinus puber (Brachyura, Portunidae)

2 Polycyclic Aromatic Hydrocarbons in engine sump oil. Mutagenicity, bioavailability and environmental fate.

3 The regional impact of the European Community's Common Fisheries Policy.

Applications are invited from graduates with good honours degrees in subjects appropriate to the proposed area of study and from undergraduates expecting to fulfil these requirements this summer For project 1, preference will be given to candidates with diving experience.

Research Assistants are expected to register for a higher degree (CNAA). Appointments are for periods of 2 years with possibility of a third year (fixed term contract)

Application forms — to be returned by Friday 31 May 1985 — and further details from Personnel Office, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA (Tel: (0752) 264639).

(6400)P

Plymouth Polytechnic

UNIVERSITY OF NOTTINGHAM
University Park
Nottingham, NG7 2RD
DEPARTMENT OF ZOOLOGY
Applications are invited for a
POSTDOCTORAL ASSISTANTSHIPS (Grade IA)

to investigate cellular oncogene activity in primary cultures of chicken embryo cells. This project is funded by the Cancer Research Campaign and will be for 2½ years. A cell or molecular biologist with experience of tissue culture and blotting techniques would be suitable for this post. Starting date 1st October 1985; salary at first point of IA scale Application letters, plus a curriculum vitae and the names and addresses of two academic referees, should be sent directly to Dr D I de Pomerai at the above address by 1st June 1985. (6372)P

SEMINARS and SYMPOSIA

INTERNATIONAL SYMPOSIUM
ROLE OF DNA IN BRAIN ACTIVITY
Ravello, Italy — May 27-29, 1985

TOPICS: Chromatin structure in neurons. Neuronal hyperploidy. Brain DNA turnover in learning and sleep Enzymology of DNA replication and repair. Brain-specific transcripts. Axoplasmic mRNA Cloning and expression of nucleic acid sequences coding for brain peptides, hormones, receptors and transmitter enzymes. Transcription and manipulation of human genes in neurological diseases

SPEAKERS: Barnard (London), Bernocchi (Pavia), Brown (Toronto), Davies (Oxford); De Gennaro (Martinsried), Ginzburg (Rehovot); Giraudat (Paris); Giuditta (Naples), Giuffrida (Catania); Hahn (Denver); Herbert (Eugene); Hyden (Goteborg), Kaplan (Pittsburgh); Kuenzle (Zurich); Lim (London); Mallet (Paris); P Mandel (Strasbourg); J.L. Mandel (Strasbourg), Mares (Prague); Milner (La Jolla), Morrison (Dallas); Reinis (Toronto); Richter (Hamburg); Roberts (New York), Scheller (Stanford); Soreq (Rehovot), Subba Rao (Hyderabad); Sutcliffe (La Jolla)

Late registrations will be accepted up to a maximum number of 120 participants. Registration fee 150 US Dollars Younger investigators 50 Dollars. No fee required for students Ravello is located at about one hour's drive from Naples

Further information from Prof. A. Giuditta, I.I.G.B., Via Marconi 10, Naples, Italy Tel 081-7257243. (W1842)M

CONFERENCES and COURSES

UNIVERSITY OF LEICESTER
SCHOOL OF BIOLOGICAL SCIENCES
DEPARTMENT OF BOTANY
BIOTECHNOLOGY SHORT COURSE CO-ORDINATOR

Applications are invited for the post of Biotechnology Short Course Co-ordinator from those with current research interests in the field of plant biotechnology. This post has lecturer status The person appointed will assess the demand for post-experience courses in biotechnology and subsequently in other areas of biotechnology. He or she will be expected to pursue a research programme within the Department of Botany.

This appointment will be for five years, supported by a grant from the DES under its PICKUP programme.

Initial salary will depend on qualifications and experience on the scale £7,520 to £14,925 (under review).

Further particulars and application forms can be obtained from the Registrar, University of Leicester University Road, Leicester, LE1 7RH, to whom applications should be returned by 31 May 1985. (6280)C

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APPOINTMENTS WANTED

advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of 25p a word. (Personal Box Numbers £2). Advertisements must be pre-paid and sent to: Nature Classified (Appts Wtd), 4 Little Essex Street, London WC2R 3LF. (890)B

STUDENTSHIPS

MEDICAL RESEARCH COUNCIL

Institute of Hearing Research
Nottingham

RESEARCH STUDENTSHIP

Applications are invited for a 3-year MRC studentship from graduates holding, or expecting to obtain, an upper second or first class honours degree in any biological subject. The successful candidate will study structural and functional development of the auditory system, with emphasis on abnormal development processes leading to deafness.

Applications should include a cv and names and addresses of two referees. Further details may be obtained from Dr K P Steel, MRC Institute of Hearing Research, University of Nottingam, Nottingham NG7 2RD. Tel: 0602-223431. (6406)F

UNIVERSITY OF LIVERPOOL

Institute of Medical and Dental Bioengineering

RESEARCH STUDENTSHIPS

A small number of studentships is available in this interdisciplinary institute for research leading to a higher degree. The precise project will depend on the academic background and interests of the student but will be in one of the following areas:

Blood flow in arterial prostheses. Interactions between metals and tissues Polymer degradation. Tissue reactions to implanted particles.

Candidates should expect to obtain a good honours degree in an appropriate biological or physical science (eg materials science, engineering, chemistry, biochemistry, cell biology).

Applications, together with the names of two referees, should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool, L69 3BX.

Quote Ref: RV/263/N (6353)F

THE ROWETT RESEARCH INSTITUTE

Bucksburn, Aberdeen, AB2 9SB

RESEARCH STUDENTSHIP IN ANIMAL NUTRITION

Applications are invited for a Studentship leading to a PhD (Aberdeen University) for research into the factors which influence rumen development and hence intake of dry feed in young calves. The Project, sponsored by British Sugar PLC, will investigate how the physiological/biochemical mechanisms of the developing rumen respond to changes in the nature of the carbohydrate source supplied in weaner rations.

Applicants should possess or expect to obtain this year a good honours degree in agricultural biochemistry, nutrition or any related subjects.

Applications, including CV and the names of two referees should be addressed to the Secretary, Rowett Institute, Bucksburn, Aberdeen.

Closing date 30 May 1985. (6336)F

THAMES POLYTECHNIC

SCHOOL OF BIOLOGICAL SCIENCES AND ENVIRONMENTAL HEALTH

SERC RESEARCH WORKER IN PLANT MOLECULAR BIOLOGY

Applications are invited from recent honours graduates or postgraduates to work with Dr R Yon on 'Protein structure and gene organisation of pyrimidine-pathway enzymes in higher plants'. Practical experience in protein and/or nucleic acid biochemistry would be of value. The post is tenable for three years and registration for a higher degree will be considered.

Salary: £6,219 per annum inclusive.

SERC CASE STUDENTSHIP

to investigate into the purification, properties and biosynthetic regulation of a novel bacterial aryl dehalogenase An upper second class honours degree, or better, in biochemistry or biochemistry with chemistry is required, preferably with experience of enzyme purification. The post is tenable from 1 October 1985 in collaboration with PHLS-CAMR, Porton Down, under the joint supervision of Dr ARW Smith and Dr AV Quirk.

Further particulars and application form from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to be returned by 4 June 1985.

Thames Polytechnic is an equal opportunities employer. (6407)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

SERC-CASE STUDENTSHIP

Applications are invited for a SERC-CASE studentship to investigate the effects of synthetic plant growth regulators on sterol and gibberellin biosynthesis in whole plants and tissue cultures The project will be conducted in collaboration with Dr J Lenton, Long Ashton Research Station, Bristol. The interrelationships of the sterol and gibberellin pathways will be examined to determine if their regulation is a factor in the control of plant cell divisions and cell elongation. The student will gain experience in GLC and HPLC, mass spectrometry, plant cell culture, radiochemical and microscopy techniques. Applicants will be expected to have or to obtain, a first or upper second class Honours degree in Biochemistry, Chemistry or Botany. Further information can be obtained by contacting Dr L J Goad.

Applications including curriculum vitae and the names of two referees should be sent as soon as possible to The Registrar, The University, PO Box 147, Liverpool, L69 3BX.

Quote Ref: RV/267 (6380)F

QUEEN MARY COLLEGE

(University of London)

DEPARTMENT OF CHEMISTRY

RESEARCH STUDENTSHIPS

Applications are invited for the following Research Studentships for higher degree work under the supervision of Professor R. Bonnett. Research is expected to commence in August/September 1985.

1. Geochemical Studies on Porphyrins in relation to Coalification (supported by the British Coal Utilization Research Association Ltd.)

2 Synthesis of Porphyrin Sensitisers for Tumour Phototherapy (supported by Efamol Ltd.)

Applicants should have, or expect to obtain this year, a degree in chemistry with at least upper second class honours. Conditions for these studentships follow SERC guidelines. Application forms from Dr J R C Light, Chemistry Department, Queen Mary College, Mile End Road, London, E1 4NS to be returned by 17th June. (6352)F

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CHEMISTRY

BIO-ORGANIC CHEMISTRY

SERC CASE STUDENTSHIPS

Applications are invited from graduates in Chemistry for two CASE Studentships Applicants must hold a first class or second class (Division 1) degree and will study for the degree of PhD.

(i) Anti-Cancer Uses of Synthetic Nucleoside Phosphonates in collaboration with Amersham International plc

The project involves the chemical synthesis of new derivatives of adenosine 5'-diphosphate, ADP. These are designed (i) to resist cleavage by phosphatase enzymes in vivo, (ii) to act as specific ligands for technetium-99m, and (iii) to penetrate tumour cells selectively.

The main part of the programme will be the synthesis of organic phosphonates of novel constitution and their incorporation into biological molecules; particular use of multinuclear high-resolution NMR and of MS facilities will be required.

(ii) Enzyme Inhibitors of the Shikimate Pathway in collaboration with ICI (Pharmaceuticals Division).

The project is directed towards the synthesis of antibacterials which function by blocking steps in aromatic amino-acid biosynthesis. Modelling will be based upon knowledge of reaction mechanisms and the application of computer graphics.

Applications or requests for further information should be made by writing respectively to (i) Dr G M Blackburn or (ii) Professor E Haslam at the Department of Chemistry, University of Sheffield, Sheffield S3 7HF. Quote ref: R258/G. (6365)F

WYE COLLEGE

(University of London)

DEPARTMENT OF BIOCHEMISTRY, PHYSIOLOGY AND SOIL SCIENCE

SERC CASE STUDENTSHIP

Applications are invited for a SERC CASE Studentship for research into the incorporation of foreign compounds (drugs, pesticides and other chemicals) into lipids, and the toxicological consequences of this phenomenon. The research will be carried out at Wye College, under the supervision of Dr P F Dodds, and at Shell Research Ltd, Sittingbourne, under the supervision of Dr D H Hutson.

Applicants should have, or expect to obtain, a first or upper second class Honours Degree in biochemistry or a related subject. An interest in lipid biosynthesis or drug/pesticide metabolism would be an advantage. The successful applicant will be required to register for the PhD degree at the University of London. Applications, comprising a full curriculum vitae and the names of two referees, should be made to The Registrar, Wye College (University of London), Wye, Near Ashford, Kent, TN25 5AH, from whom further information may be obtained (6349)F

THE UNIVERSITY OF WARWICK

DEPARTMENT OF BIOLOGICAL SCIENCES

POSTGRADUATE STUDENTSHIPS

Applications are invited from persons possessing, or expecting to obtain in June 1985, a suitable upper-second class Honours degree for the following eight studentships:

1 "The physiological basis of oligotrophy - morphological adaptation and selected cell type expression", Supervisor: Dr C S Dow; NERC Quota

2 "Carbon metabolism and growth of Marine Synechococcus species", Supervisors: Professor N G Carr/ Warwick and Dr I R Joint/NERC Institute for Marine Environmental Research, NERC CASE.

3 "Bioactalytic properties of ligninase from Phanerochaete chrysosporium"; Supervisor. Professor H Dalton, SERC Biotechnology.

4 "Molecular biological studies of infectious bursal disease virus"; Supervisor Dr M A McCrae, MRC Biotechnology.

5 "Reverse mutagenesis of Cowpea Mosaic virus", Supervisor. Dr D Evans/Warwick and Dr G P Lomonossof/John Innes Institute, SERC CASE.

6 "Neutralisation of influenza virus by IgM"; Supervisor Dr N J Dimmock/Warwick and Dr G C Schild/NIBSC; SERC CASE.

7 "Toxic product removal during microbial biotransformations"; Supervisors: Professor H Dalton/ Warwick and Dr P J Geary, Shell Research Centre; SERC CASE

8 "Ricin B chain as a carrier of antibodies into cells as a contribution to a more effective means of treating chemical poisoning"; Supervisors. Dr L M Roberts/Warwick and Dr A F Wright/ICI; SERC CASE

Applicants should indicate the project(s) of interest and send a cv and the names of two referees to the Executive Officer, Department of Biological Sciences, Warwick University, Coventry CV4 7AL Closing date 23rd May 1985. (6383)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

RESEARCH STUDENTSHIP

Applications are invited for a studentship, leading to the degree of PhD on "Endogenous and exogenous regulatory factors of protein degradation in normal and abnormal skeletal muscle", funded by the Muscular Dystrophy Group of Great Britain and Northern Ireland. Applicants should possess or expect to receive a 1st or upper 2nd class degree in Biochemistry, Molecular Biology or a related subject

Applications, together with the names of two referees, should be received as soon as possible by the Registrar, The University, PO Box 147, Liverpool L69 3BX

Quote Ref: RV/266 (6378)F

Medical Research Council Research Studentship (Partnership Award)

University Laboratory of Physiology, Oxford and the National Institute for Medical Research, Mill Hill, London

A 3-year M R.C. Studentship leading to a D.Phil. is available from 1st October 1985, for a project investigating contractile mechanisms in 'skinned' muscle fibres employing 'caged' ATP and the fluorescently labelled derivative of the calcium sensitive regulatory protein, troponin (TnC-DANZ). The training will involve the combination of physiology and biochemical methods, particularly the use of novel optical and laser techniques in muscle research, introduced by Dr C C Ashley and Dr. D.R. Trentham, F.R.S., the co-supervisors of the project.

The overall aim is to correlate mechanical and biochemical experiments on skinned muscle fibres with those on intact fibres in order to provide a clearer insight into the basic contractile mechanism and its regulation.

Applicants should have or expect to obtain a 1st or upper 2nd class honours degree, and have a background in physiology, biochemistry or physical sciences.

Applications including a CV and the names and addresses of 2 referees should be sent to Dr C.C. Ashley, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, as soon as possible. (6312)F

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF ANATOMY AND BIOLOGY AS APPLIED TO MEDICINE,
Cleveland Street, London W1P 6DB
RESEARCH STUDENTSHIP IN PATTERN FORMATION

Applications are invited for a research studentship in the area of the cellular or molecular basis of pattern formation from October 1, 1985. Applications, or further enquiries, to Professor L Wolpert at the above address. (6394)F

UNIVERSITY OF LONDON
Institute of Neurology
DEPARTMENT OF NEUROCHEMISTRY
RESEARCH STUDENTSHIP

Applications are invited from undergraduates expecting to graduate with a 1st or upper 2nd class honours degree in biochemistry or molecular biology to undertake research with Dr F S Walsh. The project involves an analysis of the structure and function of the cell adhesion molecule N-CAM in skeletal muscle using a combination of cell and molecular biology techniques. For further information see EMBO J 4 623 (1985).

Applicaitons, containing the names and addresses of two referees, should be sent to Dr F S Walsh, Institute for Neurology, The National Hospital, Queen Square, London WC1N 3BG. (6390)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF BIOCHEMISTRY
SERC CASE STUDENTSHIP
Drug Membrane Interactions

Applications are invited from students in physics, chemistry or biochemistry for an SERC Case Studentship in association with the Smith Klyne and French Company, Welwyn, to apply biophysical techniques such as laser flash photoplysis and FTIR spectroscopy to the studies of drug-membrane interactions.

Applicants should have (or expect to have) a First or Upper Second Class Honours degree or an equivalent qualification.

For further information please contact Professor D Chapman, Department of Biochemistry and Chemistry, Ground Floor, RFHSM, Rowland Hill Street, London, NW3 2PF (01-794-0500 Extn 3246) to whom applications giving the names of two referees should be sent as soon as possible. (6357)F

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF PHYSIOLOGY
MRC RESEARCH STUDENTSHIP

Applications are invited from individuals holding, or expecting to obtain, a good honours degree in Physiology or a related subject for a Research Studentship tenable in the Department of Physiology, St George's Hospital Medical School.

The successful applicant will work on one of several possible research projects. Further details are available from Professor J G Widdicombe, Department of Physiology, St George's Hospital Medical School, London SW17 0RE. (Tel 01-672 1255 x 4651), to whom applications with CV and names of two referees should be sent. (6377)F

UNIVERSITY OF LIVERPOOL
BOTANY DEPARTMENT
SERC/CASE RESEARCH STUDENTSHIP
Ion transport in a marine fungus

Applications are invited for a PhD research studentship to study ion transport in a marine fungus by tracer flux analysis and electrophysiocal techniques on intact hyphae and protoplasts. The studentship involves collaboration between the Botany Department (supervisor Professor D H Jennings) and the Marine Biological Association Laboratory at Plymouth (supervisor Dr C Brownlee). Candidates should have (or expect to have) a First or Upper Second Class Honours Degree or an equivalent qualification.

Applications with the names of two referees should be received as soon as possible by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

Quote Ref: RV/262/N (6354)F

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF MICROBIOLOGY
SERC CASE STUDENTSHIP
MEDICAL MICROBIOLOGY

An SERC CASE award is available in association with Glaxo Laboratories to study the phenomenon of 'tolerance' to penicillin in streptococci. Applications are invited for the studentship, which is expected to lead to the degree of PhD The student will be concerned with investigating factors influencing expression of the tolerance phenotype; its biochemical basis; its prevalence in clinical isolates; and its possible therapeutic implications. Applicants should possess, or shortly expect to obtain, a first or upper second class honours degree in microbiology and have a special interest in medical microbiology

Applications, with full curriculum vitae and the names of two referees, should be submitted to Dr David Greenwood, Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, not later than 31st May 1985. (6396)F

UNIVERSITY OF OXFORD
BOTANY SCHOOL
RESEARCH STUDENTSHIP
Calcium and Calmodulin in Higher Places

Applications are invited for a three year Studentship for studies on the regulation and localisation of the calcium-dependent regulator protein calmodulin in higher plants. The successful applicant will be expected to use immunological and related techniques and will work in a small group studying similar topics. Closing date for applications May 31st 1985.

For further details please contact: Dr D E Evans, The Botany School, South Parks Road, Oxford OX1 3RA. (6362)F

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF BIOCHEMISTRY
SERC/CASE STUDENTSHIP

Applications are invited for the above studentship tenable for three years from October 1st, 1985, and leading to a PhD degree. The project is concerned with 'Procollagen processing in the control of fibril formation' and will be supervised by Professor M E Grant & Dr D J S Hulmes (University of Manchester) and Dr C H J Sear, G D Searle (Research & Development), High Wycombe, Bucks. Applicants should have an interest in enzymology, electron microscopy and/or the application of physical techniques of biochemistry to the study of connective tissue disorders. They should have, or expect to obtain, a first or upper second class honours degree in biochemistry or biophysics. Applicants should write by May 17th, 1985, to Professor M E Grant, Department of Biochemistry, University of Manchester Medical School, Oxford Road, Manchester M13 9PT, enclosing a detailed curriculum vitae (in duplicate) and the names of two referees. (6399)F

UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY
SERC-CASE STUDENTSHIP
with
Shell Research Ltd
Sittingbourne

Applications are invited for the above position which involves a joint project between Dr D J Bowles at Leeds and Dr R L Lyne as the industrial collaborator. The research will concern the characterization of markers of embryogenesis for use in barley anther culture.

Candidates should have or expect to obtain a good Honours degree in Biochemistry, Biotechnology or Plant Sciences

Further information may be obtained from Dr D J Bowles (0532 431751 ext 7506). Applications with curriculum vitae and the names of at least two referees should be sent, by 31st May 1985, to Dr D J Bowles, Department of Biochemistry, University of Leeds, Leeds LS2 9JT (6344)F

THE QUEEN'S UNIVERSITY OF BELFAST
DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE
RESEARCH STUDENTSHIP

Applications are invited for an MRC Partnership Award Research Studentship to undertake the chemical synthesis of analogues of fragments of peptide hormones and to study their biological and immunological properties. The studentship will be tenable from 1st October, 1985 for a period of 3 years provided that satisfactory progress is maintained. Candidates should have or be expected to have by July, at least a second class (upper division) honours degree in biochemistry or chemistry. Applications with curriculum vitae should be sent to Professor D T Elmore, Department of Biochemistry, Queen's University, 97 Lisburn Road, Belfast, BT9 7BL by 24th May, 1985. (6374)F

UNIVERSITY OF ABERDEEN
DEPARTMENT OF PLANT SCIENCE (BOTANY)
RESEARCH STUDENTSHIPS

Applications are invited for:

i. a SERC CASE studentship (with The Rowett Research Institute): Immunochemical studies on the plant cell cytoskeleton.

ii. a NERC CASE studentship (with ITE, Banchory Research Station): Early changes in soils under birch and heather.

iii a limited number of additional studentships which may become available in the fields of plant photophysiology, plant morphogenesis, ultrastructure of plant cells, stomatal physiology, recognition of plant chemotypes by insects, and plant cell and protoplast culture.

For further details write to Professor C H Gimmingham, Department of Plant Science, University of Aberdeen, St Machar Drive, Aberdeen AB9 2UD preferably before May 10th 1985. (6389)F

UNIVERSITY OF BRISTOL

DEPARTMENT OF MICROBIOLOGY

Applications are invited for

TWO SERC/CASE STUDENTSHIPS

ach of which is tenable for 3 years. ne project, in conjunction with fizer Central Research, will investiate adhesion of E coli to mammalian ceptors mediated by K88 pili. The cond project, in conjunction with nperial Chemical Industries, will vestigate expression of penicillin nding proteins in E coli.

Applicants should have, or expect obtain, a first or upper second class onours degree in a biological ience. An interest in molecular netics and/or bacterial cell mem-anes would be an advantage. urther details of both projects may e obtained from Dr Ian Chopra, epartment of Microbiology, The edical School, University Walk, ristol BS8 1TD to whom applitions, including a curriculum vitae nd the names of two referees should e sent. Applicants should indicate hether they wish to be considered r one, or both of the projects. losing date for applications May 1st 1985. (6361)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF SURGERY

Applications are invited for a

POSTGRADUATE STUDENTSHIP

work on an experimental study (in ivo and in vitro) of the pathways volved in the regulation of gall ladder emptying with special mphasis on cholinergic mechanisms.

Candidates, who must have a first lass or 2 1 honours degree, will be xpected to register for a PhD.

Applications, stating age, academic ualifications and experience, ogether with the names of two eferees, should be received as soon s possible by The Registrar, The niversity, PO Box 147, Liverpool 69 3BX. Quote ref RV/265/N. (6386)F

ST. MARY'S HOSPITAL MEDICAL SCHOOL (University of London) Norfolk Place, London W21 1PG

SERC CASE STUDENTSHIP

pplications are invited for a SERC ase Studentship in collaboration vith Shell Research Ltd (Dr D H Hutton) for research (leading to a PhD legree) into the effect of environmental conditions on the metabolism of hemicals by fish.

Applicants should have, or expect o obtain, a first or second class ionours degree in biochemistry or hemistry, and have an interest in hemical and environmental oxicology.

Applications, including the names of two referees should be sent as soon is possible to Dr P Millburn, Department of Biochemistry, at above address, from whom further particulars nay be obtained (tel 01-723-1252 ext 118) (6277)F

UNIVERSITY OF WALES COLLEGE OF MEDICINE

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS IN ASSOCIATION WITH MERCK SHARP AND DOHME RESEARCH LTD

SERC RESEARCH STUDENTSHIP

Applications are invited for the above 3-year studentship from candidates with or expecting a good Honours degree. Our preferred candidate will have an interest in experimental in vitro pharmacology, computer on-line data collection and interfacing, mathematical data analysis and modelling and have significant undergraduate experience in at aleast one of these.

The project will investigate the intrinsic modulation of neurotransmission in autonomically innervated tissues using microcomputer techniques to analyse drug effects on responses to field stimulation.

Applications, in the form of a curriculum vitae with the names and addresses of two referees, should be sent to the Registrar and Secretary, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, from whom more detailed information is available (Tel No 0222-755944 Ext 2296). (6360)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY & EMBRYOLOGY

Applications are invited for a

SERC STUDENTSHIP

to work on the organisation of the visual cortex using anatomical, physiological, immunohistochemical and computer imaging techniques. Applicants should have, or expect to receive, a good honours degree and register for the PhD of the University of London

Applicants should write to Professor Zeki, Department of Anatomy & Embryology, University College London, Gower Street, London WC1E 6BT, from whom further details may be obtained. (6395)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ZOOLOGY

SERC CASE STUDENTSHIP

Trematode larvae and the snail immune response

Applications are invited from recent graduates or those about to graduate in a Biological Science for a CASE studentship leading to a PhD. The project, in collaboration with Dr V R Southgate (British Museum, Natural History), will investigate the cellular basis of host-parasite interactions between the blood fluke Schistosoma spp and the snail Bulinus spp both in vivo and in vitro.

Applications with a curriculum vitae and the names of 2 referees should be sent to T M Preston, Zoology Department, University College London, Gower St, London WC1E 6BT from whom further information may be obtained. (6393)F

UNIVERSITY OF CAMBRIDGE

SERC CASE RESEARCH STUDENTSHIP

Development of Laser-Ionisation Mass-Analysis (LIMA) as an Organic Microprobe in Chemical and Biological Systems

Applications are invited for an SERC CASE Research Studentship in collaboration with Shell Research Ltd, Sittingbourne, Kent, leading to the PhD degree.

The project is of a highly interdisciplinary nature, entailing the interpretation of organic mass-spectra, the preparation of biological materials, modification of computer software and an interest in physical instrumentation. Most of the work will be done on a new Cambridge Mass Spectrometry Ltd. LIMA instrument in the Department of Metallurgy and Material Science, Cambridge, with facilities for comparative SEM, X-ray microanalysis and FAB-MS work at Shell Research Ltd, Sittingbourne.

Enquiries are invited to Dr M J Southon, Department of Metallurgy and Materials Science, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, from whom further particulars may be obtained (tel 0223 65151). (6335)F

UNIVERSITY OF LEEDS

DEPARTMENT OF PURE AND APPLIED ZOOLOGY

MAFF STUDENTSHIP

The above studentship is available from October 1985 for up to 3 years on the following topic.

The effects on the environment of pesticide use on grassland.

Applications are invited from graduates or final year students in agricultural zoology; applied biology or a related discipline.

Informal enquiries may be made to Dr J S Bale (Tel 0532 431751 ext 7423).

Applications in the form of a curriculum vitae with the names and addresses of two referees should be sent to Dr J S Bale, Agricultural Sciences Building, The University, Leeds LS2 9JT by 20 May 1985. (6345)F

UNIVERSITY OF READING

DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

Applications are invited for an

SERC/CASE STUDENTSHIP

shared between this department and the Tolworth laboratory (MAFF). The project is on the use of esterases as indicators of the exposure of birds to pesticides

Candidates should have a good honours degree (1st or 2(i)) in Biochemistry or a related discipline, and some interest in field work.

Further details may be obtained from Mr C H Walker, Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ, to whom applications and the names of two referees should be sent. (6385)F

UNIVERSITY OF KEELE

RESEARCH DEPARTMENT OF COMMUNICATION AND NEUROSCIENCE

RESEARCH STUDENTSHIPS

Research studentships leading to the degree of PhD are available in this interdisciplinary postgraduate research Department in the following areas of study:

1 Auditory physiology: frequency selectivity, pitch, intensity, animal models of deafness, tinnitus, clinical applications, rehabilitation (Professor E.F. Evans).

2. Visual neurophysiology: retina, cortex, pattern, texture, motion, relative motion; neural correlates of after-effects (Dr P. Hammond).

3 Speech processing: human perception, computer synthesis and recognition, lip-reading aids (Dr. W A Ainsworth).

4 Visual psychophysics: pattern recognition, motion acuity, mathematical modelling, colour vision, clinical applications (Dr. D H Foster).

5 Neuroanatomy: ultrastructure of auditory system in normal and deaf animals, dye tracing of physiologically identified neurones (Dr. C.M Hackney)

6. Visual evoked potentials: information processing in human visual cortex (Dr. D A Jeffreys).

7. Clinical auditory psychophysics and evoked responses: diagnosis, prevention and amelioration of sensorineural hearing loss (Dr G.F. Pick).

8 Auditory psychophysics: pitch and space perception, frequency-sweep adaptation, frequency analysis, cochlear echoes, tinnitus (Dr. J.P. Wilson).

Interested students with a first or upper second class honours degree in an appropriate discipline are asked to write to:

Dr. D. .H. Foster,
(Postgraduate Admissions),
Department of
Communication &
Neuroscience,
University of Keele,
Keele,
Staffordshire ST5 5BG.

(6347)F

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF GENETICS

SERC/CASE STUDENTSHIP

Applications are invited for a SERC/CASE studentship leading to the degree of PhD. The project is concerned with molecular genetics and gene cloning in the methylotrophic yeast Hansenula polymorpha and will be carried out in association with Unilever plc. The successful candidate will be expected to spend a period in Unilever's laboratories in Holland. Applicants should possess, or expect to obtain, a good honours degree in a relevant subject.

Applications, to arrive before 31 May 1985 (including a full curriculum vitae and names and addresses of two referees), should be sent to Dr P E Sudbery, Department of Genetics, the University, Sheffield S10 2TN, from whom further details may be obtained (telephone 0742-78555 ext 6186). Quote ref: R261/G. (6403)F

FELLOWSHIPS

UNIVERSITY OF GUELPH
UNIVERSITY FELLOWSHIPS

The College of Biological Science is emphasizing its Research and Graduate Programs and invites applications from individuals interested in competing for NSERC University Fellowships Candidates are reminded that they would be eligible for consideration for any tenure track position available following their appointment
The following are the areas of research emphasis in the various departments·

BOTANY
Growth and development of plants
MICROBIOLOGY
Microbial pathogens of fish
Fermentation technology
Molecular biology of virus reproduction
MOLECULAR BIOLOGY AND GENETICS
Cell and molecular biology of gene expression
Development biology
DNA replication and genetic recombination
Human genetics
NUTRITION
Nutritional immunology
Nutritional toxicology
Nutritional biochemistry
ZOOLOGY
Dynamics of marine invertebrate communities
Developmental and reproductive biology
SCHOOL OF HUMAN BIOLOGY
Human physiology
Physical anthropology
Ergonomics
Quantitative biomechanics/bioengineering
Human morphology and human and medical genetics
Successful candidates would have the opportunity of participating in both the undergraduate and graduate teaching programs.

Applications, including a curriculum vitae, and the names of three referees should be forwarded, by August 1st, to: Bruce H. Sells, Ph.D., Professor and Dean, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1.
(NW1739)E

NUFFIELD FOUNDATION
ONE YEAR SCIENCE RESEARCH FELLOWSHIPS

The Nuffield Foundation is offering to University Lecturers in Science and Applied Science Departments a number of one-year research fellowships to be awarded competitively at the end of 1985 and to be held during the academic year 1986-87. The objective is to enable younger university lecturers to be freed from teaching and administrative duties for one academic year. Preference will be given to applicants under the age of 40. Awards will cover the cost of replacement teaching (up to the fourth point on the lecturer scale) and will include an allowance for research expenses.

The closing date is 11th October 1985 and further particulars may be had from the Director, Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS.

This scheme is only open to applicants from Institutes within the United Kingdom. (6332)E

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY
POSTDOCTORAL FELLOWSHIP IN THE GENETICS OF ENERGY TRANSDUCING SYSTEMS OF ESCHERICHIA COLI

A two-year postdoctoral research fellowship is available in the laboratory of Dr I R Booth from I October, or as soon thereafter as possible The SERC funded project is to study genetic and bioenergetic aspects of the role of the eup gene product in the transduction of energy in E coli.

Salary on the Range 1A scale for Research and Analogous Staff, £7,520 to £12,150 per annum, with appropriate placing

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 7 June 1985. (6387)E

UNIVERSITY OF WATERLOO, DEPARTMENT OF BIOLOGY
NSERC UNIVERSITY RESEARCH FELLOWS

The Department of Biology, University of Waterloo, invites applications for NSERC University Research Fellowships in the following areas of research interest (i) population genetics with emphasis on the genetic structure and dynamics of real as opposed to theoretical populations, (ii) protein chemistry or biochemistry (including immunology). Applications in other areas will also be given serious consideration Successful candidates will be expected to establish a vigorous research program and participate in teaching both undergraduate and graduate students.

A curriculum vitae including names of three referees should be sent to Dr. J.E. Thompson, Chairman, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1. Prospective candidates should examine the NSERC Awards Guide 1985-86, paragraph 196 on page 54 for details In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. (NW1740)E

POSTDOCTORAL FELLOWSHIP

Positions are available in an NIEHS training program for multidisciplinary research at Yale Medical School in environmental toxicology and physiology in the following areas· biochemistry, epidemiology, pathology, pharmacology, physiology, and toxicology The positions are open to U S citizens or permanent residents with M.D , D V M., Ph D., or equivalent degrees Applicants should send curriculum vitae with two references, and specify their area of interest to. **Arthur B. DuBois, M.D., John B. Pierce Foundation Laboratory, Yale School of Medicine, 290 Congress Avenue, New Haven, Conn 06519.** *Affirmative Action/Equal Opportunity Employer.*
(NW1730)E

MASSEY UNIVERSITY
Palmerston North,
New Zealand
UGC POST-DOCTORAL FELLOWSHIPS

Applications are invited for Postdoctoral Fellowships in 1986 to be funded and awarded by the University Grants Committee and tenable within Massey University

Awards may be made for one year or for three years.

Applications from candidates wishing to work in any department of the University will be considered, but in making the awards the University Grants Committee will give priority to current growth research areas such as biotechnology, genetic engineering, electronics, engineering, chemistry etc

The emolument payable will be at a rate to be determined but not exceeding the lowest step on the University scale for lecturers (currently NZ$23,622 per annum).

Applicants should have completed the requirements for the award of the degree of Doctor of Philosophy.

Further details of the position, together with conditions of appointment, may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University, with whom applications close on 31 July 1985 (W1843)E

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Additional information can be obtained from Professor S R Naborski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road Leicester, LE1 7RH, UK, (0533 551234, Ext 361) to whom applications, including CV and names of two referees, should be sent. (6306)E

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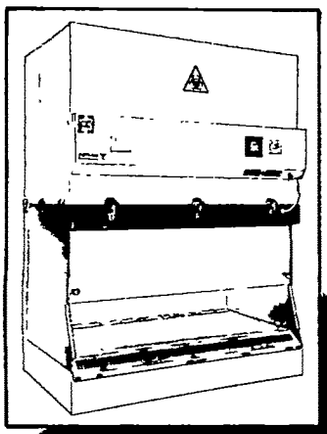
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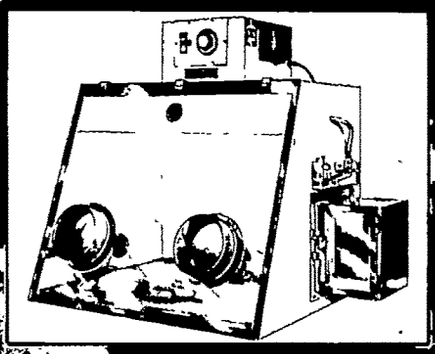
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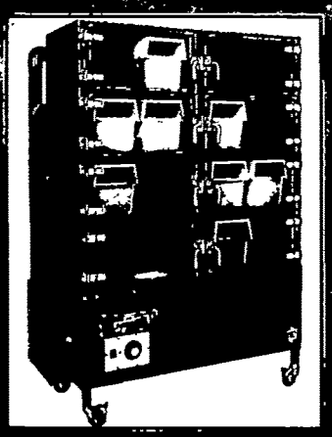
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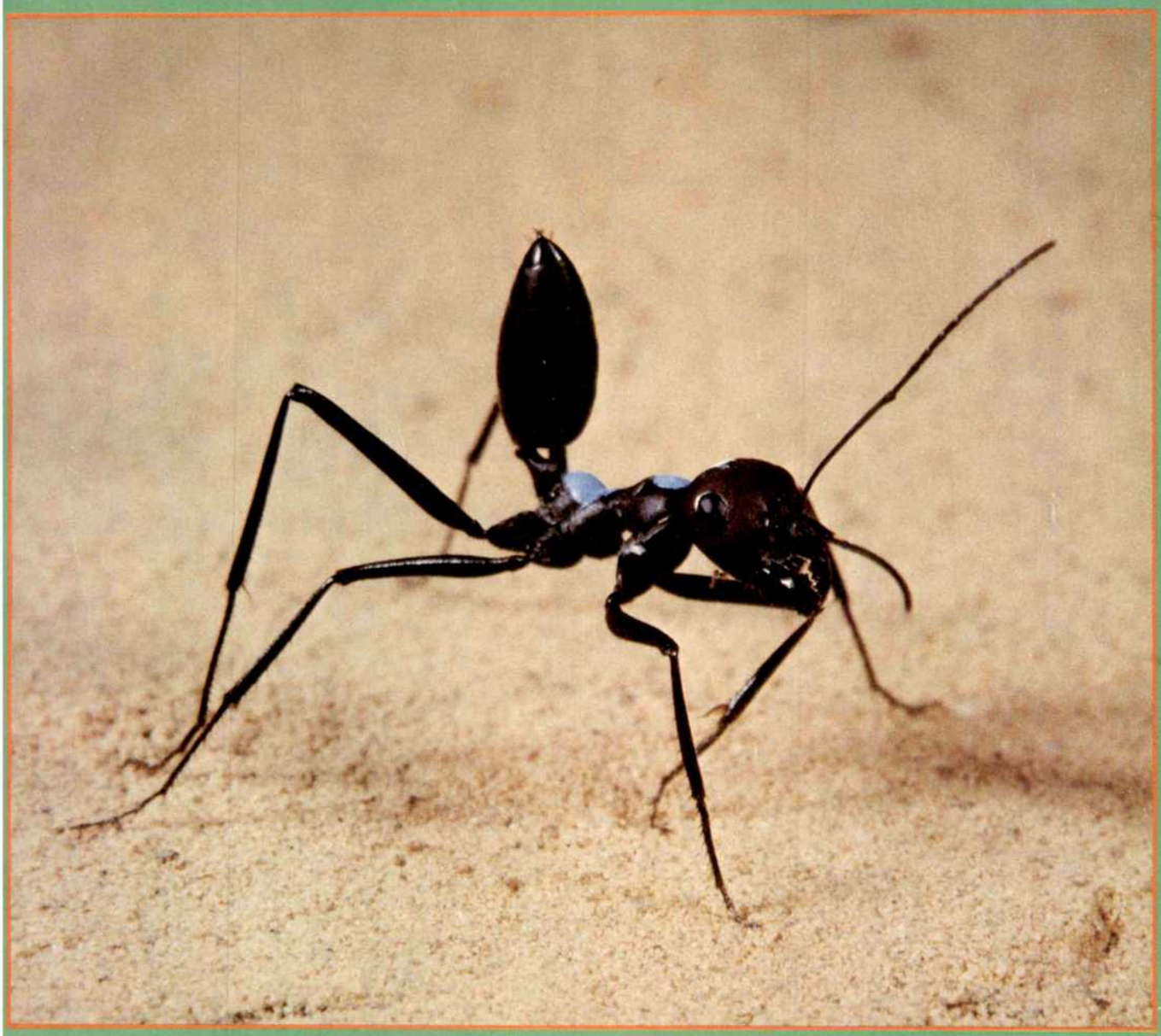
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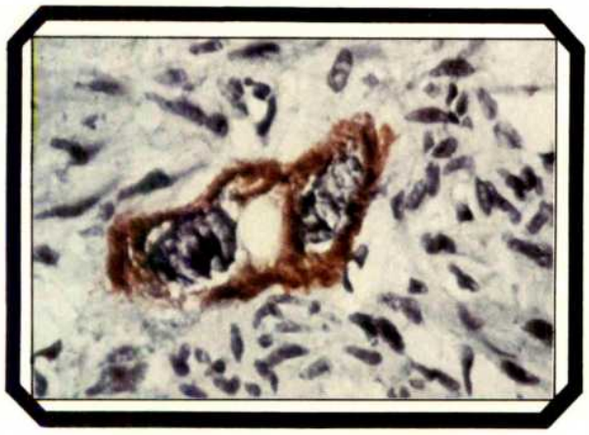
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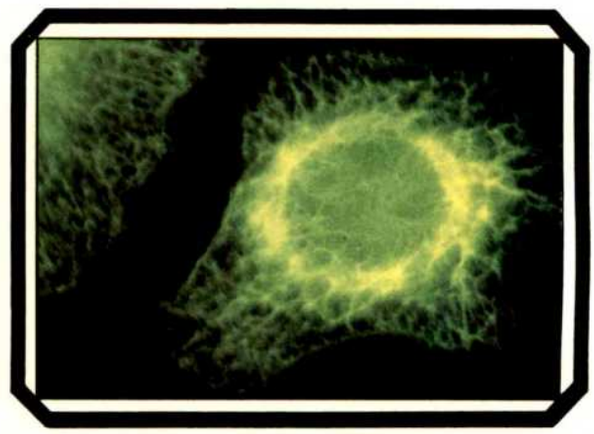
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Two TPA positive cells from a transitional cell carcinoma of urinary bladder epithelium invading a deeper layer of the bladder wall. PAP/haematoxylin staining. Microphotograph by courtesy of Dr P. Oehr, University Clinic, Bonn.



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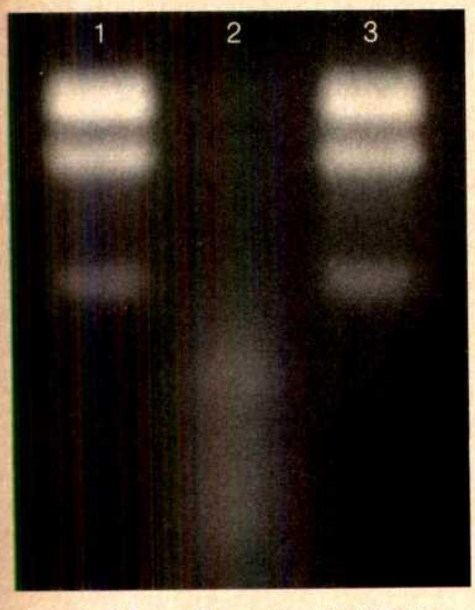
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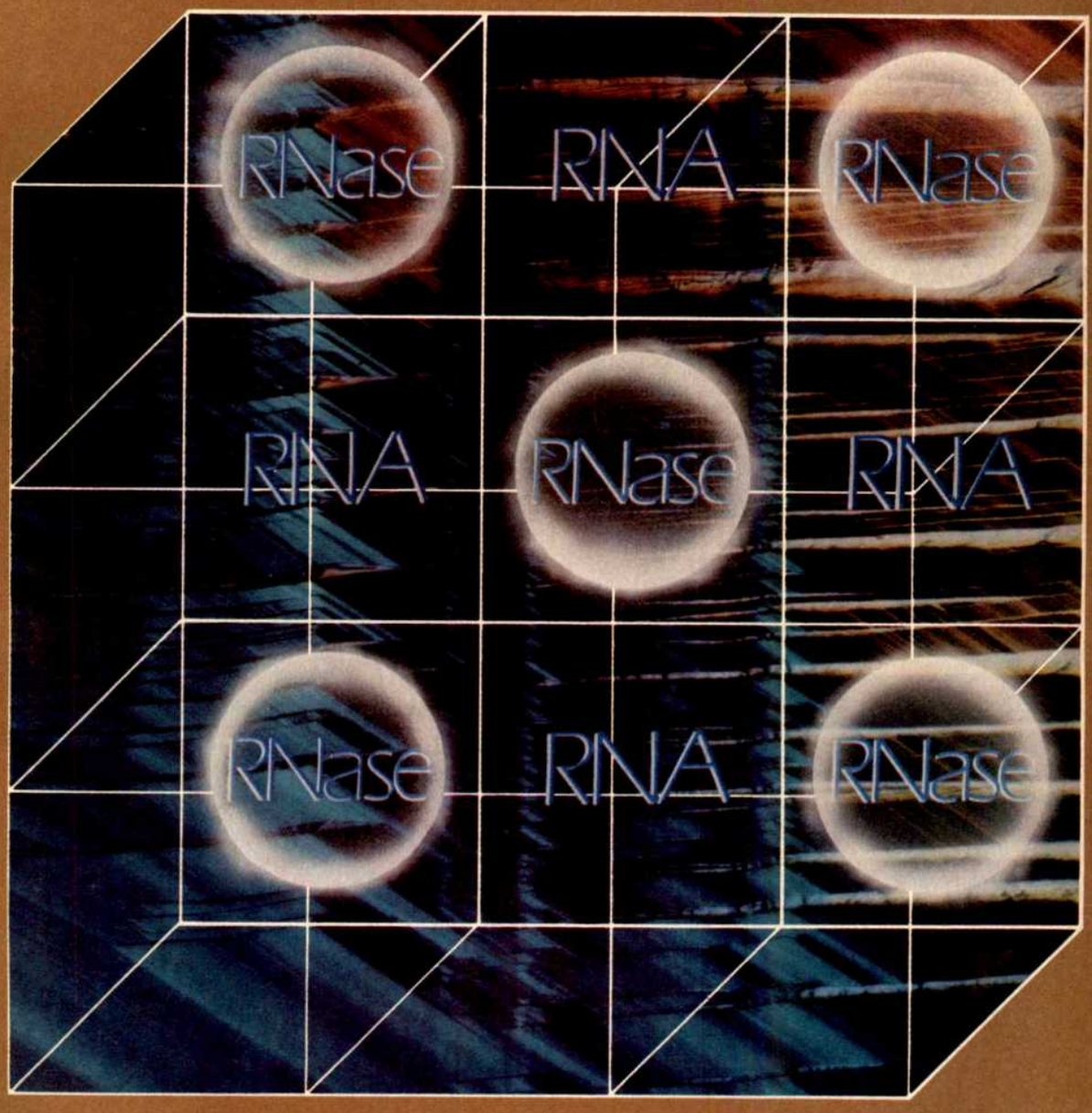
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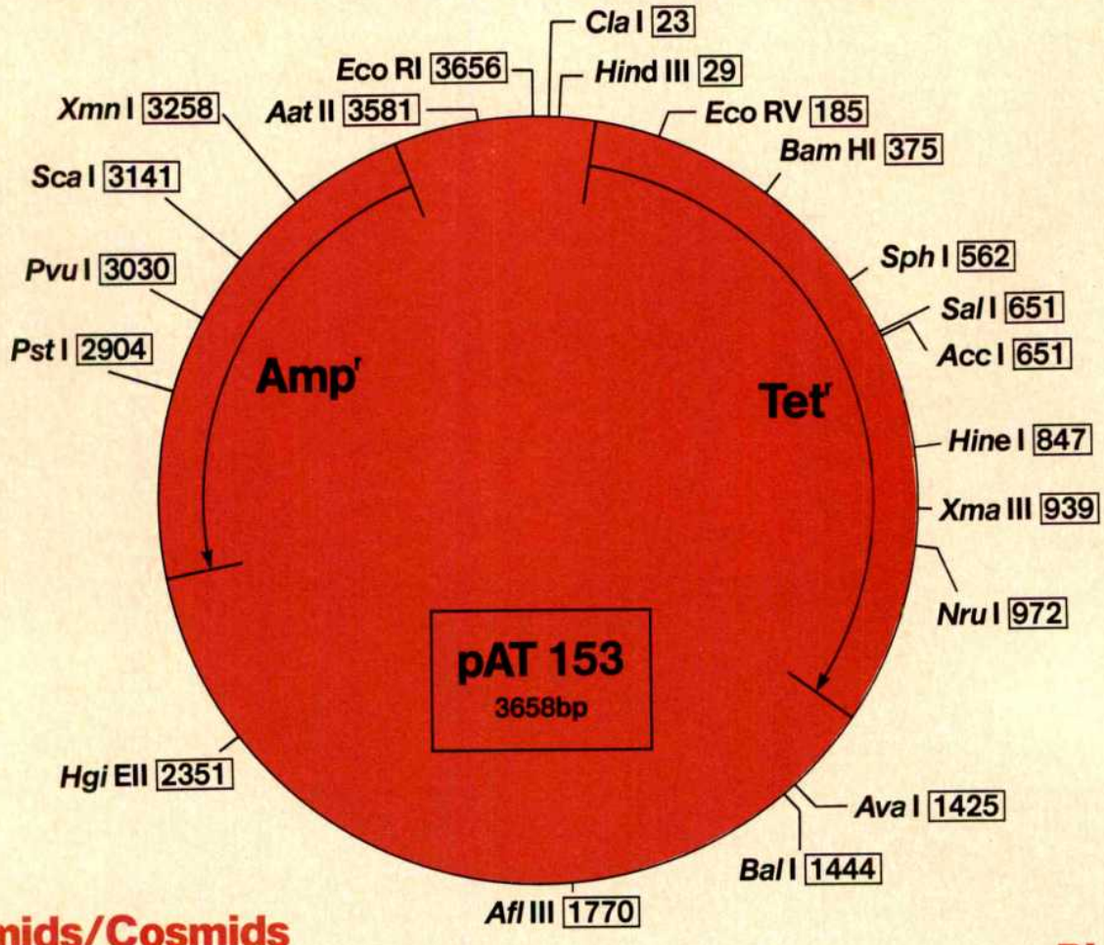
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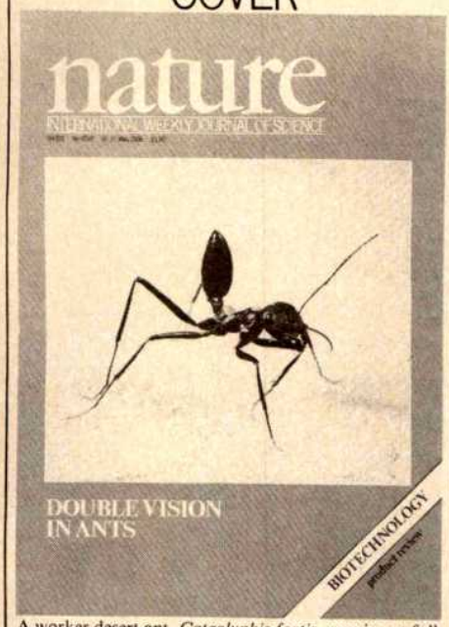
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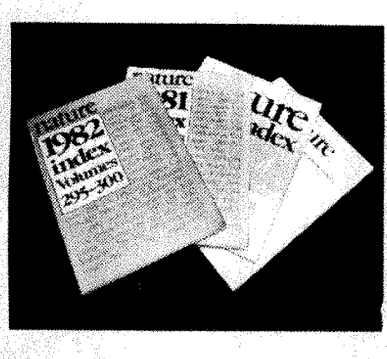
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- Recurrent radio variation
- Transgenic histocompatibility genes
- Transgenic β-globin regulation
- Cortical visual pathways
- Malarial parasite chromosomes

GUIDE TO AUTHORS

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NATURE VOL. 315 16 MAY 1985

President Reagan and Mr Perle

The US administration seems to be speaking with two voices on arms control. Mr Richard Perle, the hard man from the Pentagon, should be the one to opt for moderation.

THE assumption that statesmen have no time to read the newspapers seems to be gaining ground. That, at least, is the simplest explanation why President Ronald Reagan (at Strasbourg) and Mr Mikhail Gorbachev (in Moscow) used the fortieth anniversary of the ending of the Second World War in Europe to tell their separate audiences of the other's malevolence. Although much of the rhetoric may have been directed at Western Europe in general, and not at the European Parliament (Mr Reagan) or a celebratory parade in Moscow (Mr Gorbachev), both declarations will have helped chiefly to reinforce the belief that nobody is in much of a mind to win agreement at the negotiations of strategic arms soon to resume at Geneva. But if the negotiations are again a failure, people may well remember what the superpower leaders were saying last week.

As when a bridge-player can win only when some vital card is on his left, he had better play on that assumption, so it is prudent to suppose that the rhetoric masks a more benign state of affairs. But then the question arises of the remarks of Mr Richard Perle, assistant secretary at the US Department of Defense, who was explaining to a congressional committee again last week why, in general, arms control agreements favour the Soviet Union rather than the United States, why the Soviet Union can be relied upon to break any agreement that may be signed and why, in particular, the United States need not be too meticulous in observing the spirit of the SALT II treaty, the unratified agreement which limits the numbers of strategic missiles which each side may deploy, and which has been broadly followed since it was signed in 1979. Mr Perle, who knows well enough that other people read the newspapers, has never hidden his belief that arms control is a fruitless pursuit. But making such a song and dance about it is bound to give Soviet readers the impression that it is a part of the US administration's policy, which is presumably untrue.

Mr Perle's views nevertheless deserve attention. There is a sense in which agreements favour the Soviet Union. The US system is open to public scrutiny, much of it relentlessly vigilant, while the Soviet system is, by comparison, secret. So, in principle, the Soviet Union can sign an agreement and then promptly break it. That is what Mr Perle suggests. The snag is that the Pentagon's attempts so far to prove Soviet cheating on arms control agreements are thoroughly unconvincing. Allegations of the proxy use of toxic chemicals in South-East Asia are dubious. The allegations of breaches of the threshold test-ban treaty are only marginally convincing, given the uncertainties of seismic interpretation. The surest transgression so far established is the Soviet construction of a phased-array radar at Krasnoyorsk in central Siberia, apparently a breach of the anti-ballistic missile treaty of 1972 (which has been ratified). The United States has a perfect right to demand a better explanation than has been given so far. What Mr Perle leaves out of his calculations is the certainty that the Soviet Union, secretive though it may be, would nevertheless be seriously harmed if it were shown unambiguously to have cheated on a solemn agreement.

In reality, the Soviet Union (like the United States) has a creditable record on compliance with agreements on strategic arms, and did not complain that the US underground test that vented radioactivity to the surface was a violation of the oldest (1953) agreement on nuclear weapons. The agreement not to place nuclear weapons ("weapons of mass destruction") in space has been kept. If anything, the Soviet Union has been more meticulous than the United States in observing the provisions of the Non-Proliferation Treaty (1968) that its partners in the civilian trade in nuclear materials would not be allowed to fashion nuclear weapons from them. Not that either of the superpowers would relish the emergence of other nuclear weapons states. The difference between them is merely that the United States has a more vivid interest in the commercial benefits of the trade, which are often then frustrated by US domestic legislation.

A more accurate version of Mr Perle's conviction that the Soviet Union will always cheat is therefore that the Soviet Union will always honour the letter of its agreements, but will exploit to the full the opportunities it is not formally denied. This, it might be held, is why western Russia was thickly populated with SS20 missiles in the late 1970s, in defiance of the spirit but not the letter of the SALT I treaty (1972), which says nothing about missiles of intermediate range.

Mr Perle now says that the new Soviet missile, called the SS25, which has begun to make its appearance on the ground, is a violation of SALT II because it is an essentially new device, which would be forbidden by the treaty, even though its external dimensions do not differ much from those of the much earlier SS13. Mr Perle's difficulty is that while the treaty allows each side to deploy one new type of long-range missile, the definition of what is new and different is determined (in article IV.8) primarily by the external dimensions (plus or minus 5 per cent). The Soviet Union, which appears to have offered to retire as many old missiles as the new versions it may introduce in the months ahead, may be legally right to claim the SS25 is within the treaty. The issue matters to the United States, which will have to decide in September whether the sea-trials of the new *Trident* submarine will require it to retire an ageing *Polaris* boat. What Mr Perle might gracefully acknowledge is that the faults lie not primarily with the Soviet Union, but in the language of SALT II, which has been overtaken by events in just seven years. Geneva, now, should be partly about the redrafting of this clumsy treaty and partly about its extension to missiles of intermediate range. This should preferably be done before the treaty runs out at the end of December — a deadline specified on the assumption that, by then, there would be more meaningful treaties on the books.

SALT II is therefore not a treaty that people should struggle to see ratified. If that were Mr Perle's case, he would be generally applauded. Where he goes wrong is where he goes further, in saying that the existence of this informal scrap of paper is of no

Biological manuscripts

From the beginning of next week (20 May), Miranda Robertson, Biological Sciences Editor of *Nature*, will be based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees). □

significance — that it counts for nothing more than the anti-ballistic missile treaty, now threatened by the star wars programme. The assumption that these documents count for nothing is a psychological mistake. The most striking evidence to the contrary is that the two superpowers, while knowing that the treaties lack the force of law, have nevertheless chosen to behave as if the treaties were binding on them. But to the extent that the states of Western Europe are interested parties, these scraps of paper are the only assurance they have that events will not get out of hand.

It would serve Mr Perle's purpose just as well, and that of the US administration at the same time, if he were to turn the other cheek and challenge the Soviet Union by demanding that the US Congress should ratify the threshold test-ban treaty in its present form, which forbids the testing even underground of nuclear warheads exceeding the equivalent of 150,000 tonnes of TNT. This, broadly speaking, is the practice to which the superpowers have kept since 1972. Congress in its present mood would sign with very little persuasion. (Only the Senate would matter.) The benefits would be that the Soviet Union would be required to produce a stack of seismic data with the help of which suggestions of violations could be more accurately assessed, even retrospectively. If Mr Perle's suspicions are correct, they would be confirmed. If they are not, sweetness and light would spread. Either way, the fear would be dispelled that the US administration is divided on arms control, showing up at Geneva diligently while thinking like Mr Perle. □

Football squalor

The deaths at a British football game should be an invitation to economic realism.

THE British government is in a tragic dilemma over the game of soccer, otherwise association football. Last Saturday, 53 people were killed (with more than a dozen people still missing) when a wooden structure in which several thousand had been sitting caught fire and burned to a cinder in the Yorkshire city of Bradford. But for most of the winter, the government has been worrying not simply about the British economy but about the phenomenon of soccer hooliganism, the way in which bands of football supporters attack each other, or the other side's players, or sometimes the officials who try to ensure fair play. Football crowds have been a worry for British governments ever since 1972, when even more people were killed than last weekend during a stampede at a football ground in Glasgow.

The causes of last weekend's fire and of the long-standing hooliganism of football crowds are linked. British professional football, long since a spectator sport, is by legend the poor man's entertainment. So it was before the invention of the television, since when attendance at football matches has been falling steadily. Now, it is more accurately a poor professional's living. All but the best of football teams scrape by on a shoestring. Their grounds are squalid places. Sitting room is limited and expensive. Most have to stand for a couple of hours at a stretch, often crowded together in a way almost calculated to provoke violence. Yet so parlous is their financial condition that more than a third of the football grounds in England and Wales have been exempted from the safety regulations made law in 1975.

The simple question prompted by the death of 53 people in a tinderbox is whether it makes sense for a British government which has other things to worry about to shelter from economic reality commercial organizations which cannot afford to protect their customers from avoidable risks, let alone provide them with an environment which induces them to watch the game and not attack each other. Whether a country of 50 million people needs more than 100 professional football teams in the top flight (counting those in Scotland) is a matter that should be determined by market forces and not the government. If the outcome were that some were at a loose end on Saturday afternoons, and forced as a consequence actually to play the game they would otherwise be watching, that might be no bad thing. □

Patent rights

Two years late, British academics are to have title to patents arising from inventions.

Two years have passed since the British Prime Minister, Mrs Margaret Thatcher, promised publicly that inventions generated with public support would no longer have to be offered for exploitation to the National Research Development Corporation (NRDC), but only this week has the government decided that the time for changing the present system has actually arrived. From now on, universities will be free to exploit their own inventions, whether or not they arise from research grants awarded from public funds. NRDC will remain in being (as part of what is now called the National Enterprise Board), and may paradoxically be even more ready in the future than in the past to exploit inventions offered by academic researchers, not least because there will be competition. Individual researchers are likely to be excited (and surprised) by the details of the new arrangements, which should provide them with a powerful incentive to market their discoveries, but their institutions will also benefit, on paper at least. So will British academic research now be further transformed by people's ambitions to get rich?

To a first approximation, the answer is no. The new arrangements for the exploitation of British inventions are no different in principle from those in force in the United States since 1972. Before British academic researchers and their institutions start counting their golden geese (to mix two metaphors), they had better reflect on the frequently disappointing experience of US universities, which have often found that the right to the independent exploitation of innovations is often exercised only with difficulty, while the duty to do so (implied by the US legislation) may be onerous. For what many US universities have discovered is largely what NRDC has been saying almost since its foundation in 1947 — that only a tiny proportion of academically-based patents become money-spinners. A large national organization, with a monopoly right of first refusal, can hope to cover the cost of protecting ideas taken onto its books by the proceeds from a few money-spinners. Smaller organizations, even whole universities, are by definition less able to cover their risks.

Even so, it is right that there should be a change in the arrangements in force in Britain. Although NRDC has never managed to demonstrate the touching belief of its founders that Britain was awash with patentable inventions going to waste, and while its work over the decades has been creditable, it has also accumulated a rich fund of ill-will among academic researchers who consider, rightly or wrongly, that their bright ideas have been too often overlooked. The truth, of course, is that no single organization can hope to make consistently wise decisions in such a vast field. The best approach is bound to be one in which different organizations make independent appraisals of novel developments and then agree to back those they fancy with tangible resources, both money and management. With the persistent pressure on university research funds, and the change in general attitudes towards the exploitation of new ideas, ending the corporation's monopoly right will do much more good than harm.

The consequences of this change for the character of what happens in British academic laboratories is unlikely, in the short run, to be profound. To be an acknowledged success as a scientist or engineer will continue to be an important if no longer a paramount consideration. Moreover, NRDC will retain an active interest, and indeed is plainly planning (with government help) to strengthen its links with university researchers. The most likely change is that there will be other venture capitalists scouring the academic laboratories for exploitable innovations, while established companies will have a further incentive to forge academic links, perhaps with particular departments, by offering help with patent protection. The danger, probably not at this stage serious, is that some universities or parts of them may become so immured in particular relationships that they will cease to be impartial sources of help and advice for industry at large. That is an issue to which university administration should urgently pay attention. □

US–Soviet exchanges

Academy divided over new agreement

Washington

THE US National Academy of Sciences (NAS) is coming under increased criticism for negotiating a renewed scientific exchange agreement with the Soviet Academy of Sciences last January. Details of the agreement, which is still awaiting ratification by the Soviets, are being kept secret by the academy, which has intensified the uneasiness of groups working on behalf of Soviet refusniks and dissidents.

Shortly before the NAS annual meeting last month, a letter, signed by Christian Anfinsen, Paul Flory and Arno Penzias, was sent to all academy members, challenging both the wisdom and the morality of the move to reestablish ties. (The letter was subsequently published in *Science* **228**, 530; 1985). Anfinsen *et al.* asked what had changed "to explain the about-face from the moral stance of 1980", when NAS broke off relations with the Soviet Academy in protest at the internal exile of Andrei Sakharov.

The three said Sakharov's condition had in fact worsened, and, citing NAS's own Committee on Human Rights, that there had been many new arrests of scientists. "To have held the negotiating meeting in Moscow", they concluded, "makes it hard to avoid an unfortunate hat-in-hand image."

A sharper challenge has come from Sakharov's son-in-law, Efram Yankelevich, who is living in the United States. After seeing Dr Frank Press, president of NAS, Yankelevich wrote an impassioned letter, taking Press and the academy to task in the strongest terms for having abandoned his father-in-law. The letter has been widely circulated within the scientific community.

Finally, Richard Perle, the hard-line Assistant Secretary of Defense for international security policy, broke government silence on NAS's actions with a strong criticism of his own. Speaking at a press forum arranged by the Scientists Institute for Public Information in Washington, Perle said that he was "disappointed that NAS is plunging ahead" with renewing exchanges. He argued the familiar administration line that the Soviets gain more from such exchanges than does the United States.

There is little doubt that Press has the support of the NAS membership, but these attacks have put him on the defensive. Press said last week that the differences between him and his critics amounted to a disagreement over tactics for aiding the refusniks and dissidents. He argued that, without face-to-face contacts, NAS's leverage is "non-existent". He said that "sending telegrams that are never even acknowledged may ease people's consciences, but there's no proof they've done any good".

Asked about reports from refusniks that their situation has worsened since he signed the agreement in Moscow (see the letter from Solomon Al'ber in *Nature*, 2 May, p.10), Press said that he had heard "contrary information" from his sources. And a source familiar with the negotiations said that NAS had demanded considerable concessions, including mention of the Helsinki accord in the text of the protocol. Academy officials have also let it be known that a part of the NAS delegation that went to Moscow met with refusniks there; Press himself did not, however.

As for Perle's criticisms, Press said that "he should have asked me what happened in Moscow before he made those remarks", remarks Press characterized as "intemperate".

Press said that Perle had not taken advantage of the opportunity to be briefed about the agreement (as were State Department officials and the US Ambassador in Moscow).

Press also rejects complaints about the secrecy of the document. He said that it is still in draft form and cannot be made public until negotiations have been completed and the text has been agreed by the Soviets.

Stephen Budiansky

Correction

IN the graph illustrating the percentage of approved extramural research grants that are awarded by the National Institutes of Health (*Nature* May 9, p.88), the units on the right-hand axis were incorrectly specified. The range covered should have been from 30 per cent to 60 per cent, not from 3 per cent to 6 per cent. □

Sakharov

Resignation from Soviet academy

Ottawa

THE Ottawa review conference of human rights performance by the 35 signatory countries of the Helsinki Final Act opened last week to a flurry of rumours that Academician Andrei Sakharov and his wife, Elena Bonner, have received permission to emigrate.



These rumours, which did little to defuse the procedural hassles that delayed the formal opening of the conference by several hours, so far remain unconfirmed.

Almost simultaneously came the news, confirmed by Dr Efrem Yankelevich, Mrs Bonner's son-in-law, who acts as Dr Sakharov's personal representative in the West, that Dr Sakharov had announced his intention of resigning from the Academy of Sciences of the USSR, with effect from 10 May, if his conditions of exile are not improved, and if Mrs Bonner is not allowed to travel abroad for medical treatment.

Although the Soviet authorities have, on several occasions, tried to pressurize the academy into expelling Dr Sakharov, these efforts have been resisted even by party hard-liners in the academy as liable to set a dangerous precedent. Dr Sakharov's voluntary resignation, however, would clearly not be in the interests of the authorities, in view of the considerable comment it would arouse internationally.

Moreover, Dr Sakharov, who will be 64 on 21 May, is past Soviet pensionable age, and the loss of his academy stipend, which he has still been permitted to draw, would not necessarily put him at risk of a charge of "parasitism" (being without visible means of support). Dr Sakharov has previously been reluctant to leave the Soviet Union, feeling that his presence there provides valuable moral support for the human rights movement and for victims of oppression. Hence in 1975, he did not collect his Nobel peace prize in person for fear that he would not be allowed to return to the Soviet Union. Since he was exiled to Gor'kii in January 1980, he has been effectively prevented from giving any assistance to his distressed fellow-citizens, and in 1983 he accepted an invitation from the Norwegian government to settle in Norway — provided, of course, that he is allowed to leave the Soviet Union.

Prospects for the Sakharovs to emigrate are not, however, promising. According to Dr Yankelevich, Mrs Bonner, who was sentenced to three years' internal exile to Gor'kii in August 1984, was excluded from the amnesty proclaimed in honour of the fortieth anniversary of the end in Europe of the Second World War. Dr Sakharov's own exile, of course, is the result of an administrative decision and, technically speaking, has no legal validity.

Vera Rich

Genetic engineering

RAC stakes its claim

Washington

IN the face of proposals to create a new layer of bureaucracy to oversee genetic engineering, the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) has begun to assert itself with a new vigour. At the meeting of the committee on 2 May, members voiced concern that other agencies moving to assert jurisdiction over recombinant DNA — in particular the Environmental Protection Agency (EPA) — lack the expertise that RAC has accumulated in its 10-year existence and that basic research could suffer as a consequence.

The focus of concern is the lengthy proposal of the Office of Science and Technology Policy (OSTP) for the establishment of a "coordinated framework" for regulating biotechnology (see *Nature* **313**, 85; 1985). Under that proposal, a Biotechnology Science Board, a sort of "super-RAC", would be created to address generic issues and to settle interagency disputes; in addition, each agency that deals with biotechnology (US Department of Agriculture, National Science Foundation, EPA, Food and Drug Administration and NIH) would have its own RAC to provide advice within the agency.

Although most of this proposed bureaucracy would address only applications for marketing or development of commercial products and would not involve any new statutory authority, EPA has indicated its intention to claim some authority over research. The agency has already said that field tests of microbial pesticides produced by genetic engineering come under the Federal Insecticide, Fungicide, and Rodenticide Act (nothing new), and (a definite departure) that the usual 10-acre exemption would not apply. Under interim rules announced by the agency, anyone who wants to field-test a genetically engineered microbial pesticide on a plot of any size must notify EPA; the agency can then demand a formal application for an "Experimental Use Permit".

EPA also intends to view the DNA in novel microbes as "new chemical substances", subject to the notification and safety-testing requirements of the Toxic Substances Control Act (TSCA). Under that act, EPA must be notified 90 days before the manufacture of such a new substance commences; EPA can demand additional data and with cause can restrict or even ban the product.

In a letter from RAC chairman Robert Mitchell to OSTP, the concern of RAC about the proposal was spelled out; RAC members say that EPA and other agencies appear to be reinventing the wheel, raising questions that RAC was able to lay to rest years ago. And there is strong feeling within RAC that all laboratory research at least must remain the business of RAC alone.

In discussion at this month's meeting, members made it clear that a major function of the committee since its inception — in the committee's own view, that is — has been protecting basic research from overregulation, a feat accomplished largely by providing a forum for defusing public concern and heading off demands for regulation *per se* through a demonstration of cautious self-regulation.

Although EPA's TSCA proposal raises the possibility of EPA's regulating even the laboratory research phase of certain commercial biotechnology products, RAC in fact faces little challenge to its claim to having exclusive oversight over laboratory experiments. The major grey area at this point is environmental testing. The RAC staff, still staggering under the paperwork and legal burdens of the Lindow-Panopoulos proposal for field-testing genetically altered ice-nucleating bacteria, would like to turn it all over to EPA. A proposal by the staff, on which RAC decided to defer action at the last meeting, would allow RAC at its discretion to accept the review of a proposal by other agencies as meeting RAC's requirements. The partial victory by anti-genetic-engineering activist Jeremy Rifkin in the Court of Appeals (see *Nature* 7 March, p.6) has opened the way for continual court challenges of the adequacy of RAC's approval of Lindow's experiment and other environmental-release experiments, another headache that the staff would as soon avoid.

At least a part of the RAC membership, however, is less ready to concede that jurisdiction to EPA. RAC has adopted an informal document to guide those submitting proposals for environmental-release experiments (*Points to Consider for Submissions Involving Testing in the Environment of Microorganisms Derived by Recombinant DNA Techniques*); it was noted at the recent meeting that RAC enjoys the flexibility easily to amend this document with experience; the regulatory agencies face a much more ponderous process.

Stephen Budiansky

US education

More engineers needed

Washington

THE chronic shortage of engineering faculty at US universities has arisen largely because engineering professors are paid $10-12,000 a year less than their counterparts in industry, according to a $900,000 study of engineering education* published last week by the National Research Council (NRC). And although the number of engineering PhDs awarded each year will increase over the next few years to 4,000 in 1988 (from 2,800 in 1983), this will still not be enough to meet the increased demand from industry and academic institutions.

According to NRC, 8.5 per cent of engineering faculty positions are unfilled, and 6,700 new appointments would have to be made to restore student/staff ratios to the levels common in the mid-1970s. The situation is unlikely to improve greatly unless a university career is made more attractive for talented researchers. An associate professor in his 30s, for example, will typically earn less than $38,000 a year.

NRC calculates that as many as 30 per cent of all US engineers work for the government either directly or indirectly, and uses this statistic to argue that both government and industry would be working for their own benefit in making advanced engineering studies more attractive. Legislation should be introduced that would "facilitate" gifts of laboratory equipment to engineering colleges. And the federal government should be prepared to match dollar for dollar funds raised for new buildings.

NRC makes surprisingly little attempt to analyse future demands for different engineering skills, but does predict that the number of engineers needed will increase. The lack of hard numbers is blamed on the poor quality of existing databases, and NRC asks in passing that the National Science Foundation (NSF) should do something about it. One somewhat sensitive problem, however, is that more than 40 per cent of graduate students studying engineering in the United States are foreigners on temporary visas, many of whom will return to their own countries after graduating. NRC wants to see more US citizens in the pool.

The NRC study is critical of the way that most federal support for engineering is channelled into a relatively small group of "first tier" colleges specializing in graduate education. More than half of engineering graduates with bachelor of science degrees graduate from the disadvantaged second tier colleges, however.

NRC is laconic when it comes to what exactly should be taught, however. The study concludes, on the basis of verbal arguments, that it might be advantageous to delay specialization in some arcane fields in order to improve management and communications skills, apparently often lacking in engineering graduates; again, NSF is asked to experiment with novel courses.

The NRC study was supported by NSF, the National Aeronautics and Space Administration, the armed forces and industrial corporations.

Tim Beardsley

*Engineering education and practice in the United States: foundations of our techno-economic future. National Academy Press, 1985.

Star wars

Franco-German accord?

THE disagreement at last month's summit meeting between France and West Germany over participation in the US star wars programme seems to be blowing over. President François Mitterrand seems to have offended Chancellor Helmut Kohl at the Bonn meeting by saying that France would not take part, and seems himself to have taken offence at West Germany's willingness, which was taken as a sign of disinterest in the alternative French proposal of a European programme of high-technology research, called Eureka. It is now expected that these differences will melt away when Mitterrand visits Bonn later in the month, but meanwhile it has come to light that two major French companies are planning to go ahead with star wars research in any case. So what exactly did Mitterrand's gesture mean?

M. Pierre Aigrain, research director of Thomson-CSF, one of the two French companies suspected of star wars complicity, said last week that he "didn't know". And others, pointing to the electronics and armaments company's continuing heavy losses, suggested that if Thomson-CSF could (quietly) find a slice of the $26,000 million star wars budget, nobody in France was going to complain too much.

Aigrain, himself an ex-minister (of research), agrees that, on the whole, Mitterrand is right to be wary of arrangements in which Europe would act merely as subcontractor to the United States. But would Thomson-CSF itself consider such deals? "That's something we would look at on a case by case basis", said Aigrain. His company is among the world leaders in higher power vacuum-tube technology. "If we were asked to develop a special high power tube, I don't know whether we'd say no. I think probably we'd consider it if the funding's all right and so on. A company will always react by thinking about its own profit." When it comes to research funding from abroad, however, a company would need government approval.

Aigrain considers, as does the French government, that in the Strategic Defense Initiative (SDI or star wars) programme, the United States "is at least as much looking for a way of advancing technology as it is in developing an active weapons system". In Europe, this technology should be developed by European cooperation, Aigrain believes, thus defending President Mitterrand's proposal of a major civil programme, Eureka, to match those aspects of SDI capable of civil application.

"The big problem is that until now, with the exception of the European programme of research in information technology (ESPRIT), Europe has been spending extremely little on joint technology programmes". Even ESPRIT is costing only one-fiftieth of the SDI budget. Thus Mitterrand's Eureka "is a way to say we've no option but to spend a lot more money on advanced technology. As far as this is concerned I can only say I'm in favour of it", said Aigrain.

The money should not come from extra taxes, but from rechannelling existing, poorly-spent sums. In telecommunications systems, Europe spends more on research and development than the United States and Japan put together, according to Aigrain. But much of the spending is duplicated and "rather ineffective".

The problem with SDI for Europe, says Aigrain, is that the programme has been put under the military. "It's true in every country in the world that the defence establishment is usually the most efficient user of technological advance. One may not like it but it's true. The problem in Europe is that we don't have a military establishment at European level. but I don't think we should have this until we have a much greater level of political unity."

The most important spin-off from SDI will be in communications and systems, Aigrain believes. "It's obvious — people are always speaking of the basic weapons part of SDI but a much bigger challenge is the automatic collection of information, processing the data, making an intelligent system, says Aigrain. He believes the development and civilianization of such technology may be the biggest effect of SDI.

Robert Walgate

Royal Society

All change ahead in London

THE Royal Society of London seems to be well launched on a period of organizational change, with a new management structure, new people in top posts and with ambitions to exert more influence on public policy. The immediate occasion for the change is the retirement of Dr R.W.J. Keay from the post of executive secretary of the society with effect from 20 May.

Keay is to be succeeded by Dr Peter T. Warren, deputy secretary since 1977. The society has taken this as an opportunity to equip itself with a middle management structure. The post of deputy secretary has been abolished, but instead there will be three officials responsible for national, international and financial matters.



Sir George Porter.

Mr Peter Cooper, a member of the society's staff for the past ten years, will be the assistant secretary responsible for national affairs, Mr S.J. Cox (now at the British Embassy in Washington) will look after international affairs while Mr N.B. Parfitt, now the society's finance officer, will be in charge of finance and administration. One of the officers of the society says that the need for the new structure has been appreciated for some time, but that the change has been delayed by lack of funds.

Among the new directions in which the society is embarking is that of providing a more systematic commentary on the condition of British science than has been possible by means of the *ad hoc* studies carried out by voluntary committees, which have nevertheless been a growing part of the society's work in the past five years.

The plan now is to set up a small unit, comprising perhaps three professional people, to compile a database describing the pattern of British academic research and eventually to produce analytical papers which, it is hoped, will guide the often acrimonious public debate about the scale and mechanisms of research support in Britain. To ensure the independence of this unit, whose acting head is Dr Peter Collins, the Royal Society plans to meet the costs out of its own funds, not from those supplied by the central government.

Meanwhile, the Royal Society is also preparing for one of its quinquennial changes at the top. Sir Andrew Huxley, president of the Royal Society for the past five years, will retire on 30 November and be succeeded by Sir George Porter, director of the Royal Institution since 1966, who shared a Nobel Prize in 1967 for his work on the use of lasers in the study of rapid chemical reactions. Porter plans to retire from the Royal Institution, but a date has not yet been fixed.

The effects of this change are, as always, unknowable. Sir Andrew Huxley, thought before his election as president to have been shy of public controversy, has been outspoken on a variety of issues, ranging from the Soviet treatment of scientists and the rights and wrongs of cladistics to the British government's regard for basic science. Dogged determination has been the watchword in the past five years.

Porter is more mercurial than Huxley, and has earned a reputation as a popularizer by means of his work with television, which fits in with some of the society's ambitions to improve the public presentation of science. □

Japanese environment

Rediscovery of pollution

Tokyo

PUBLIC outcry over pollution has almost vanished in Japan as the once notorious Tokyo smog becomes just a memory of the 1970s: the feeling is that the really big problems have been beaten.

The White Paper (policy statement) just released from the Environment Agency thus comes as something of a shock, detailing a host of new and more insidious problems.

There is no doubt that major battles have been won in the control of air pollution. Ten years ago, photochemical smog warnings were issued on close to 300 days of the year and some 5,000 victims a year were being registered in Tokyo alone. In 1965, things got so bad in Yokkaichi, a refinery port close to Nagoya, that all primary school children in the most polluted parts of town were issued with special face masks — not that they appeared to be effective. Now, nitrogen dioxide levels in the air are steadily declining in most parts of the country, despite increased traffic, and smog alerts are down to some 130 days a year.

Where new problems are arising is in the pollution of rivers and lakes and in the mass movement of population to new satellite cities with an infrastructure ill-prepared to receive them. Japan's largest lake, 674 square kilometre Lake Biwa, provides a classic example. The population of Shiga prefecture, in which the lake is located, is growing very rapidly as people move out of the nearby Osaka and Kyoto conurbations in search of a better environment and industries are relocated where land is cheaper.

The resulting effluent pouring into the lake, coupled with acid rain from the huge industrial areas nearby, is now rapidly turning it into a graveyard. The shallow southern half of the lake is in a state of almost total eutrophication, and the northern half is plagued by "red tides" — freshwater algal blooms. Attempts to cut the input of phosphates, by banning sale and use of phosphate detergents, for example, began in 1980 but came too late; it is unlikely that the lake water will show major improvement this century.

Similar problems are seen in many other lakes and rivers, and pollution is spreading into underground waters. Analysis of water in wells shows that some sites are contaminated by carcinogenic organo-chloro compounds at 600 times the levels recognized as safe by the World Health Organization, a serious problem given that 30 per cent of Japan's drinking water comes from underground sources and that the sources of pollution are virtually untraceable with present understanding of underground water flow.

All this highlights the way in which movement of population from the countryside and giant cities into suburban satellite cities, accompanied by an increasing reliance on automobiles, has not been accompanied by adequate attention to sewerage and water supplies. "A glut of Walkmans and a shortage of sewers" was how experts from the Organisation for Economic Cooperation and Development recently summed up Japan's emphasis on private rather than public expenditure. Noise, traffic jams and garbage disposal problems are also becoming more severe in the new cities as they inherit the crises of the larger cities, and with Japan's urban population expected to reach 70 per cent of the total by the end of the century, there is little hope of a let-up in the pressure. But creating new urban infrastructures, or the greater dispersal of population and industry that the white paper calls for, cannot be achieved without massive expenditure that the government seems unwilling to make.

To cope with deterioration of lake and marshland, the Environment Agency has introduced legal limits on the levels of phosphorus and nitrogen effluent that can be discharged by industry. Japan is the first nation to attempt to control both factors. Initially stringent limits were, however, reduced after opposition by the Ministry of International Trade and Industry (MITI), highlighting the Environment Agency's own lack of power. Although the agency was originally intended as a single comprehensive pollution control body, when it was set up in 1971 it found that ministries with regulatory powers over pollution, particularly MITI, were unwilling to relinquish them. The result is that the agency is a watchdog without teeth: it can plan and coordinate policies but is not responsible for enforcing them, except in the case of wildlife protection.

In this area, the Environment Agency has just shown a willingness to respond to international concern by setting up a new "Headquarters for Wildlife Protection Measures". Severe criticism of Japan from abroad, reinforced by the visit of Prince Philip, Duke of Edinburgh, in his capacity as president of the World Wildlife Fund (see *Nature* **311**, 698; 1984), for failing to obey the Washington Convention prohibiting the import of endangered species, was the chief stimulus to the establishment of the new office. The number of customs houses through which wildlife can be imported has been reduced to nine and officials are given training in spotting illegal imports.

The white paper itself, however, pays scant attention to international issues. Although it charts the destruction of tropical rain forest in South-East Asia — destruction in which Japanese logging companies and Japan's voracious demand for timber have played a large part — it does not call for any action other than membership of standard international environmental organizations. **Alun Anderson**

French universities

New watchdog barks well

Paris

UNDER a dozen glittering candelabra that must each weigh a ton, beneath the frescoed ceilings of the Elysée Palace and surrounded by much of the academic élite of France, President François Mitterrand last Friday gave his full dignity to the latest effort to reform the French university system, the establishment of the Comité National d'Evaluation des Universités, or CNEU.

The purpose was serious, and the guests were hopeful, especially the director-general for the universities at the ministry of education, M. Jean-Jacques Payan, whose cheerful face has been proclaiming lately that he thinks his job now done. The 75 French universities, many created shortly after the 1968 student revolt, and mostly egalitarian and left-wing in spirit, have been opened up to once-hated industry and now, through CNEU, to competition among themselves.

The committee "responds to an evident need" said President Mitterrand on Friday. "It signifies for our country an innovation of major importance." It represents the increasing autonomy of the French universities (sweet words to the relatively powerless university presidents) whose counterpart is "the objective evaluation of their strengths and weaknesses" (rather more bitter words for the feebler institutions).

CNEU will be "entirely free" to make its own judgements and will report each year to the President. M. Mitterrand said, "I expect reports to have concrete effects" on the decisions of the French administration. (CNEU will not directly control and divide funds as does, say, the British University Grants Committee.)

The French universities, described by M. Henri Tézenas du Montcel, who resigned as president of the University of Paris (Dauphine) to write a critical book on his *alma maters*, as those "foggy and smoky" places, are now required, said M. Mitterrand, "to be more transparent and to develop the best among them".

The universities must obey three principles, he said: autonomy, which the President expanded to mean obedience to the recently-introduced system of research contracts agreed between the ministry of education and each university, assessed *a posteriori*; "emulation", a soft word for "competition" in which each university must attempt to emulate the best elsewhere; and quality, "the condition of success in scientific competition... and world technology, which greatly depends on the success of our universities and scientists". On this success the French people depend. And the President depends on CNEU to help increase the quality of the univer-

sities "and therefore the chances for France".

M. Laurent Schwartz, like M. Tézenas du Montcel an old critic of the French universities, is presiding over CNEU, but had raised himself from his sick-bed to deliver his opening address last Friday. He pronounced himself "sensible to the solemnity" that the French President was giving to the installation of his committee, and promised to make of CNEU "an instrument fundamental to the success of the French universities".

According to Schwartz, French universities have been subjected until now to little real objective evaluation, either internally like the US universities, or externally, like the British. In France, there has been only piecemeal evaluation by the research councils, or by the national university promotions committee (the Conseil Scientifique des Universités). CNEU would introduce global evaluation, something "much more ambitious".

"The question is to make known to each establishment, to the minister of education and to public opinion how the universities are doing branch by branch and globally." The better a university does in its evaluation, the more autonomous it should expect to become. CNEU would not distribute money directly, as that might result, in the long term, in "equipartition".

The regulations defining CNEU give it an "entirely new" freedom of movement, said Schwartz. It will have "absolute independence". Many organizations in France are governed by strict rules penned out in minute detail. But "the decree that establishes our committee leaves us on the contrary complete freedom to evaluate as we wish". If only, dreamed Schwartz, this atmosphere of confidence and liberty could extend to other organizations — such as the universities themselves.

"We must combat taboos and corporatism", said Schwartz. "We must understand and make understood: that demands courage and objectivity. We will force ourselves to have them. The grandeur of our task is impressive but is also fascinating."

The 15 members of CNEU will not carry out all the evaluations, but will appoint outside independent experts, said Schwartz. CNEU will use international experts as much as possible "as is done throughout the world". CNEU will not evaluate individuals, but will work at the level of groups and above. It will consider both research and teaching together, "for to separate research and teaching would kill both".

"CNEU will not behave like a policeman" said Schwartz. "Evaluation is totally contrary to Napoleonic control. We shall not be checking that the universities follow all the rules. On the contrary, we shall see if the universities can innovate, if they can launch audacious projects: we shall support initiative and not conformism or retreat."

Robert Walgate

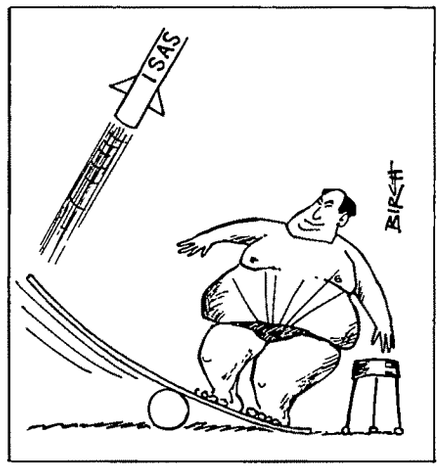
Japan in space

David keeps Goliath guessing

Tokyo

THERE seems no limit to the ambitions of Japan's Institute of Space and Astronautical Science (ISAS), the tiny academic cousin of the government-run giant National Space Development Agency (NASDA). Despite a shoestring budget and home-grown solid-fuel rocket technology, the agency is determined that Japan should be the third country to send a satellite to the Moon.

ISAS has just revealed that it is drafting a detailed programme to launch Japan's first Moon observation satellite in fiscal year 1989. Under the plan, a satellite will be placed in Earth orbit and, after a day for accurate orbital alignment, will be blasted off for the Moon by a "kick motor". On its first pass, the satellite will be slung past the lunar surface at an altitude of about 6,000 km, one month later reaching an apogee of well over 1 million



km. Thereafter, it will be catapulted back towards the Earth-Moon system and, after a second "near miss" of the Moon, will enter a highly eccentric Earth orbit stretching across the Moon's path. Two months and four Earth orbits later, the satellite will then whip off on another two-month loop around the Moon, or so the theory goes, but according to ISAS's "gunnery lieutenant" Hiroki Matsuo, professor of orbital dynamics, much more detailed calculations will be required before he can predict exactly where Japan's first Moon shot will end up.

Given ISAS's resources, it is a wonder that this organization can even contemplate a lunar mission. Japan is unique in having two space organizations, each equipped with its own satellites, rockets and launch sites. The larger NASDA, affiliated to the Science and Technology Agency, is in charge of the development of applications satellites and their launch vehicles, while ISAS, affiliated to the Ministry of Education, Culture and Science and operating on a fraction of NASDA's budget, carries out research and development of scientific satellites and launch vehicles. NASDA uses liquid fuel rocket technology developed almost entirely under US licence but ISAS has its own "home-made" solid fuel launchers.

In the 1960s, when ISAS was part of Tokyo University rather than a national institute, the Japanese government asked the institute to limit the size of its rockets, to avoid competition with NASDA and to prevent the academic organization getting too big for its boots or so it said. By the early 1970s, ISAS had already reached the 1.41-m limit on the tail diameter of its rockets, since when its MU-series rockets have been getting taller and taller but no wider, at least at the base. With the Moon shot, however, ISAS is reaching the limits of ingenuity in what it can do with its present launchers.

To kick the lunar probe out of Earth orbit and off to the Moon, ISAS plans to add a 500-kg fourth stage to the MU-3SII used in the successful launching of the Halley's comet test satellite, *Sakigake* (see *Nature* 17 January, p.175). Add to this the 170-180 kg weight of the satellite and the payload capabilities of MU-3SII will be stretched to the full, whence ISAS's cunning plan makes gravity do most of the work. Launch dates and total budgets for the programme have not yet been agreed but Y160 million ($0.6 million) has been approved for systems design in the current fiscal year.

Unlike previous programmes, the lunar programme, codenamed MUSES (MU Space Engineering Satellite), is aimed largely at engineering studies of control of the speed and orbit of the satellite and, according to Professor Matsuo, no scientific instruments will be carried on the first satellite, MUSES-A. However, a possible successor, MUSES-B, may carry a scientific package to be dropped to the lunar surface.

But the big question in the minds of the institute's members is what more can be done with present launch facilities. Among the projects under consideration are a solar physics project for observations of solar flares that would, ideally, be carried out at the next solar maximum in 1991, but which would clash with a plan, in conjunction with the US National Aeronautics and Space Administration, to launch a satellite from the space shuttle which would follow a similar profile to MUSES-A. So the solar physics project will probably be scheduled for 1992.

Also under consideration is a project to examine diffuse X rays following on the successful series of X-ray satellites, while the institute also has more grandiose ambitions to send satellites to the planets which would be beyond the capabilities of MU-3SII. Using NASDA launchers would help, but NASDA has its hands full while ISAS cherishes its independence.

David Swinbanks

French research

End to growth in sight?

MORE is not enough for the French national research council, CNRS (Centre National de la Recherche Scientifique). Despite a real increase in its spending since 1981 of over 35 per cent, a figure only to be dreamed of on the other side of the English Channel, CNRS is pressing the ministry of research for a further increase this year. And that increase should be double last year's, at least in support for laboratory equipment and materials, and approach a real 10-12 per cent annually for the next three years, CNRS director-general Pierre Papon is claiming.

But research minister Hubert Curien, whose new three-year plan for the future of French science is now before the prime minister Laurent Fabius, has warned CNRS that it may have to be content with what it has. And with the French government facing a possible trade deficit this year as opposed to a planned surplus, and Fabius refusing to win votes in next year's parliamentary elections with a spending spree, Curien may well be right.

Papon, however, says this may mean cuts in the CNRS 5-7 year plan announced recently (see *Nature* 2 May, p.5), in which 20 research areas were selected for special effort. That plan itself required some hard choices, claims Papon, and to develop it requires the re-equipment of some laboratories, particularly in minicomputing and other "middleweight" expenditure.

But has the period of plenty at CNRS, which amounted to an average real increase of 5.5 per cent a year in total budget for the five years 1981-85, not left the organization sitting pretty, with cash to spare? Not according to some outside observers, who judge that President Mitterrand's policy of "solving the economic crisis" by spending on science and technology has left basic science, the fields mostly supported by CNRS, protected from cuts, but not truly expanded. The 5.5 per cent "real" increase against French domestic inflation has been swallowed up by a falling French franc against harder currencies in which most scientific equipment and materials must be bought (typically dollars, marks and Swiss francs).

Nevertheless, CNRS is attempting to share equipment costs, and technicians, by creating "federated institutes" around well-instrumented laboratories, linking three to five existing groups around genuine joint research objectives. This will have the double advantage of reducing costs and of unifying an often fragmented French research effort. But still "money is the big problem" Papon says, thus shedding a disturbing light on those research councils elsewhere whose budgets have remained fixed if not falling.

Innovations other than more money that CNRS would like to see in the new plan, which should go before parliament in July, include an increase in the number of studentships for people studying for one of the new PhD degrees, more incentives to mobility of scientists, and the establishment of more CNRS-linked laboratories in the *grandes écoles*, the élite engineering schools which tap the best talent emerging from French secondary education.

Although these schools produce some 12,000 engineers a year, only 5-7 per cent go on to do a PhD (or the previous equivalent, "third cycle" diplomas), according to CNRS figures. This lack of scientific training is a "real handicap" for French industry (of which the graduates of the *grandes écoles* often become leaders), according to Papon.

Robert Walgate

CNRS spending

Over the period 1981-95 inclusive, the spending of the French CNRS (which supports 10,000 of its own scientists, 25,000 engineers and technicians, and many more scientists by means of grants at universities) is estimated to have increased (in real terms) at average annual rates of:

- **2.9 per cent in nuclear and particle physics (excluding international subscriptions).**
- **5.4 per cent in other basic physics and mathematics.**
- **8.4 per cent in physics for engineering.**
- **5.1 per cent in chemistry.**
- **4.1 per cent in Earth sciences and astronomy.**
- **6.0 per cent in life sciences.**
- **6.0 per cent in social science and humanities.**
- **14.5 per cent in interdisciplinary programmes.** □

Charitable foundations

Wellcome Trust plans asset sale

THE British medical charity the Wellcome Trust, whose only substantial asset consists of 100 per cent of the shares in the Wellcome Foundation, the successful pharmaceutical manufacturer, is planning to increase its income and diversify the source from which money flows by disposing of a fifth of its holding in the company.

The immediate attraction of the proposed share sale is that the trust could expect to realize a substantial sum of money from the sale which, invested in high-yielding securities, might even double its income, which is estimated to amount to £23 million during the present year. During 1984, the Wellcome Foundation earned a profit (after tax) of £48.6 million on a turnover of £806 million.

The share prices of comparable companies, such as Glaxo, are at present not very different from 20 times the net earnings per share, which would value the Wellcome Foundation at something like £1,000 million.

A statement put out by the trust earlier this week emphasizes, however, that diversification is also a large part of the trustees' motives in disposing of part of their shareholding. The trust says that the trustees have "for some time been concerned about the wisdom of having all their eggs in one basket". But the statement also says that after the disposal of 20 per cent of the shares, the trust will not sell further shares for at least two years, and that it will not allow its shareholding to fall below 50 per cent.

The chances are that the new arrangement will also benefit the foundation, whose attempts to behave as a thoroughly commercial organization have always been cramped by the relationship with the trust. The relationship has, among other things, prevented the company from rewarding its senior employees by means of share options and from growing more quickly by the acquisition of other companies in return for its own stock. The second constraint will remain so long as the trust retains a controlling interest.

The relationship between the trust (which is technically a charitable foundation) and the foundation (which is a commercial company) derives from the will of Sir Henry Wellcome, who died in 1936 leaving to the trust which he created the sole ownership of the company, originally Burroughs Wellcome Ltd. The company was renamed the Wellcome Foundation in 1926.

Over the years the relationship between the trust and the foundation has been curiously ambivalent. Each has been jealous of its independence from the other, with the company repeatedly in difficulties in explaining to its dependent charity why its annual share of the commercial profit has to be substantially less than 100 per cent. (In 1984, the trust received £17 million of the total profit of £48 million, most of this by way of payments under deeds of covenant allowing the company to economize in tax payments.)

Even so, the trust is now the largest of the private foundations in the United Kingdom.

In a quite separate development, Sir John Vane, research director at the Wellcome Foundation for the past eleven years, announced last week that he is to leave the company so as to devote himself to his scientific interests. Vane, who was awarded the Nobel prize for his account of the mechanism of action of aspirin, said last week that he has no immediate plan to do something else. □

UK agricultural research

Penury brings radical reappraisal

ADVERSITY (mostly financial) has persuaded the most threatened of the British research councils, the Agricultural and Food Research Council (AFRC), to canvass a radical plan of reorganization. One proposal would reduce the number of separate institutes from about twenty (with seven separate Scottish research institutes) to perhaps half that number.

It is also proposed that there should be a management board for agricultural research to which would belong research council officials and a representative of the Department of Agriculture and Fisheries, Scotland, as well as the "ten or fewer" institute directors.

The discussion document, *A long-term view*, says that the number of research institutes would be reduced "by successive amalgamation and restructuring", and that wherever possible institutes will be sited on or near a university campus. Under such a regime, institute directors would have a greater degree of independence in the direction of management of the research for which they were responsible. The document suggests that it should be possible, within such a framework, to give research groups more flexibility, by specifying "the questions to be answered rather than the areas of science to be studied".

Part of the council's management problem is that many of the research institutes for which it is the chief source of financial support are not formally directly under its control, but are historically independently constituted. The Rothamsted Experimental Station is one of these. Thus, in the discussion document, AFRC suggests that people whose salaries it pays should in future be its own employees, which would entail both a change of status and, for some people, a greater degree of insecurity. No doubt the council would benefit through the greater freedom it would have to move people from one place to another.

The discussion document says little about its immediate origin, the proposed reduction of funds for research commissioned by the Ministry of Agriculture, Fisheries and Food of £10 million in the current financial year and twice as much during 1986-87. The question is to be considered by the priorities board for research in agriculture at a meeting later in the summer, but it appears that there is very little prospect of relief from the Treasury edict that these substantial sums of money should be lopped from what has become, in effect, part of the council's budget.

Among the other proposals put forward in the discussion document is the suggestion that the need to avoid duplication of research effort within Europe may make it necessary for the council to withdraw from some fields of research.

But the council is plainly also hoping that it would win research support from elsewhere for some of the research that it carries out at present. The document also repeats the council's expressed intention, during the past several years, of increasing its income from research contracts with private industry as well as from other government departments than those responsible for agriculture.

On people, the council promises to consult trades unions on the reorganization outlined in the document, and says that it acknowledges the need that creative scientists should have a secure environment in which to work. But it also says that it needs to plan for change, for which reason it will continue to recruit many of its staff on short-term contracts.

Flying a more adventurous kite, the council says that part of its case for continued existence is that AFRC is responsible for the "largest non-medical biological research capability" in Britain, which will be widely taken as a reference to the fondness of Sir Ralph Riley, the recently-retired secretary of the council, for the creation of a new research council with responsibility for basic biological research, medical and non-medical.

Comments on the discussion paper are invited by 15 July. □

Tumour necrosis factor

Drug agency jumps TNF gun

Washington

IN an unusual move, the US Food and Drug Administration (FDA) has allowed human tumour necrosis factor (TNF) produced by recombinant DNA technology to be given to a cancer patient. FDA had previously maintained that there are insufficient animal data on the effects of TNF to approve clinical trials, but it has now allowed Dr Herbert Oettgen of Memorial Sloan-Kettering Cancer Center in New York to treat a gravely ill patient with TNF manufactured by the Asahi Chemical Industry company of Japan.

In the normal course of events, FDA grants a so-called Investigational New Drug exemption — permission to enter clinical trials of a putative therapy — only when there are sufficient data to provide a reasonable expectation that the therapy will be safe and effective. FDA allowed a compassionate exemption for the Sloan-Kettering patient on condition that Sloan-Kettering's institutional review board accepts full responsibility for the case, since FDA had no prior information on the TNF used, and the data submitted with the compassionate exemption request covered only basic safety requirements such as pyrogenicity and sterility.

FDA itself appears to be confused about the exemption. Even after granting Sloan-Kettering permission to use TNF on its patient, FDA officials were insisting that the substance was not being used on patients in the United States.

The Sloan-Kettering patient is said to be a member of the staff of the institution. The request to FDA for a compassionate exemption was approved unanimously by over 30 members of Sloan-Kettering's institutional review board on 9 April; according to Dr John Lewis, chairman of the board, FDA was provided with a treatment protocol, a detailed clinical assessment of the patient's condition and "voluminous" information about TNF.

Dr Doris Hutchison, head of the institute's investigational drug committee, said that FDA's bureau of biologics had been "very co-operative" in dealing with the request.

FDA not infrequently grants Investigational New Drug exemptions on compassionate grounds when the relevant animal data have been submitted for FDA's consideration but are held up in the bureaucratic pipeline. Patients with acquired immune deficiency syndrome (AIDS) have been treated under compassionate exemptions with drugs approved for other conditions, for example. Compassionate exemptions have also been granted for trials on seriously ill patients who fall just outside the age range specified in the protocol for a clinical trial. It is, however, less common for FDA to approve what amounts to a clinical experiment when the data are as fragmentary as they are for recombinant human TNF. Some authorities on medical ethics speculate that FDA might feel justified in making an exception if the patient concerned has a sophisticated understanding of the risks and benefits of the treatment.

Dr Lloyd Olds, associate director of scientific development at Sloan-Kettering, is one of the original researchers on TNF, first produced from mouse serum. Dr Olds is being supplied with genetically engineered human TNF for research purposes by Genentech Inc.

Although this material is being used only for animal and *in vitro* studies, according to Genentech, Asahi's TNF is undergoing clinical trials in Japan. After the publication of scientific reports detailing the cloning and expression of the human TNF gene (*Nature* **312**, 721 and 724; 1984 and **313**, 803; 1985), researchers at Genentech and Asahi were deluged with enquiries from anxious cancer sufferers and their families about where TNF could be obtained.

It is still unclear how soon organized clinical trials of TNF will start in the United States, or whether the Sloan Kettering case will serve as a precedent for future compassionate exemptions in similar cases.

Tim Beardsley

Halley's comet in ancient times

SIR — In his comment on the Babylonian observations of Halley's comet[1], C.B.F. Walker states that in Mesopotamia "almost all the early texts are concerned with astrology rather than astronomy"[2]. Walker continues by noting that the earliest astronomical observations known to Ptolemy in *circa* AD 150 dated "from the time of the Babylonian king Nabonassar (747–734 BC)". Both these points deserve comment.

First, although doubts have been expressed as to the link between astrology and astronomy in Babylonian times (for example, ref. 3), there is no firm evidence that the powerful temple priests of Mesopotamia made any distinction between what we call by the separate names of "astrology" and "astronomy": facility in expounding the former necessitated skill in understanding the latter.

Second, Sawyer and Stephenson showed in 1970 (a hypothesis confirmed by Muller and Stephenson in 1975)[4] that what was in all likelihood a total solar eclipse was observed during daytime at Ugarit on 3 May 1375 BC. It seems fair to assume (although the details were not known to Ptolemy in AD 150 and are still not known fully to us now) that astronomical observations took place in and around Mesopotamia for a considerable period of time before 747 BC.

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1 Stephenson, F R , Yau, K K C & Hunger, H *Nature* 314, 587 (1985)
2 *Nature* 314, 576 (1985.
3 Clagett, M. *Greek Science in Antiquity*, 11 (Books for Libraries Press, Plainview, New York, 1971
4 Stephenson, F R and Clark, D H *Applications of Early Astronomical Records* (Adam Hilger, Bristol, 1978), 43-44

Embryo research

SIR — Professor J.A. Davis (*Nature* 25 April, p.666) wonders whether a "legally or morally valid distinction" can be made between negatively interfering to stop a natural process, for example by procuring the abortion of a fetus *in utero*, and simply refraining from "positive intervention required for its survival", by not putting an egg fertilized *in vitro* into a woman, without which it must inevitably die.

But there is at any rate a *legal* difference between intentionally killing someone and doing nothing to save him. You are under no legal obligation to rescue a drowning man, and if you feel morally obliged to jump in to help him that is a matter for your own conscience, and no concern of the law. But to push him in deliberately is certainly a criminal offence, and probably murder if he drowns.

Professor Davis rightly understands that the argument in my last letter (*Nature* 14 March, p.126) could not be used to justify aborting an embryo that had already started on its normal development *in utero*, but that it might allow experimentation with embryos generated *in vitro* which, undoubtedly human as they are, cannot of themselves realize their potential for further development without some positive act of intervention from outside.

The Warnock Committee (*Report*, para. 11.22) proposed a limit of 14 days for keeping an embryo alive *in vitro* (otherwise than frozen), whether or not is was used for experimental work, and nobody seems to have suggested that this should be much extended. But it should be noted that experiments with living fetuses that have been legally aborted before the age of viability, that is to say up to something over 20 weeks gestation age, are not prohibited under English law. These are by definition doomed to die but they can be kept alive for at least several hours, and considerable publicity was given some years ago to attempts at Cambridge to develop an artificial placenta, using a human fetus which from press photographs appeared to be of 16–18 weeks gestation age. Although the Warnock Report (p.64) has suggested that the law ought to be reconsidered in this respect, such work is at present still quite legal, even if it is not for the squeamish.

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Artificial hearts

SIR — Your leading article "Hearts in the wrong place" (*Nature* 21 March, p.206) classifies you among the modern legal luddites, glorifying form over substance and accepting the idea that the lawyer-regulators know best, regardless of the consequences of their actions.

You admit that Dr Copeland's patient would clearly have died without the use of the artificial heart. In the light of this, neither the patient nor the patient's family was harmed by the heart's insertion and important information was obtained which may well allow the effective use of such hearts or improved models in the future.

There was also the possibility, albeit remote, that the artificial heart might have preserved the patient's life for a long enough time to allow for a successful implantation of a new human heart.

Thus the only potential results of the use of the heart were beneficial, even your editorial writer being unable to discover anyone who had been harmed. The use of available technology in such situations should be encouraged and not barred. In view of the admitted benefits and lack of harm to anyone, Dr Copeland deserves commendation not condemnation.

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Value-free science

SIR — Mark Diesendorf (*Nature* 10 January, p.92) questions the notion of value-free science, which I discuss in my letter of 27 September 1984 (p.294). Unfortunately, his comments suffer from the very confusion of categories that I tried to clarify. Since this error has marred much of the discussion of the subject, and since my explanation was excessively brief, it seems worthwhile to consider further what we mean by "science" and by "value-free" or "objective".

The word "science" is ordinarily used to refer to three things: a methodology, a resulting body of knowledge and the ensemble of activities of those who professionally use that methodology. The methodology has evolved a set of canons, and a social framework, designed to ensure maximal objectivity and reliability of the resulting knowledge. On the other hand, the activities of scientists are indeed filled with subjective value judgments: by the individual (what problem to work on, how to approach it, how to present the results), and also by social organizations (what to support, what to accept for publication, how to translate scientific knowledge into technological practice). But despite this subjective element, if the science is honest the practitioner must try to make its *content* — the data and the logical inferences drawn from them — as objective as is humanly possible. He may not succeed perfectly, but at least he should not deliberately inject ideological or other biases.

Diesendorf further calls attention to such presumbably value-laden terms as "fashionable" and "confusion" in my letter. I would suggest that these terms are actually capable of being objectively assessed. But more fundamentally, value-laden terms would not be out of place. For discussions of the nature of science constitute still another category, to be distinguished from the three noted above: they are metascience, presenting philosophical judgments rather than objective knowledge.

Finally, I agree with Diesendorf that problems involving social value judgments should not be treated as though they were purely technological, and his example of planning how to generate electricity fits. But I believe he errs in similarly categorizing the risk of fallout from nuclear weapons. It is a technical question, whose answer then becomes part of the broader question of what to do about these weapons. With the growth of technological innovation we face an increasing number of such questions (for example, the dangers of biotechnology), and we impair our analysis if we do not try to separate their technical from their social aspects.

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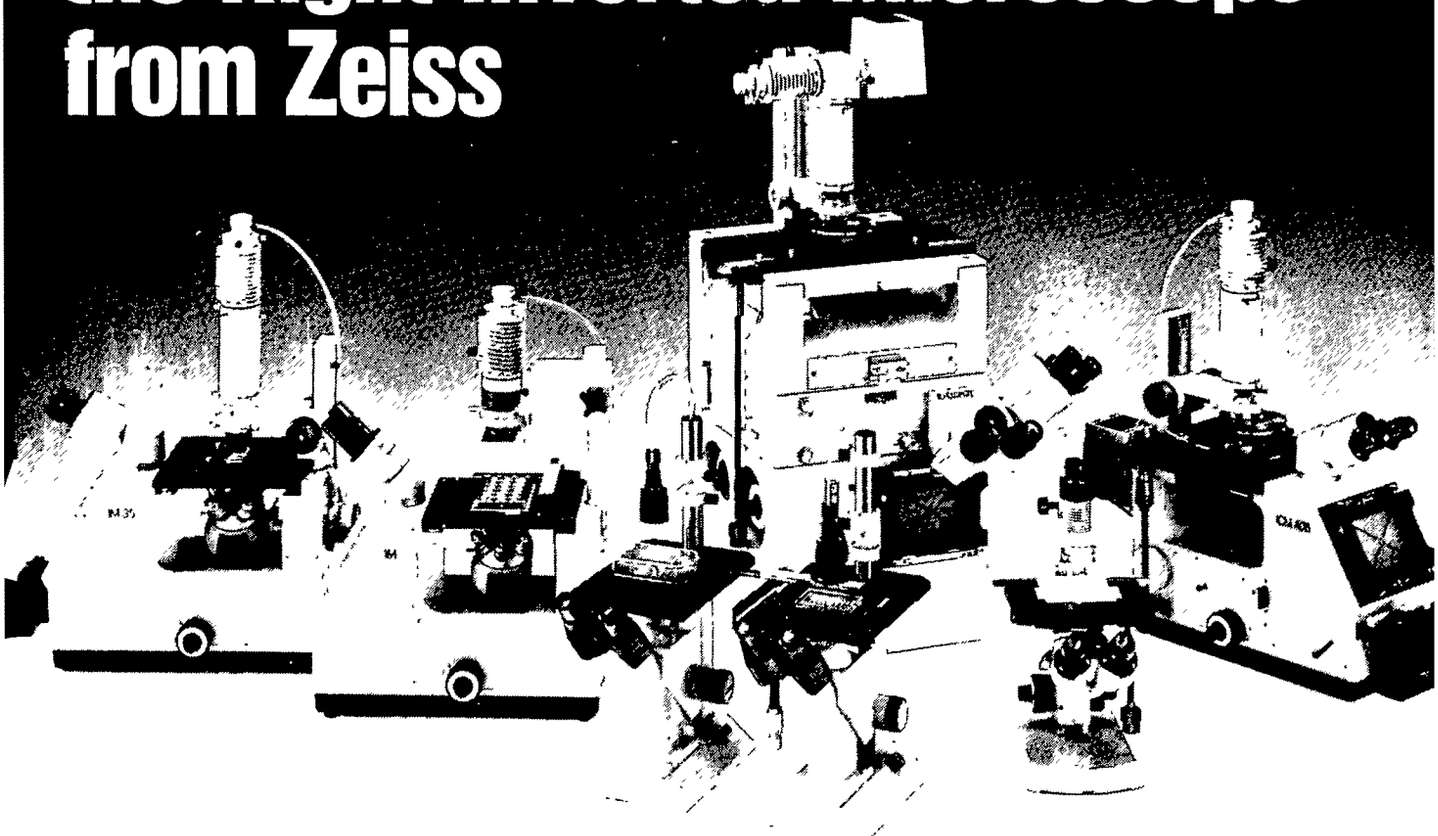
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New ways with microscopes

The range of the phenomena usable in the construction of microscopes continues to increase. Neutrons, for example, are now being used in place of photons.

If you have a particle or a field of some kind whose interaction with material objects varies with their constitution, the chances are that you will be able to fashion a microscope from the phenomenon. That truth is nicely illustrated by the versatility of microscopy based on the electromagnetic fields corresponding to optically visible radiation. Contrast between different elements of an object can depend on reflectance, on absorption during transmittance through a thin section, on rotation of the plane of polarization or on variations of refractive index, as in phase-contrast microscopy. The texture of an image is determined by the contrast, but a useful microscope also requires a means of magnification equivalent to the lenses of the familiar microscope. Two new developments this week suggest that people's ingenuity is far from exhausted.

The report by a group of Spanish molecular biologists and Switzerland-based IBM researchers on p. 253 of this issue is an application to a biological problem of a technique of microscopy previously used for the measurement of the surface structure of semiconductor materials and others used in the construction of electronic devices, and which may itself be the only application to microscopy of a strictly quantum phenomenon. Electrons, the textbooks say, will tunnel through a potential barrier if there is a state of identical energy for them then to attain. What is called the scanning tunnelling microscope is for practical purposes a device for arranging that the tip of a fine electrode will physically follow the profile of a surface at a distance of 10 ångströms or less.

In the usual application to the measurement of the profiles of semiconductor surfaces, the voltage between the electrode and the semiconductor is maintained at a value sufficient to promote an electron from the filled valence band to one of the energy states of the largely unfilled conduction band (usually a mere fraction of a volt), but which is less than the work function of the material of which the electrode tip is made, and which then serves as the potential barrier. The electrode tip can then be made to follow the surface profile by means of circuitry, including a piezo-electric crystal, designed to keep the tunnelling current constant, and at a value which may be no more than a few nano-amperes. For all the world, the device is the electronic analogue of the engineering devices for measuring successive profiles of a surface by amplifying electro-mechanically or otherwise the displacements perpendicular to the surface of a small ballbearing that is rolled along in contact with it.

The sensitivity of the quantum tunnelling effect is vividly illustrated by the way in which Baró *et al.* show a series of profiles along a surface of pyrolitic graphite which indicate a step-like structure corresponding to the abrupt termination of a couple of layers of the carbon atoms accounting for less than 7 ångströms of thickness.

Indeed, the resolution of the microscope is reckoned to be of the order of 1 ångström perpendicular to the surface and between 5 and 10 ångströms laterally. Baró *et al.* explain clearly enough how they have been able to correlate the known shapes (from electron microscopy) of bits and pieces of the bacteriophage ϕ29 dried on a graphite surface with the measured tunnel profiles, but their promised account of the mechanism of conduction through these biological molecules could be of great interest in its own right. Their technical achievement, however, is to have made the microscope work in air at ordinary pressure, which should make this type of instrument more versatile even in the measurement of semiconductor surfaces.

The other new development is the first realistic account of a neutron microscope, a goal long advocated by the Soviet physicist I.M. Frank but now realized by a group at the Technical University of Munich and the Laue-Langevin Institute at Grenoble (Herrmann, P. *et al. Phys. Rev. Lett.* **54**, 1969; 1985). This microscope uses "ultra-cold" neutrons from the source at Grenoble, while the microscope itself consists of a pair of mirrors — a large parabolic mirror 20 cm in diameter and a smaller (5 cm) mirror which is convex and spherical.

Ultra-cold neutrons are neutrons moving very slowly, some 6 metres a second in this case. Their interest for would-be neutron microscopists is that they can be reflected from suitable surfaces as if they were photons, which is a simple consequence of the circumstance that, at speeds like this, their wavelength is greater than the internuclear spacing in materials such as nickel-58 (used for coating the "optical" elements in the Munich design).

The snag is that the trajectories of neutrons moving so slowly will be affected by gravity, which is why the two mirrors are arranged in a vertical direction, with the secondary mirror above the primary (and each of them fixed in a horizontal plane with a precision of 10^{-4} radians). Even so, neutrons moving vertically will be decelerated, so that neutrons starting out with slightly different speeds will, left to themselves, come to a focus at different points, which is the equivalent in this field of what those who design optical instruments know as chromatic aberration.

It turns out that the chromatic aberrations of the two mirrors will cancel out only if the neutrons reflected from the parabolic mirror are allowed to reach the peak of their trajectory, at a height of 2 metres or so, and then allowed to fall back onto the secondary mirror, whose reflecting convex surface must therefore point upwards, like the larger primary.

The system has been designed to yield a magnification of 50, with the object placed near the optical focus of the parabolic mirror. The image is formed after reflection from the secondary mirror some 1.5 metres above the primary, whence neutrons are reflected sideways (and downwards, so that they can pick up a little speed) into a neutron detector, which simply counts the numbers arriving. Herrmann *et al.* say that a suitably faceted mirror deflecting neutrons from different parts of the field into different detectors would allow almost instantaneous observations.

So far, they have only the most humdrum observation to report, that of the profile of the neutron intensity across a slit placed in the object plane. The measured resolution of this neutron microscope is 0.1 mm, but that, the authors say, is determined by the counting errors with the low-intensity source of ultra-cold neutrons now available. (Grenoble plans 100 times as much intensity soon.) Aberration with the present arrangement should limit resolution to about 0.02 mm, but preselection of the velocity of the neutrons should allow that to be reduced.

But to what end? The authors' dream is that the reasons and techniques that have made the scattering of thermal neutrons a powerful means of telling the distribution of hydrogen in matter of all kinds can be translated simply into neutron microscopy. But one guesses that a large part of their motivation must be a little like the familiar reason for climbing Everest. Nobody else has made a neutron microscope function at a reasonable magnification, although Frank's Soviet group is well on the way.

John Maddox

Quasi-crystals

Respectable icosahedral symmetry

from Paul A. Heiney

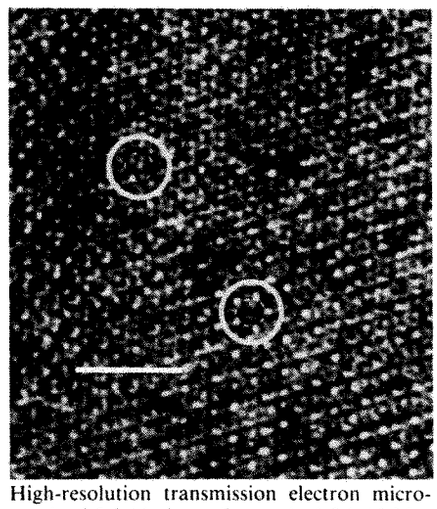
THE structure and properties of icosahedral 'quasi-crystals' were among the topics discussed at the recent meeting of the American Physical Society (Baltimore, 25-29 March, 1985). Quasi-crystals seem to represent a new state of matter in which icosahedral point symmetry is combined with a modified form of translational order. The clue to their structure may lie in the properties of Penrose tilings of a plane (see ref. 1).

Icosahedral ordering was first observed (D. Shechtman, National Bureau of Standards) in rapidly quenched alloys of Al-Fe, Al-Mn, and Al-Cr (ref.2). Dendritic microcrystals up to 2μm in size show the sharp electron diffraction patterns more usually generated by crystals, but with icosahedral rotational symmetry (see figure). Elementary geometry shows that icosahedral symmetry is forbidden for crystalline solids. The usual explanation of electron diffraction peaks which are crystallographically disallowed involves multiple scattering from twinned microcrystallites, but in the present case, the twinning hypothesis appears to be denied by both convergent-beam electron microscopy and dark-field imaging, which are consistent with local icosahedral symmetry on length scales as short as 200Å.

Much of the more recent work has centred on optimizing conditions for the growth of large-grain pure samples in the Al-Mn system (R.J. Shaeffer, National Bureau of Standards and L. Bendersky, Johns Hopkins University). The icosahedral phase is produced either by quenching a stream of molten alloy on a rapidly spinning copper wheel or by sweeping an intense electron beam across the surface of the alloy. If the cooling rate is too great, there are fewer icosahedral grains and they are smaller; if it is too low, ordinary crystals of various Al-Mn compounds are formed. The stability of the icosahedral phase is enhanced by a small amount of Si. An unexpected new 'T' phase, formed at higher Mn concentrations, may be intermediate between the icosahedral phase and a normal crystal.

High-resolution X-ray powder diffraction measurements (P. Bancel, University of Pennsylvania) provide data for quantitative tests of various theories of icosahedral order. The X-ray diffraction peaks are described by an icosahedral indexing scheme with six Miller indices. It is inferred that the positional correlation lengths of several hundred angstroms, extracted from peak widths, are reduced by defects which nevertheless allow orientation order to be maintained across grains with dimensions of 1 μm. High-resolution transmission electron microscopy (R. Gronsky, Lawrence Berkeley Laboratory and L. Bursill, Arizona State University) shows details of the atomic structure. Images formed from the electron diffraction patterns by optical Fourier transformation are remarkably similar to those produced by the quasi-crystal models now developed; 5-fold atomic packing symmetry and defects such as dislocations and stacking faults are observed.

In the best-known theoretical model[3] for these materials, two or more unit cells are packed with unequal spacings to form a 'quasi-crystal' without normal crystalline translational symmetry (P.J. Steinhardt, University of Pennsylvania). Quasi-crystals are much more highly ordered than glasses, but have special symmetries that are impossible for crystals. Remarkably, although quasi-crystals are not periodic, a calculation of the diffraction pattern predicts sharp spots, as with a crystal. In an alternative approach[4] to icosahedral order, quasi-crystalline lattices can be generated by projections from higher dimensions (D. Gratias, CECM/CNRS); the icosahedral lattice is generated by projecting a six-dimensional hypercubic lattice onto three dimensions. This mathematically elegant approach allows a straightforward calculation of X-ray diffraction intensities.



High-resolution transmission electron micrograph of 5-fold plane of quenched 6:1 Al-Mn. Bar, 2nm (courtesy R. Gronsky and L. Tanner).

In another approach[5-7], based on Landau's theory of the emergence of symmetry during phase transitions such as solidification, the atomic structure is described as a set of mass density waves with adjustable magnitudes and phases. An icosahedral quasi-crystal is generated by six density waves directed toward the vertices of an icosahedron. This method lends itself naturally to calculations of the structural stability, thermodynamics, elasticity and the occurence of defects in the icosahedral phase. A careful treatment of dislocations (D. Levine, University of Pennsylvania) reveals that these defects are considerably more complicated than their crystalline analogues; each dislocation requires two vectors, rather than one, to describe it fully.

Conventional discussions of matter in the solid state which assume either crystallinity or almost total disorder are not easily applicable to quasi-crystals. Future research will address fundamental questions about the nature of vibrations, electron transport, mechanical strength, thermodynamic equilibrium and other properties of these new materials. The immediate challenge for experimentalists is to produce larger, more pure and better-characterized samples; and for theorists, is to develop the mathematical tools needed to deal with this intriguing new state of matter. □

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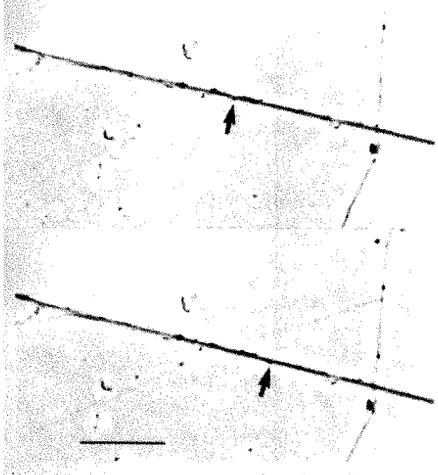
Cell motility

Fast axonal transport dissected

from Dennis Bray

ANYONE interested in cell motility likes to see things move. Electron micrographs have their place, as do two-dimensional gels. But for innocent enjoyment nothing beats a working model. Myofibrils isolated from a muscle contract explosively when ATP is added; bundles of microtubules from a sperm flagellum propagate waves and swim around. Marvellous! And now the machinery driving yet another type of cell movement — that of particle transport in the cytoplasm — has been isolated. As shown on page 245 of this issue[1], and in several other recently published papers, all you really need is a membrane vesicle and a microtubule.

For some time, it has been suspected that microtubules are involved in cytoplasmic transport. Membrane-bound organelles moving through a cell (see figure) are usually close to a microtubule, and drugs that disrupt microtubules often, but not always, paralyse these movements. Whole-cell studies of this kind have now been taken to their logical extreme by the discovery of an organism in which movement of organelles



Cultured chick embryo neurites show fast transport of organelles, some of which are visible as dense particles with phase-contrast optics. These two micrographs show a typical neurite at an interval of 10 seconds; the arrows denote an organelle which has moved along the neurite. Scale bar, 15 μm. (Courtesy of P. Hollenbeck.)

is confined to a single microtubule[2].

The organism was discovered on the sides and bottom of a freshwater aquarium in Berkeley, California. Fortunately, whoever found it must have been something of a naturalist, as they welcomed the intruder with a microscope rather than a scrubbing brush. The organism was composed of an enormously branched network of fine threads in which myriads of tiny, phase-dense particles streamed. Following a natural line of enquiry, the threads — subsequently identified as the giant freshwater amoeba *Reticulomyxa* — were examined in the electron microscope and found to resemble nothing so much as the thin processes of nerve cells. Like axons, each cytoplasmic thread contains aligned microtubules, and some are so thin that they contain but a single microtubule.

Correlating their light- and electron-microscope observations, Koonce and Schliwa were able to show that the phase-dense organelles, most of which are mitochondria, can move even in threads that contain only one microtubule[2]. Moreover, they can move in either direction, despite the fact that the microtubule is certain to have a distinct structural polarity. The authors conclude that explanations of organelle movement based on the beating of myosin crossbridges in muscle, or of the dynein side-arms in a cilium, both of which produce unidirectional forces, are not to be accommodated wholesale.

But any cell, no matter how attenuated, is still something of a black box. Three years ago, a far more accessible system was described that allows the rapid transport of vesicles to continue in the absence of an outer cell membrane[3]. Axoplasm is extruded from the giant axon of a squid and then examined by video-processed polarization-based light microscopy. With this exciting and powerful method, it is possible to see swarms of tiny vesicles, 0.2 μm or less in diameter, scurrying about their business in the axoplasm. If the axoplasm is spread even more thinly the vesicles can be seen moving along individual fine filaments, the nature of which forms the subject of two papers recently published in *Cell*[4,5].

Organelles ranging in size from mitochondria down to the smallest vesicles all move along 'transport filaments' in the extruded axoplasm at steady rates of about 2 μm s^{-1}. Unlike the movements in intact axons, large organelles travel as quickly as small ones, and the movements are smoothly continuous, lacking the saltatory, stop-and-go motion long considered the hall-mark of cytoplasmic particle transport. The difference, presumably, is that in the intact axon many organelles are obstructed by other components of the cytoskeleton. Most importantly, the transport filaments, although no more than 25 nm in diameter, are clearly revealed by the high-technology light microscope to be microtubules. Treatment and examination of the filaments by immunofluorescence and by electron microscopy shows that they contain tubulin (and not actin) and that they have the unmistakable substructure of microtubules.

Given these observations, what experiment would you like to see performed next? If your answer is to test whether movement is regained when separated vesicles and microtubules are reconstituted, turn to the paper by Gilbert *et al.* on page 245. Rather than try to separate microtubules from axoplasm, they used the ready-made parallel bundles of microtubules in the flagella of sea-urchin sperm. Freed of their outer membrane by detergent and dialysed against low-ionic strength solutions to remove most of their dynein side-arms, these readily available bundles present smooth straight tracks of uniform (and known) polarity. Gilbert *et al.* show that vesicles recovered from the squid axon travel smoothly, rapidly, without interruption and (as in both the giant amoeba and isolated axoplasm) in either direction along these tracks. Because movements proceed in the absence of most of the dynein arms, and because they ignore the intrinsic polarity of the ciliary axoneme, it is unlikely that the vesicles are moving by the same machinery that drives the cilium. More and more it seems that each organelle must have its own power source, like an outboard motor, that can drive it in either direction along a microtubule. The motor does not resemble that driving muscle contraction or ciliary beating; so what might it be?

Close on the heels of all these studies comes yet another instalment in the unfolding story. Vale and collegues[6] have now developed a reconstituted transport system in which the components are split not just two ways, but three. Membrane organelles from squid axoplasm constitute one part, and isolated microtubules the second — in this case purified from squid optic lobe tubulin. But the third component is the most exciting: a soluble, heat-labile, trypsin-sensitive extract without which nothing moves. Addition of this extract enables not only organelles but also polystyrene beads to travel along microtubules. It even causes the microtubules themselves to glide over the surface of a glass coverslip, looking like miniature snakes. Any speculation as to the nature of the mechanochemical protein in the soluble extract is clearly redundant; the answers are surely already in the press. □

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Thunderstorms

Wind shear and aviation safety

from Edwin Kessler

THAT aircraft should avoid thunderstorms is advice as old as flying itself. Although airborne weather radar provides a means of inflight evaluation of the weather, flight through echo-weak areas of thunderstorm complexes has led to several fatal and highly publicized accidents. All but three of the twelve such accidents between 1940 and 1970 followed a catastrophe at substantial altitude. With subsequent emphasis on the use of radar for avoiding storms, the incidence of accidents initiated at altitude declined remarkably. Since 1970, US aircraft have been involved in seven more fatal thunderstorm-related accidents, but five of these occurred during takeoff and landing and have been attributed to strong wind shears, mostly associated with thunderstorms near airports. There have also been other disturbing incidents during aircraft operations near terminals. The focus of research has accordingly shifted to the study of both thunderstorm downdrafts and outflows (downbursts) and of the strong variations of horizontal wind that accompany intense warm fronts[1].

Whereas aircraft used to bounce around a lot in bad weather, like leaves in a storm, today's planes are more sensitive to larger-scale atmospheric phenomena. The typical 1950s propeller-driven transport had a relatively low wing loading: it flew about 300 m.p.h at altitude and about 100 m.p.h on landing. The reciprocating engines responded within about 2 seconds to pilot inputs. Today's commercial transports fly at about 600 m.p.h at altitude and 150 m.p.h at landing and takeoff, and their engines take up to 7 seconds to respond fully to pilot inputs. Thus, during takeoffs

and landings, the 1950s aircraft would have flown about 300 feet before the engines responded; today's aircraft fly about 1,500 feet. And today's runways, about 10,000 feet long, twice the length required for a typical piston-engine aircraft, indicate the extended exposure of modern commercial aircraft to boundary-layer phenomena during takeoff and landing.

The variation of wind divided by the distance over which the variation occurs is the wind shear: mathematically, the shear is a tensor whose nine components correspond to the three orthogonal directions in which the wind variations can occur, combined with the three directions from which the wind can be blowing. The pilot is most interested in wind and its variations in the directions of flight, and in the vertical wind, because these produce the principal lift forces. Small-scale shears, termed turbulence, can produce discomfort, but if an aircraft enters a region of much reduced headwind during takeoff or during approach to landing, loss of lift is a serious safety concern. This is exacerbated when a downdraft is encountered along with a sudden unfavourable change of horizontal wind speed. In a severe downdraft outflow, with wind variation around 20 m.p.h per 1,000 feet, decline of headwind and downdraft can cause irreversible loss of altitude to the point of a crash. This is most likely during takeoff or landing when a thunderstorm is over or near the airfield, but can also occur with solitary atmospheric waves.

As a result of several incidents beginning in the late 1960s, the Federal Aviation Administration (FAA) issued a series of advisory circulars on the hazards of thunderstorms and wind shear, and developed new instrumentation for use at airports. Until the late 1970s, surface winds at air terminals were reported by a single centrally located anemometer. Guidance from a single device is inadequate in the jet age, especially when winds vary by 50 m.p.h or more from one side of an airport to the other, and such variations can even occur in clear air and without local visual clues. In 1976, the FAA developed the Low Level Wind-shear Alert System — a system of multiple anemometers with a display and alarm system that notifies the control tower operator when any one of the anemometers indicates a wind speed different from the centre field wind[2]. The efficacy of the system is suggested by the fact that only one fatal accident (in July 1982 at New Orleans) has been attributed to wind shear in an airfield with the equipment. About 60 airfields now have the basic system, and there are plans for installation at 50 more. The installations at Denver and New Orleans are being upgraded by adding extra, more closely spaced anemometers.

Substantial study continues to refine our knowledge of low-level wind-shear events, their individual and statistical characteristics, climatology and predictability. Downbursts and microbursts are being studied in the Chicago area[3,4], at laboratories of the National Center for Atmospheric Research and the National Oceanic and Atmospheric Administration (NOAA) at Denver, Colorado[5], and at NOAA's National Severe Storms Laboratory at Norman, Oklahoma[6,7]. Downdraft outflow phenomena are unusually frequent and intense at ground level in the Denver area. There they are often abetted by a layer of rather dry air on and near the ground, having a steep lapse rate of temperature. Such a boundary layer tends to amplify any thunderstorm or shower downdraft, especially with the strong evaporative cooling that accompanies precipitation from scattered showers. Findings from the Denver-based projects JAWS (Joint Airport Weather Studies) and CLAWS (Classify, Locate and Avoid Wind Shear) have both theoretical and practical implications. Downdrafts strong enough to be classified as microbursts around Denver are often 0.5-1 mile in diameter, and typically last 5-15 minutes, although occasionally they develop significantly within one minute. The difference between peak winds on opposite sides of outflow on the ground at the time of maximum development is about 55 m.p.h. In summer, there is on average one microburst a day within 14 miles of Denver airport.

Further work seek to develop cost-effective systems, both for observing hazardous microbursts in appropriate detail and for communicating the information to pilots at the time of landing and taking off, so that accidents will be avoided and economies are achieved in the use of time and fuel. Observed atmospheric extremes are also being programmed in flight simulators to evaluate aircraft control strategies for mitigating effects of extreme shear during unforeseen encounters.

Other atmospheric phenomena hazardous to flights at low altitudes include solitary waves, strong vertical shear of the horizontal wind and vortices rotating around vertical axes along gust fronts. Solitary waves can retain significant intensity while propagating for hundreds of miles in a stable boundary layer; when the air is dry they may be practically invisible, but under suitably moist conditions they are marked by a line of cloud that may have a deceptively innocuous appearance. They have been especially targeted for research in north-eastern Australia, where they are known as 'morning glories' and are quite common before noon[8]. In the United States they sometimes develop from thunderstorm gust fronts that propagate in a stable air layer ahead of the originating disturbance[9].

Intense warm frontal shear — a variation of the horizontal wind with height — especially when accompanied by poor visibility in rain and fog, can cause a descending aircraft to land short of the runway — as happened on 17 December 1973 at Logan Airport, Boston[10]. This outcome of extreme conditions can usually be averted by timely advice to the pilot on wind speed and direction at different altitudes. Doppler radars can provide this and other detailed wind information routinely, at intervals of a few minutes[11,12].

Future weather-surveillance systems for airports will probably include surface anemometers and Doppler radar, and possibly a lightning-locating system. It will be essential to collect data at a central location and to process them there for communication to pilots. As technology advances, we can expect an improved match between timely displays of processed meteorological data, actual wind and weather, aircraft capabilities, and pilot performance[13-15]. □

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100 years ago

Paradise Found. The Cradle of the Human Race at the North Pole. A Study of the Prehistoric World. By William F. Warren, S.T.D., LL.D., (London: Sampson Low and Co.)

IT has come to be an understood thing that when geologists or biologists propound theories as to past stages of life on the earth, and these theories attain to a certain popularity, some theologian shall twist the words of the Book of Genesis into a new interpretation, to show that this was what the inspired author meant all the time. A fresh musician has set Moses to dance to a new scientific tune. Since the publication of well-known modern views as to the diffusion of plants and animals from the Polar Region, it was to be expected that we should have a book proving that man was created in an Arctic Paradise with the Tree of Life as the North Pole; and here the book is. Other ancient cosmologies, such as the Greek and Indian, are made to bear their not always willing testimony. The commendatory letters published from Professors Sayce, Tiele, and Whitney do not at all imply that these eminent scholars countenance the Polar Paradise doctrine. The author seems to have sent them a paper on "Ancient Cosmology and Mythical Geography," their acknowledgements of which they are now perhaps hardly delighted to find as certificates in a "Paradise Found."

From *Nature* **32** 28, 14 May 1885.

Neurophysiology

Potential challenge from glia

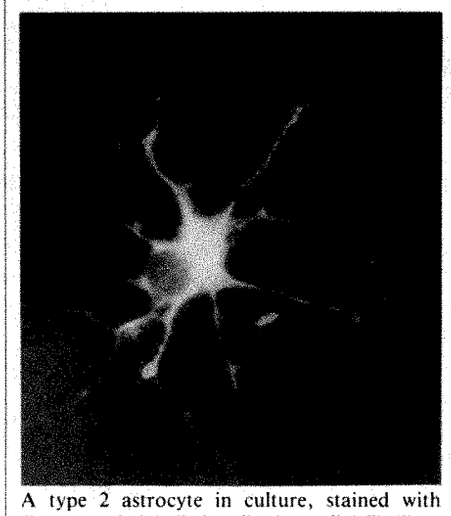
from A.R. Gardner-Medwin

THE neurones in our brains are required, as we all know, for the complex information-processing tasks of everyday life. Roughly an equal amount of space in the brain, however, is devoted to glial cells, chiefly astrocytes and oligodendrocytes, whose functions are diverse and poorly understood. Glial cells offer many challenges in understanding the overall function of nervous tissue, just one of which is their intriguing electrophysiological behaviour. Several recent papers[1-5], including one on page 229 of this issue by Bevan and Raff[1], show that glial cells in culture sometimes have hitherto unsuspected membrane characteristics, including high permeability to potassium, higher membrane conductance than resting neurones, direct electrical connections with one another, and the ability to respond metabolically to depolarization induced by local neuronal activity, all of which add to their fascination. How far these properties are characteristic of glial cells *in situ* is still a matter for conjecture.

The electrophysiological study of glial cells in tissue culture began in 1958 with work of Hild, Ching and Taskai[6] who showed that morphologically identified glial cells do not display the action potentials that are characteristic of most neurones, yet have resting potentials that are sensitive to potassium. Over the years, with contributions from many people working on both isolated cells and intact tissues, a picture has emerged in which some glial cells have very remarkable properties[7-10], including probably the most extreme selectivity of membrane permeability for potassium over sodium of all known biological cells.

One aspect that is beginning to be well characterized is the role of glial cells in stabilizing extracellular potassium levels in the nervous system[11-15]. Many questions remain, even in this highly specific area. For example, the relative importance of potassium dispersal through glia and of net uptake into glial cells remains controversial. The principal mechanisms of net uptake are uncertain and the significance of all these mechanisms for normal neural function are largely subjects for speculation. These current issues would tax one's ingenuity enough in designing experiments to settle them, even without the addition of the surprising new data from studies on isolated glial cells in culture.

The special advantages of work in tissue culture are that the cells can be studied in a simplified context and with the widest range of modern cell biology techniques. These include antigenic characterization with monoclonal antibodies and 'patch' electrophysiology, in which a pipette is sealed tightly to the outside of the cell membrane. Because glial membrane conductance is largely a potassium conductance in most preparations, it is gratifying that K^+-selective single channels (with, interestingly, a variety of different sizes) have been identified in oligodendrocytes[2]. The observed channels open and shut intermittently, although any channels that are constantly open may perhaps have escaped detection for technical reasons.



A type 2 astrocyte in culture, stained with fluorescently labelled antibody to glial fibrillary acid protein (courtesy of Dr. J. Cohen).

First of the particularly novel glial characteristics to emerge are sodium-selective[3] and calcium-selective[4] conductances, revealed with various pharmacological interventions. Astrocytes treated with barium and tetraethylammonium ions can even produce action potentials, involving Ca^{2+} channels[4] and with some resemblance to action potentials of heart muscle. Voltage-dependent sodium[5] and potassium[1] conductances, comparable with those normally found in neurones, have now been demonstrated in apparently normal, well-characterized astrocytes in culture (see figure). Despite many electrophysiological studies of cultured astrocytes[7], such potentials have not previously been reported, so there would seem to be substantial variation in properties between glial cells in different laboratories, and even to a certain extent among cells prepared in a single laboratory, with consistent techniques[5]. The reasons for these differences are obscure, but an important consideration may be that the cells for which results are illustrated in Bevan and Raff's study[1] seem to have much higher input resistances (100-150 MΩ) than have been found before (usually 1-10MΩ). It is not clear whether this could be explained by morphological differences, variations in the extent of coupling between cells or aspects of the recording conditions that may affect the membrane conductance. The new findings[1-5] do show convincingly, however, that some glial cells are capable of producing the molecules, presumably proteins, responsible for various different channels found in membranes.

At least three suggestions can be made as to the possible functional significance of voltage-dependent channels in cultured glial cell membranes. They might have no functional significance at all, appearing only as a consequence of particular culture conditions; they might exist in mature glial cells *in vivo* and play some physiological role; or they might exist only transiently in glial cells and play some role in the development or maintenance of neurones[5]. Because the channels are opened only when the membrane is depolarized above about −40mV, they could function solely in conditions leading to extreme glial-cell depolarization.

Such extreme disturbances are known to occur only in two pathological conditions: spreading depression, a condition believed to occur in some migraine attacks[16], and anoxia. The potasium conductance of glial cells may play a significant role in preventing spreading depression[17,18], which would indeed be assisted by a conductance increase on depolarization, but none of the studies *in vivo* has yet revealed a class of glial cells with non-linear conductance[7-10]. The recent studies on cultured glial cells may spur attempts to tackle the substantial technical problems that will be encountered before such cells can be identified with certainity *in vivo* and distinguished from neurones. □

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Erratum

In the article by Jonathon Bacon 'Why flying locusts do not crash' (*Nature* 9 May, p. 94), the fourth paragraph in colunm 3 should begin "The descending neurones make only weak direct connections with flight motor neurones but have strong inputs..."

Atmospheric physics

Predicting the ionosphere

from D.G. Cole

THE status of the ionosphere — modelling, measurement techniques, applications and forecasting of the ionized upper atmosphere — was discussed recently at a conference held under the auspices of the Australian Ionospheric Prediction Service in Sydney*. Regarded by some as an anachronism, ionospheric research has enjoyed a resurgence of interest to cope with a demand for high-speed digital data traffic on ionospherically propagated paths, increased high frequency (3-30 MHz) radio communications in developing countries and trans-ionospheric propagation problems.

The fundamental tool of the ionospheric forecaster is an ionospheric model. The present status of such modelling was reviewed by J. R. Dudeney (British Antarctic Survey). At present the most widely accepted model is the International Reference Ionosphere (IRI) (eds Lincoln, J.V. and Conkright, R.O. NOAA Report, Boulder, 1981) which is distributed on magnetic tape. IRI gives profiles of electron and ion density, temperature and relative ion composition; a user supplies location, time of day, season and solar activity. The model is used widely, even though it is inapplicable to auroral zone regions and times of very high solar activity, and does not consider irregularity structures.

The two regions that still provide the greatest problems in any modelling or prediction schemata are the equatorial and polar/auroral zones. Of these, the equatorial region is the easier to model. Indian researchers have gathered much decisive data by widespread low-cost satellite-beacon scintillation and electron-content studies. The IRI model has been shown to underestimate considerably the low-latitude F-region — the ionospheric layer of maximum electron density at about 300 km above ground level — daytime plasma density scale-height and total electron content. Disagreement with the topside ionosphere is bad, which is relevant to transionospheric systems and, through vertical plasma transport, to HF ionospheric systems.

Total electron-content predictions can be improved by using theoretically calculated profiles, but this is prohibitively time-consuming and expensive even with present-day large computers. D.N. Anderson (US Air Force Geophysics Laboratory) and coworkers have recently made considerable progress with a semi-empirical low-latitude ionospheric model that uses only coefficients from such theoretical profiles to form the basis of a fast portable computer code to produce realistic low-latitude F-region electron-density profiles.

The polar region and the behaviour of irregularities still remain a world-wide problem, requiring concerted intensive global data collection and analysis. An exercise which can be regarded as a forerunner of such a study, project SUNDIAL, was conducted late in 1984. The project arose because there was no prediction capability for day-to-day variations in ionospheric structure and it highlights the fact that the various ionospheric domains are not isolated subsystems, but are indeed interactive elements in the entire solar-terrestrial network. The project looks to progressive development of a genuinely predictive ionospheric specification capability under both quiet and disturbed conditions. Such a capability would add direct solar and magnetosphere inputs as well as latitudinal and longitudinal coupling to the fundamental ionospheric model, and requires near real-time updates from a responsive network of ionospheric monitoring stations.

The experimental period of project SUNDIAL was based on an eight-day flight of the space shuttle carrying a 1.28-GHz ground imaging radar in October 1984. This aperture-synthesis radar requires phase-path constancy through the ionosphere to obtain maximum resolution. During this period the international ground-based ionospheric programme (coordinated by E.P. Szuszczewicz, Science Applications International, Virginia) collected ionogram, electron content and scintillation data from equatorial, mid-latitude and both polar domains, later supplemented with data on solar, magnetospheric, geomagnetic and interplanetary activity. Two workshops are planned in 1985 to analyse these data and to issue guidelines, recommendations and suggest follow-on programmes. Future studies will emphasize new programmes and also the appropriate coordination to ensure that diverse existing ionosphere measurement facilities are fully used. Interestingly, the USSR Solar Terrestrial Physics group was the first to propose an intense global ionospheric study.

A final note of interest is the detection of meteor echoes by the Australian Project Jindalee, an Over-the-Horizon Backscatter Radar near Alice Springs. Conventional radar meteor studies, starting with the early work at Jodrell Bank in 1945, have always used only the direct line-of-sight return signal. It seems now that, for the first time, significant rates of meteor echoes have been detected by various ionosphere (and ionosphere-ground) reflection modes. R.M. Thomas (Project Jindalee team) also suggests that the steerable, narrow beam-width antenna pattern, coupled with the frequency, agility and sophisticated processing available to the system, can be used to optimize the radar for a particular meteor radiant by changing the appropriate parameters. Such a facility could significantly revitalize interest in delineating the faint-field meteor influx and improve the management of polar high-speed meteor forward-scatter links of interest to military and other circles. □

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*The conference on the ionosphere and radio wave propagation was held at the University of Sydney, 11-15 February 1985, sponsored by the International Union for Radio Science.

Evolution

The point of a toucan's bill

from J.S. Jones

MODERN evolutionists are beguiled by random change. Recent theories about DNA sequence divergence suggest that natural selection does not determine the fate of most genes and that genetic drift is the main agent of evolution, with selection acting only to remove harmful mutations. It is therefore important to remember that not all evolution happens by accident; that natural selection is a potent force for adaptive change, and that its products can be seen all around us. Some of the most spectacular examples of the intensity of natural selection and its ability to produce rapid adaptation have emerged from recent studies of birds ranging from the mundane sparrow to the outrageous toucan.

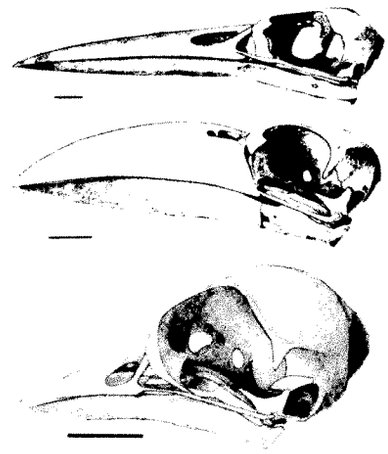
House sparrows were introduced into the United States in 1852, and are now widespread. Since their introduction, American sparrow populations have undergone considerable morphological evolution, and northern birds are larger and have relatively shorter extremities than do those in the south[1]. These differences are maintained in the face of dispersal sufficient to produce relative uniformity in the frequencies of genes coding for soluble enzymes[2]. They probably represent an adaptive response to climatic selection: birds with short limbs survive in cold weather considerably better than do those with long. In one particularly harsh Kansas winter a 25 per cent difference in mean fitness was associated with humerus length[3]; a difference related to the extent of heat loss through the extremities. Larger males, but small females, are better at surviving the winter — an effect which might result from an advantage of more dominant males and more submissive females in conditions where fighting is metabolically costly. Differences in food items selected by birds of different body size may also help to ex-

plain the greater sexual dimorphism found in northern populations[4].

Natural selection appears to be an effective agent in producing rapid adaptation in these introduced populations. There is no information on the genetic basis of morphological variation in sparrows, but transfer experiments with other American birds suggest that some geographical changes in body form are a direct response to the environment[5]. Consequently, estimates of heritability are needed before the evolutionary significance of such changes in morphology can be assessed. As recently discussed by Paul Harvey[6], information of this kind is now available from new work on Darwin's Galapagos finches[7].

Patterns of differentation in their soluble enzymes suggest that the finches have occupied the islands for less than a million years and have diverged rather rapidly from the emberizine finches that are their mainland ancestors[8]. There are striking morphological differences between species, from the long slender bill of *Certhidia olivacea* to the stout and powerful beak of *Geospiza magnirostris*. Assuming there is no strong correlation between genetic and environmental variation in wild birds, family studies suggest that the heritability of morphological characters, such as body size or beak dimensions, is high[7,9]. For example, three quarters of the overall variation in *G. fortis* body size is due to genetic variation. There is therefore abundant genetic variation on which natural selection might act. Periodic droughts, such as those on Isla Daphne Major in 1977, 1980 and 1982, can lead to mortality rates of 85 per cent. Much of this is selective, and adult survivors have substantially larger bodies and beaks than those who die, probably because they can feed better on the hard seeds that persist through the drought. A selective shift of six per cent in morphological traits can occur in one year[10].

Selection of this intensity, acting on highly heritable characters, has the potential to generate rapid morphological change. A new study of the numbers of finch species on the various islands and their patterns of differentiation in beak shape in relation to food availability shows that coexisting species always differ in bill dimensions by at least 15 per cent. Such differences can be explained only by interspecific competition and by local adaptive radiation into a variety of feeding niches[11]. The role of competition for food in driving adaptive radiation is particularly manifest when comparing the morphology of species present in sympatry and in allopatry on different islands. The bills of *G. fortis* and *G. fuliginosa* diverge from each other to a much greater extent in mixed populations than in single-species populations. Comparing of feeding behaviour with availability show that this character displacement results from exclusion from particular food items in mixed populations, leading to a change in optimal beak size for each species[12]. Natural selection on beak form appears to be intense enough to bridge the gaps between existing species within only a few years. This evidence that selection is the directing force in evolution on the Galapagos contrasts with the emphasis placed on accidental change in evolutionary studies of Hawaiian *Drosophila*[13].



Adaptations in skulls of Coraciform and Piciform birds. Top to bottom: *Alcedo atthis* (kingfisher); *Selenidera langsdorffi* (toucan); *Sasia abnormis* (piculet). Scale bars, kingfisher and piculet, 10mm; toucan, 5mm (from ref.17).

Adaptive radiation has the potential to obscure patterns of taxonomic affinity if unrelated organisms, living in ecologically similar circumstances, evolve body forms sufficiently alike to produce an appearance of common ancestry[14]. This is true to some extent among Galapagos finches[15], but is far more striking among Australian birds. The Australian passerines include many species with names (wrens, crows, babblers and honeyeaters) reflecting their similarities in structure and behaviour to birds in other parts of the world. Until recently, this diversity was thought to reflect a series of invasions of Australia by a number of unrelated ancestors. Now, however, the use of DNA hybridization to measure genetic affinity suggests that it results from an explosion of adaptive radiation from one endemic ancestor within the past 60 Myr[16], to fill the niches occupied by wrens, warblers, flycatchers and chats in other parts of the world. Unique forms such as bowerbirds, butcherbirds and bellbirds have also evolved. Once again, natural selection in diverse habitats has led to a rapid adaptive response.

Comparisons of structure and of ecology have recently been combined to illustrate the forces of natural selection that have led to an exuberance of form in a group of birds far more diverse than sparrows, finches or Australian passerines[17] (see figure). The Coraciiformes and Piciformes include 15 families. Some are familiar — kingfishers, bee-eaters, woodpeckers and toucans; others are less well known — todies, cuckoo-rollers, jacamars, barbets, puffbirds and piculets. Despite their name, most kingfishers do not fish. Instead they sally for terrestrial prey, which may include small snakes. The beak is large and stout — which is perhaps not surprising given that some species nest in tunnels excavated by flying directly at a river bank. Bee-eaters pursue insects on the wing, and can catch up to 350 in a single day. Their bill is less heavily constructed than the kingfisher's but is not as lightly built as that of the hoopoe, which probes the soil for insects. The huge beak of the hornbill is used to crush dangerous prey and to peel fruit; food items, once prepared, are tossed into the air and caught in the bird's open mouth. Honeyguides have a short but robust bill and can feed on wax. The huge casque of the toucan is used to peel fruit, but may also be useful in intimidating other birds when robbing their nests, and as a social signal. Finally, the wrynecks, piculets and woodpeckers feed on insects obtained by probing or excavating with a straight chisel-like bill. This diversity of skull form is accompanied by an equivalent radiation of the muscles of the head and neck, which are reduced in bee-eaters, but massively strengthened in woodpeckers. Other specializations include the extremely small tongue of kingfishers and the brush-like tongue of the toucan, which is among the largest in any bird. This rare combination of information on structure, ecology and behaviour in a widely distributed and highly variable group gives an unusual insight into the power and opportunism of selection in leading to adaptive change.

In 1825, Sydney Smith wrote of the toucan: "To what purpose is a bird placed in the forest of South Cayenne, with a bill a yard long, making a noise like a puppy dog, and laying eggs in hollow trees? The toucans, to be sure, might retort — to what purpose were gentlemen in Bond Street created? ... There is no end to such questions. So we will not enter into the metaphysics of the toucan." Smith was hasty in his dismissal of this enigmatic bird: modern ornithology reminds us that molecular biology is still little more than an account of the structure of the genome, and that we should be wary of abandoning natural selection as an agent of change until we can describe the functional anatomy of molecules in as much detail as is now possible for beaks, muscles and feathers. □

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Plant geology

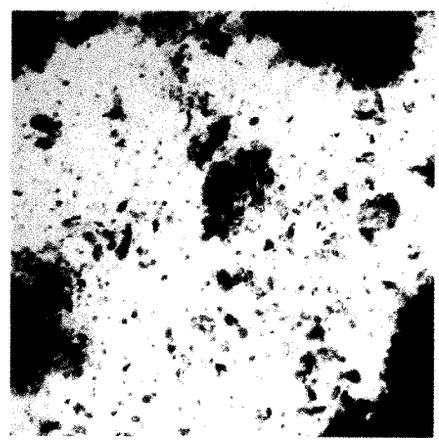
Changing weight of photosynthesis in the ocean

from I. Gilmour

ON PAGE 216 of this issue Arthur *et al.*[1] provide further evidence for the debate on what controls the carbon isotope composition of organic matter in marine sediments. The common photosynthetic pathways discriminate against the heavy carbon isotope (^{13}C), mainly as a result of a kinetic isotope effect inherent in carbon fixing reactions. As carbon dioxide dissolved in seawater contains more ^{13}C than atmospheric carbon dioxide, marine organic carbon is generally isotopically heavier — that is, ^{13}C enriched — than most land plants or terrestrial carbon. This has led to a general belief that the variations in the carbon isotope composition of organic matter in marine sediments provide a measure of the relative amounts of marine and terrestrially derived organic matter[2,3]. Arthur *et al.*, however, now report an apparent reversal of this general trend in some marine sediments of Cretaceous and older ages[1] (see figure). Using a second, independent, geochemical parameter to evaluate the source of the organic matter, they report carbon isotope values for rocks apparently containing mainly marine organic matter that are lighter (that is, more ^{13}C-depleted) than those containing mainly terrestrial organic matter.

The parameter that Arthur *et al.* have measured is the hydrogen content of the organic matter, determined using the Rock-Eval pyrolysis method, which provides a 'hydrogen index' related to the H/C ratio in the organic matter[4]. Terrestrial organic matter has a lower H/C ratio compared with marine organic matter because of the presence of lignin. This material, which gives land plants their 'woody' characteristic but is not found in marine algae, has a low H/C ratio, reflecting its more aromatic chemical structure. Arthur *et al.*, by plotting the carbon isotope composition of the organic matter against the 'hydrogen index' or H/C ratio, relate organic carbon isotope values to the chemical composition of the organic matter, which in turn is dependent on the relative contributions of marine or terrestrial material. Using this approach, they are able to demonstrate that for Cretaceous and older organic rich sequences a negative correlation is commonly found, suggesting that the lightest carbon isotope values are related to chemical compositions that indicate the greatest contributions of marine organic matter. For the more recent Cenozoic sediments, they observe either a positive correlation or no correlation, reflecting the 'normal' isotope variations between marine and terrestrial materials and purely marine organic matter, respectively. Thus, pre-Cenozoic marine organic matter is anomalously depleted in ^{13}C by around 5 to 7 parts per thousand compared with marine organic matter in more recent sediments.

Anomalous ^{13}C depletions in ancient sediments are fairly common, particularly in Precambrian sediments[5-7] where different carbon cycling pathways, notably those based on methane, have been invoked to explain them. It is difficult to try to explain such anomalies in ocean sediments, where the organic matter is derived predominantly from phytoplankton that use carbon dioxide. Arthur *et al.* consider several possibilities for this anomaly. They discount diagenetic effects, which generally do not produce sufficiently large shifts in the carbon isotope composition of the organic matter. They also eliminate, although not totally, the possibility that changes in the carbon isotope composition of the organic matter reflect changes in the dominance of marine phytoplankton groups because this explanation is not supported by the Cenozoic palaeontological record. The three remaining possibilities all relate to one factor, the level and isotope composition of dissolved carbon dioxide in the oceans and its possible effect on photosynthesis. The authors eliminate a secular variation in carbon isotopes because the average Cretaceous carbonate is about 1.5 parts per thousand heavier than modern pelagic carbonates, which would result in marine organic matter being heavier, not lighter. Thus, the favoured interpretation is as follows: increasing the carbon dioxide concentration in the oceans can have a profound effect on photosynthesis. Photosynthetic carbon isotope fractionation is maximized under conditions of high carbon dioxide availability[8,9] and, as Arthur *et al.* point out, this would result in ^{13}C-depletion of organic matter. An increase in carbon dioxide availability can be accomplished by reducing the ocean temperature, which increases the solubility of carbon dioxide. But the Creteaceous oceans were as warm, if not warmer, than present-day oceans. Thus, Arthur *et al.* argue that possible higher levels of atmospheric carbon dioxide during the Cretaceous, combined with lower levels of surface productivity, would have resulted in more available carbon dioxide and increased fractionation of the carbon isotopes found in marine plankton.



Organic material from a Cretaceous black-shale sequence from the Falklands Plateau, South Atlantic, which has revealed lighter carbon isotope composition than expected. The material is predominantly the remains of photosynthetic marine algae with minor amounts of material derived from the continents of the time. (Photo courtesy of I. Gilmour.)

Are these intepretations correct? If so, then modern marine photosynthesis may be occurring under geologically unusual conditions. True or false, their approach is certainly on the right lines. By relating carbon isotope composition to H/C ratio and hence to chemical structure, the relative contributions of marine and terrestrially derived material are better understood. However, the carbon isotope values obtained still represent an average of two components, whereas ideally the marine and terrestrial material should be resolved completely. Failing this, the approach adopted by Arthur *et al.* is certainly an improvement on merely measuring bulk carbon isotopes in isolation. The implication from this work is, as the authors suggest, that carbon isotope values alone are not reliable for organic matter provenance studies, particularly in pre-Cenozoic sediments.

The possibility of fairly substantial variations in marine carbon isotope values resulting from variable photosynthetic fractionation is worth much closer investigation. Positive carbon isotope shifts in carbonates during the Cretaceous could represent increased oceanic productivity that uses up more of the lighter isotope[10]. The work of Arthur *et al.* could upset some of the widely accepted ideas for models of the carbon cycle. Clearly, more detailed work is required, but merely taking bulk carbon isotope values of organic matter in sediments in isolation is not going to help solve the problem. □

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Scientific creationism and rationality

from Michael A. Cavanaugh

Scientific creationism is a social movement of rationalists — amateur philosophers of science — whose claims upon science policy are nonetheless wholly unreasonable. This thesis may not endear the author either to rationalists or creationists, but it may illumine the movement's behaviour and the sources of its wider appeal.

ORDINARY language contrasts the rational and the social. Individuals either pursue the truth or we pursue our intersts; either we have good reasons or we are swayed by others — peers, experts or traditions. On another point of view, derived from the sociologists Durkheim and Weber, society is defined by its shared rationality. Social structures and divisions of labour can distribute, even augment, human reason (or impede it). From this standpoint, society as such is "knowledge-based; and two heads are better than one. This, if so, is so for any society.

Modern societies are knowledge-based in yet another way: they thrive upon mass education[1]. Schooling has become indispensable for even ordinary occupational roles; fairly advanced educational credentials are demanded routinely as tickets of admission to the work force[1,2]. The consequences are political and cultural as well as economic. Complex societies discover that they cannot dispense with technical expertise. And this is no less true (as in Iran and China in recent years) when the power of technicians and experts conflicts with traditional elites or newer populist authority[3,4].

Mass education

Mass education is oddly uneven education. It enlarges society's pool of intellectuals[5], but also confers academic degrees upon people contemptuous of intellect and unfamiliar with the life of the mind. Mass education democratizes educational opportunity; it also stratifies the "new class" of the highly schooled and proliferates an intellectual underworld — Pluto's Republic, *pace* Peter Medawar.

So it is that late twentieth-century society simultaneously enjoys unprecedented levels of education and technical knowledge and suffers acute self-doubt and pessimism about the very value of human reason. "Once again," as Medawar[6] has observed, "there is a rootlessness or ambivalence about philosophical thinking, as if the discovery or rediscovery of the insufficiency of reason had given a paradoxical validity to nonsense, and this gives us a special sympathy for the dilemmas of the seventeenth century."

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How apt, as our era goes through a debate more appropriate to the age of Bacon, Newton and Descartes (or perhaps, of Thomas Aquinas). Claiming support from the scientific community, highly schooled fundamentalist Protestants propose teaching a "creation model" as an alternative to evolution. Since their heyday during 1980-82, such claims and proposals have not gone undisputed. To its opponents, creationism appears as Luddite anti-intellectualism[7]; as pseudoscience[8]; as a deviant science movement[9]; and as an American abberation (rooted in the rural South)[7]. Its advocates are portrayed as equally uninformed about genuine science as about genuine religion[10]; or as "space-age fundamentalists"[11], religious traditionalists as inconsistent as the "republican" *mullahs* denouncing Western materialism into the uplifted microphones of mass-produced tape recorders.

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Religious fundamentalism is nothing if not a protest against modern secular society[12]. Yet to regard scientific creationism simply as marginal to that society is misleading. On the contrary, creationism is not the anti-intellectualism of those who have just gotten shoes; it is the counter-intellectualism of modern offspring (who understand well the rhetoric of modern political pluralism). Consider educational experiences. Unlike their forerunners of the 1920s, contemporary scientific creationists are the beneficiaries of mass educational expansion. Almost all claim bachelor's degrees, an estimated 40-60 per cent in some scientific or technical field. Around 10-25 per cent are trained in applied areas, particularly engineering, 20-30 per cent in physical science; and a few (5-10 per cent) in the life sciences, the most relevant for an anti-Darwinian movement. (Some are now completing graduate work pseudonymously.) Many claim PhDs from accredited schools, even major universities. (Some claims have been disputed[13].) Compare this with the average US citizen's 14.2 years of schooling.

In social location, the movement is not confined either to rural margins or to the American South. It thrives wherever Protestant natural theology has spread, mainly throughout the former British Empire (for example, North America, Australasia, South Africa, India, the United Kingdom itself), concentrating in North America (primarily in expanding urban areas, in longstanding fundamentalist enclaves in midwestern and western states and provinces but not in the southern United States)[14].

The geographical and occupational distribution of creationists illustrates how fundamentalism is expanding beyond its marginal enclaves. Erstwhile fundamentalists separated themselves into subcultures with unaccredited theologically-based schools[12]. Like their secular contemporaries, today's fundamentalists seek technical credentials for occupational mobility; thus they must neutralize the worldly blandishments from which their forebears simply fled[15]. *Pace* Snow's "two cultures"[16], young fundamentalists desire (and their parents encourage) technical training with minimal humanistic education. Thus the Reverend Mr Jerry Falwell (the leader of the Moral Majority) and Mrs Phyllis Schlafly (the lawyer opposed to expanding womens' rights) wish their children to become engineers. Why? Because, the parents reason[17,18], unlike law (not to mention literature, or philosophy), engineering devises simple solutions to practical problems, without seriously challenging the intellectual traditions of any subculture (religious, political, or otherwise). Questionable though their analysis of engineering may be[19,20], this does suggest why technical training appeals to fundamentalists — Christian, Jewish, Muslim — and others (in developed and developing nations alike) seeking to avoid the choices underlying modern science and technology.

Like Islamic republicanism, scientific creationism is not simply backwardness; both are cautious bargains with the devil, modernity. Although creationism's conceptual resources betray a seventeenth-century provenance, its causes (and appeal) are discernibly more recent; and it is not entirely foreign to the normal culture of science. Why does the knowledge-based society respond to creationism *redivivus*,

rather than to Taoist alchemy or Babylonian cosmology? And why are there so many Islamic republicans but no Islamic creationists? In brief, because the West has a unique history of progressively testing key religious myths for purely secular validity[4], and creationism was tested and found wanting. Because of this legacy, there are some things creationism definitely is not. For instance, it is not preindustrial Luddite irrationalism. It is not a deviant science movement like plate tectonics once was. Nor is it pseudoscience in precisely the way that "psychic archaeology", phrenology or the green cheese theory of the Moon are. Unlike them, creationism once enjoyed standing in the history of science.

Scientific method

Robust in Newton's day, creationism degenerated[21], and no longer informs any active progressing scientific research tradition[22,23]. To survey the main social and intellectual hallmarks that identify science is to discover what creationism lacks. First, knowledge is acquired through conjectures and refutations in a research process[24]. Research is institutionalized in the various scientific professions, which select and certify practitioners, and is communicated in the various journals. Therein research is subjected, first and foremost, to the judgement (and reward) of specialist peers[25]. Subsequent public acclaim or academic standing depends on this judgement.

Second, whence the coherence of science? It cannot be founded upon tenets, which change. Any single tenet may be false; most (the set of all scientific theories minus our present ones) *are* false. Moreover, the foundationalist search for substantive bedrock is now widely acknowledged to have failed[26]. Science acquires coherence, rather, through its procedural commitment to the integrity, reliability and professional governance of scientific theory-testing (and teaching) over the long haul[27]. In other words, modern science rests (like modern law) upon long-term "orthopraxy" (the integrity of method, institutionalized in professions) rather than any short-term orthodoxy of individually trustworthy tenets.

Accordingly, the unit of knowledge acquisition is the theoretical community rather than the individual scientist; thus "science" develops as the distributed property of communities of theory (research traditions), not the consolidated property of individual genius — much less, of what Everyman observes in an untutored way. (On this view, theory is not the luxurious icing on the scientific cake, but the very cake itself. Science is nothing other than the history of its theories, since a method-independent science is impossible, and the subjects of this history are the scientific collectivities that produce and change theories[28,23].)

Third, contemporary science is organized in certain ways. It is "big science"[29]. Science is now conducted typically by career scientists within large complex organizations (rather than as the loosely organized hobby of gentleman amateurs). Increased size refines divisions of labour; consequently, "science" becomes even less the concentrated property of individuals. Each may differ greatly in understanding, talent and skill, yet all may contribute (provided their labour is divided cleverly enough) to the limits of their diverse gifts. Big science is internally "democratized", which permits the fullest use of even modest talent[6,30]. (This is, perhaps, the chief difference between organized science and organized crime.)

The organizational factor makes for a complex relationship of control between science and society. On the one hand, though the typical scientist is not Einstein, he will enjoy some expert divergence from commonsense not shared by every citizen. On the other hand, science and commonsense are not totally antithetical, for in a knowledge-based society, commonsense is increasingly tutored by "packed-down science"[31]. Thus the man on the modern street "sees" the roundness of the Earth, and his common sense "knows" that the heart is not the seat of emotion and that werewolves do not exist. Such "conventional wisdom" is primarily a fact, not of individual psychology, but of membership in a certain kind of society. The organized process of gaining, and warranting, knowledge differs from the ability to use it in packed-down form, or the ability of Everyman individually to validate what he knows.

It is the division of expert labour among a network of complex professional organizations that distinguishes contemporary science from such other enterprises as armchair scepticism, interest-group politics and even the "little science" practised by amateurs (in the best sense) over the centuries past. If such are the hallmarks of science, then it is clear why creationism is not a scientific movement, not even a deviant one. As a rule (with few exceptions), its adherents do not undertake to conjecture and refute through empirical research[32]. They circumvent the normal confines of professions and journals, going "over the heads" of peer researchers and reviewers in favour of other forums such as the popular press, textbook selection committees, legislatures and courts of law[12,33]. Popular acclaim, not expert peer review, is their ultimate court of appeal.

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While it is true that (in recent decades) creationists have not tried to outlaw evolution, they have sought to establish anti-evolutionary tenets by legal fiat. And characteristically, the campaign for lasting orthodox tenets advances by a straightforwardly empiricist rejection of evolutionary theory, as if theory meant imperfect fact — "part of a hierarchy of confidence running downhill from fact to theory to hypothesis to guess"[34] — something in dire need of extra-scientific correction.

Organized scepticism

Thus, if science is inseparable from self-governing professions, the procedures they institutionalize and (perhaps most importantly) the theories they conjecture and test, then scientific creationism does not resemble science. It requires nothing more arcane than well-known philosophical and sociological demarcations to understand why public policy justly may disregard creationist demands to produce science through political rather than scientific processes.

Faced with this assessment, a sophisticated creationist might invoke his scepticism and empiricism. He might assert that creationism is highly sceptical about tautology in evolutionary theory[35], whereas evolution requires mere faith in insubstantial theories. Does this count? Old chestnuts about tautology, verifiability and naive falsification[36,37] aside, the answer must be no. Why? First, the process of criticism in contemporary science deploys the norm of "*organized* scepticism"[30] seeking cumulative growth in knowledge. Creationist scepticism occurs sporadically outside organized science and seeks selectively to prune whatever accumulated knowledge offends extra-scientific tastes.

Second, categorical "scepticism" masks an underlying foundationalism (religious fundamentalism being the limiting case). Categorical or naive doubt is not scepticism. For doubt to be intellectually credible, the specific occasions and ends of doubt make all the difference. Creationists understand this intuition, for they, too, doubt selectively; as members of the knowledge-based society, their views retain much presupposed common-sensical packed-down science (that the Earth is round; that the heart is a pump; that men do not change into werewolves; that fossils are traces of extinct forms[38,39]). So, during fieldwork[14], one informant complained about a long-distance telephone call to a fellow creationist (one of the few who is also a Flat-Earther): "I do not intend to spend $1 per minute discussing nonsense!" Like most creationists, he doubts — but only those views (scientific, or superstitious) that countermand the foundational tenets of fundamentalist Protestantism.

Creationism is radically against method. Procedure is disregarded in favour of direct access to a science-independent, oracular, God's-eye view of nature (literally so). So why not ignore normal research channels and limit oneself, as creationists do, in the worst tradition of academic scholasticism, to *post factum* pot-shotting? Likewise creationists invoke the rhetoric of scepticism and empiricism. They will assert that such-

and-such an evolutionary finding cannot be replicated in a laboratory[40], or is not the sort of thing publicly observable by the common man[41], or would not meet lawyers' tests of evidence[35], and so is scientifically invalid. But these 'sceptical' arguments are marshalled in the interests of a submerged theological agenda[42]. Thus empiricism and scepticism, ordinarily laudable scientific hallmarks, are directed toward "doxological science"[43], which is not. Like Cartesians, creationists doubt only so as to shore up science-independent foundational certitude; unlike Cartesians, a preterhuman card waits up their sleeves. Moreover, by appealing to individualistic common-sense tests in the court of public opinion, creationists simply misapprehend the ineliminably distributed property of science and scientific theory change. (One of history's little jokes is that the very principled distrust of structured authority and tradition that wed seventeenth-century Protestant rationalism to little science[44] now divorces it from big science.)

Science and parascience

Habitually circumventing the social and intellectual structure of modern science, now inseparably linked by big science as never before, weighs decisively against creationism's scientific standing. If not science, then what? Creationism is neither deviant science nor simply pseudoscience. It is best appreciated as a specialized *parascientific* movement: one whose members operate outside professional channels of research while still concerned with the criticism, use and dissemination of scientific findings. (Philosophers of science are another such group, likewise, other science analysts, scientific popularizers and amateur naturalists such as bird watchers. For various reasons, many modern enterprises are parascientifically tinged; and now even religions typically vie for legitimacy consonant with the cultural authority of science, rther than asserting independent bases of authority[45].)

creationist parascientists run the gamut from Doubting Thomists to Popper-choppers.

Behaviourally, some creationists are amateur naturalists, fossil-hunters for example (and perhaps there are more creationists in North America than in Britain just because there are fewer bird watchers). But on the whole, creationists are amateur, anachronistic philosophers of science, acting (unlike either working scientists or professional philosphers of science) to alter the content of scientific knowledge piecemeal through plebiscite and lawsuit rather than systematically through influencing professional debates and research activities. They embrace a degenerate research tradition. Their classical mode combines a scholastic preference for oracular science-independent knowledge with a quaintly early-modern mixture of Baconian empiricism and Scottish common-sense realism[12,43]. But lately, some creationists have begun to speak of models, paradigms and falsification. Thus creationist parascientists run the gamut from Doubting Thomists to Popper-choppers.

Amateur philosophy

Scientific creationists suffer a philosophy of science at odds with contemporary philosophy of science as well as with contemporary scientific practice. Their departure from science's professions, procedures and organized scepticism in favour of extra-scientific correction of scientific knowledge is at once principled and opportunistic. It is shaped by the scholastic principles of doubting Thomism, which enjoin opportunistic pruning of whatever contravenes orthodox revelation; quite inimical resources (even Popperian philosophy) will be mobilized as opportunity arises.

The terms "philosophical" and "intellectual" here confer no normative dignity, but, as readers of *Nature* will know, the view that creationism is latter-day scholasticism has been disputed. Criticizing Marsden (its main proponent[12,46]), Jukes[47] asserts that the creationist dispute "is not a polite discussion of differences in philosophy", but a fight over the worst sort of obscurantism. Would that politeness precluded obscurantism; in my research experience, creationists are on the whole polite, indeed amiable. But personality aside, to debate creationism is to quit Plato's for Pluto's Republic, and there is much evidence of Jukes' general view.

As a movement, creationism routinely flouts fundamental intellectual norms (obligations, for example, to earn expertise rather than flaunting credentials; to scrutinize allies as rigorously as opponents; to detect and punish fraud among peers; to meet critic's counterarguments; to acknowledge criticism and retract assertions if the criticism cannot be met[7,37,47,49]). This granted, has Jukes claimed any more than that creationists are ungentlemanly intellectuals and bad philosophers? Marsden's contentions that fundamentalism is not anti-intellectualism but a distinct kind of modern intellectual movement, and that creationists are fundamentally Protestant scholastics, stand on their scholarly merits. Jukes has not refuted them; it is not clear that he has even contradicted them. (It is otherwise with Marsden's view that evolutionary theory is usefully understood as "anti-supernaturalistic mythology"[46], to which Jukes also objects.)

To be sure, the entire creationist campaign is populated with figures such as the "textbook watchers"[11] who are textbook cases of anti-intellectualism[48]. But members of movement organizations tend to be counter-intellectuals, and the movement as a whole relies on them to produce its ideology. Religious intellectuals approach science in various ways[14], the chief options being scholasticism and Averroism. These are, respectively, the view that natural knowledge and revealed knowledge mutually corroborate (classically, that the self-same Author wrote the 'Book of Nature' and the 'Book of Scripture', and the view (typically post-kantian) that natural knowledge and revelation treat different domains. For scholasticism, religion is like science, in several ways. First, theology is commensurable with science (whereas, for Averroism, the two are radically incommensurable, and religion is not just lame science). Second, for scholastics, both science and theology are systems of propositional truths; deny any single truth, and the whole system is weakened, perhaps fatally.

Scholasticism inclines towards scientific realism (against conventionalism) and enjoins extra-scientific correction. Creationists are scholastics. Hence they will produce lists[50] of real truths about nature, allegedly anticipated by Biblical writers and only later verified by science. Hence the unspoken premise of creationist rhetoric: "*Even* (name any authoritative evolutionist) admits that X.. " One cannot "admit" novel knowledge, only what every Schoolman already knows. When scientific experts are found to "admit" X, Y, and Z in this manner, mere human method-beholden science is corrected in the light of superior knowledge.

How is it possible to sustain such a view? Oracular science, like oracular law, may be feasible within delimited subcultures, but it is an impossible luxury within complex societies. There, Averroism secures civil and religious peace; and even if the scientific substance of creationism had not been discredited (as it was, manifestly, during the darwinian revolution), a hearing for such a secretarian epistemology must be sought in vain within the central-societal, cosmopolitan media of communication.

Sociologically, the short answer[12,43,51] is that sectarian Protestantism (aided especially by the segmented social structure of North America) preserved older scholastic philosophy in insulated subcultural enclaves with their own schools, presses and other (peripheral) media of communications. They thus bypassed entirely Averroism and the kantian and darwinian revolutions in the more cosmopolitan media.

Creationists are scholastics . they will produce lists of real truths about nature allegedly anticipated by Biblical writers and only later verified by science.

Scholastic "proto-postivitism"[21] regards the ordinary untutored observer as the unit of knowledge acquisition. Naive empiricism is applied to a naively real world in quest of inductively established certainty; 'proof' is demanded equally of religious and scientific propositions. Thus Arthur Pierson, in *Many Infallible Proofs. The Evidence of Christianity* (1886), could say, "Nothing," either scientific or religious,

"is to be accepted unless based in good evidence," so that if the Bible is read "in a truly impartial and scientific spirit," pious "Thomistic" doubt will dispel infidel doubt[12] Religion is rational, like science; so science is like theology, comprised of certain evident propositions about a real universe, not of more-or-less probable, conventional, theory-laden conjectures.

The same pre-kantian spirit of doxological science, evident in such members of the "parson naturalist" tradition as John Ray, Gilbert White and Jonathan Edwards, and also in the writings of Newton and fellow members of the Royal Society[52,53], continues. It echoes in Paley's memorable *Natural Theology*[54] as well as in forgotten fustian titles[55-60] and their successors[61-63] Its arguments were merely rephrased when Mr Ivan Stonehocker (President, Creation Science Association of Alberta) responded[64] to the claim by the provincial minister of education that the scientific community, not public education, determined the validity of scientific claims

> The scientific community accepts on faith the assumption that the universe and life on earth had a natural beginning There is no evidence or proof for that position — it is simply a philosophy, nothing more Science can only properly deal with events which are observable, repeatable and testable (thus) all discussion of evolution should be turfed out of all courses and all textbooks

Much the same happened when, on US television, creationist the Reverand Mr James Robinson gleefully accused evolutionist Carl Sagan of relying on mere faith in unproven theories Fundamentalism turns out to be some kind of arch-rationalism

Compare Australian creationist Mr A.W Mehlert on punctuated equilibrium: " unless you believe in natural miracles the hard facts (*sic*) of science are in strong disagreement with suggested mechanisms to support the new evolution"[65] Or compare Professor H. Enoch, MA, FZS, retired chairman of the Zoology Department, University of Madras, and now a Vice-President of the Creation Science Movement: "Careful reading of the Scriptures, with an open mind, will reveal that no established scientific fact contradicts the story of creation as described in the first chapter of the Bible"[66].

> there are many men who, though knowing absolutely nothing of the subject with which they are dealing, wish, nevertheless, to damage the author of some view with which they think fit to disagree What they do, then, is not to go and learn something about the subject, (but) wind up saying that: "After all, you know, the principles and methods of this author are totally opposed to the canons of the Baconian philosophy "

Thus, Thomas H Huxley[67] on some of his contemporaries — and some of ours. If scientific knowledge (or selected offending parts of it) cannot be "proven" in a laboratory, or rests on theoretical conjecture, or falls short of inductive certainty from common-sense observation of the facts, then away with it; it is not science. As younger fundamentalists join their contemporaries in mass education, they are exposed to post-nineteenth-century philosophy, and find it just as easy to abuse their opponents with Popperian post-positivism as with Baconian protopositivism[68]

This is not Popperianism, it is Popper-chopping. Popper is chopped in two ways. First, anomalous or difficult data are held to falsify decisively Second, Popper's erstwhile scepticism about Darwinism as a "metaphysical research programme"[69] is invoked. The former, "naive falsificationism"[37], forgets that any theory will be underdetermined by the data available, so that knock-down falsification is quite uncommon. Second, although Popper said "metaphysical", he also circumspectly said "research programme" Popper has, in any case, since retracted this criticism[70,71]. Thus creationist Popper-chopping is (at best) disingenuous[72].

Opportunity structure

Influence abhors a vacuum, and opportunists require 'opportunity structures'[73] So why are creationists so influential? Not through sheer numbers, the movement's core of adherents is small, and its kernel (actual members of scientific creationist organizations) numbers no more than several thousand worldwide. Influence amplifies within an opportunity structure whereby (1) the core uses (2) a protective belt of mainstream intellectuals (willing and unwilling) plus (3) the contemporary climate of public opinion and scientific understanding.

Perhaps the disproportion of physicists and engineers in the creationist movement is no accident physical scientists are professionally disposed to puzzle over central biological concepts.

The protective belt consists of draftees and volunteers. With sufficiently loose standards of argument, the pool of draftees approaches infinity; reading creationists, "one would think that the main spokesman for the fundamentalist cause are Ernst Mayr, G.G. Simpson, Theodosius Dobzhansky and Stephen Jay Gould!"[7] Eldredge and Gould have been drafted (protests[34,74] unheeded) because punctuated equilibrium[75] challenges Darwin's own gradualist view of the tempo and mode of evolution; and whereas doubting Thomism thinks science requires foundational certainty, in principle it cannot distinguish typical intra-scientific dissent from outright repudiation of evolution Thus creationist Mehlert[65]: "Each time the differing evolutionist factions attack each other, all the profit goes to the creationists who can reap the best from both worlds." Likewise, Popper's scepticism is noted, but neither his circumscription nor subsequent retraction

Such a draft is difficult to dodge; some do not try, although there are degrees of volunteering Perhaps the disproportion of physicists and engineers in the creationist movement (and their resurraction of misapplied probability arguments[37,76,77]) is not accidental; physical scientists are professionally disposed to puzzle over central biological concepts (for example, that individuals in a species are not identical in the way that atoms of an element are, that populations and their dynamics, not individuals, are the units of selection; that consequently "in complex, historically formed systems things occur that do not occur in inanimate sytems"[78]) Beyond intra-scientific misunderstanding, lawyer Norman MacBeth[35] mistakes scientific for legal truth-testing. However, the track record of a research programme carries over from case to case (whereas that of contesting lawyers does not); and the analogy, evolution: creationism:. American prosecutorial burden of proof: defendant's presumptive innocence, is unsound.

At what point does confusion pass into wilfulness? Liberal theologian Huston Smith thinks[79] that creationism does public service in policing "the scientific establishment" lest mechanism and reductionism unweave the rainbow. Paul Feyerabend (thoroughly "against method"[80]) proclaims "Three cheers to the fundamentalists in California who succeeded in having a dogmatic formulation of the theory of evolution removed from the textbooks and an account of Genesis included"[81]. (His immediate disclaimer that a creationist establishment would also be dogmatic may strike some as small consolation.) And what of a palaeontologist, the Sorbonne's Pierre Grasse[82], whose nationalist disdain for "daydreaming" across the Channel produces. "The probability of dust carried by the wind reproducing Duerer's "Melancholia" is less infinitesimal than the probability of copy errors in the DNA molecules leading to the formation of the eye ."? Fred Hoyle propagates[83] this same elementary misunderstanding, now a creationist mainstay[84,85]. Likewise the claim that natural selection is tautologous in which Hoyle is joined by palaeontologist Colin Patterson of the British Museum (NH). Creationists take much aid and comfort here. A 1981 talk in which Patterson likened evolutionary theory to religious dogmatism, and said that "evolution not only conveys no knowledge but seems somehow to convey anti-knowledge", was transcribed and circulated (over Patterson's protest[86]) by creationist engineer Mr Luther Sunderland. This is odd, since Patterson also dubs creationism "anti-knowledge"; but he also asserts a baconian view of evidence and theory-testing. On "Proof and Disproof" (*sic*)[87], Patterson commends Darwin's misleading claim that

Fred Hoyle propagates this same elementary misunderstanding, now a creationist mainstay.

he was prepared "to give up any hypothesis ... as soon as the facts are shown to be opposed to it" and warns: "evolutionary theory is not testable in the same way as a theory in physics, or chemistry, or genetics, by experiments designed to falsify it." Finally, there is the matter of transformed cladism (which Patterson[88] champions, along with various recent contributions to *Systematic Zoology*). Its goal of theory-free taxonomy is debateable[89], but its appeal for Doubting Thomists is understandable.

Howbeit willing or unwilling, the protective belt is useful only within a particular climate of scientific understanding. Much ink has been spilt over the rediscovery in recent decades of the insufficiency of reason, and of other curiosities of post-industrial society such as the fact that many people both ride the Clapham omnibus and consult their horoscopes. Yet caution might be urged in interpreting research evidence in favour of pervasive anti-rationalism. For example, one poll taken late in 1981[90] (at the height of the furor) was widely reported as showing that 86 per cent of Americans favoured creationism. What it actually showed was that only 10 per cent believed in creationism, while 76 per cent favoured teaching both creation and evolution. A more recent survey of science students at Ohio State University corroborated, with 80 per cent in favour of both in science courses, although students with increased education in biology were less likely to concur[91]. This suggests that it was not creationism, but belief in fair play (or perhaps the belief that both are "only" theories, and thus on a par) that is at work.

Clearly, the creationists pitch their public appeal to a widespread cultural belief in fair play and equal time for the underdog[11]. And their campaign must attract support, perennially, to the extent that it is able to tap perennial conditions such as "Pluto's Republic" fed by mass education, populist distrust of professional politics in democracies, a folk-epistemology of common-sense empiricism, and simply poor scientific education (of the sort that especially afflicts America, in part because of the erosion of biological curricula by creationism). In other words, the "success" of creationism may be an artifact of the "one-eyed man effect"[92], in which fervently communicated belief fills a vacuum simply in default of any better-defended or better communicated contender. On the other hand, creationism itself is parasitic on the distinctively Western culture of science, including the packed-down science characteristic of modern common-sense knowledge. Thus, not surprisingly, there is some evidence from the counter-mobilization of anti-creationists[93,94] that (*just* because creationism is likely to appeal to those with an interest in and limited knowledge of science) members of the public initially disposed to entertain creationism will change their minds when confronted with a well-argued case for evolution.

Whither creationism?

If the events of 1980-82 appeared as a flash in the pan, the social forces conducive to the creationist movement are perennial. It may disappear from time to time below the threshold of public perception. It has not thereby gone away; though "monkey trials" may not make the headlines, creationists are still at work. Upgrading the climate, and deflating the protective belt, might curtail the disproportionate influence of the movement's core; and, if it does thrive on an outmoded philosophy of science, then including the history, philosophy, and sociology of science as an integral part of science education might exert significant deflationary pressure. Some might regard attention to the climate of "conventional wisdom" as a mere hobby or distraction from real science. However, although the conventional wisdom may be conventional, it may also embody some wisdom and produce some consequences for the support of science; and the scientific community might well ask if concerted attention now is not a small price to pay in order to avoid future generations who, having passed through higher education, agree with that 25 per cent of Ohio State students who believe that evolution is just bunk[91] — "even the scientists say so".

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Evolution of tumours and the impact of molecular oncology

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It is generally accepted that tumours arise through the accumulation of several changes affecting the control of cell growth. Recent advances in molecular biology have made it possible to define some of these changes in molecular terms and to trace the steps by which certain tumours evolve.

FEW would contest the statement that the division and differentiation of somatic cells in higher organisms are regulated by multiple controls. Because cancer develops by the emancipation of a single cell from host controls, it probably arises in several steps. This has been confirmed in a number of ways. Statistical analyses of age-incidence curves have suggested three to four successive mutation-like changes for leukaemias and six to seven for carcinomas (see refs 1, 2 for review). This must be considered the minimum, rather than the actual number, as only the rate-limiting steps will be detected by statistical analysis.

Histological, cytogenetic and experimental analyses have also confirmed that the natural history of spontaneously occurring human and animal cancers is usually a multistep process, based on the successive emergence of new subclones that replace their predecessors as a result of some selective growth advantage[3-5]. The only known exceptions are found in experimental viral oncology. Some of the retrovirus-transduced oncogenes (for example, v-*src*) can cause tumours in a single step. However, as demonstrated recently by Temin[6], serial passage *in vivo* in the laboratory puts the v-*onc* genes carried by the directly transforming RNA tumour viruses under selective pressure. Multiple genetic changes that favour transforming and/or tumorigenic activity can accumulate in a single oncogene. The same principle is illustrated even more dramatically by the discovery of an increasing number of retroviruses carrying two unlinked oncogenes that make different products with complementary transforming effects. The natural process of spontaneous tumour development is quite different. Selection acts on the cell, not on a virus. This favours single changes in multiple genes.

Foulds[3,4] was the first to analyse this process of 'tumour progression'[7]. He emphasized that the neoplastic phenotype is composed of several 'unit characteristics' capable of wide variation and able to reassort independently of each other. Thus, the same phenotypic endpoint can be reached through several alternative pathways[4]. Each progression step may reflect the activation, mutation or loss of different genes. Molecular biology has opened the door towards their definition. At least three major functional groups can be discerned already. The oncogenes represent the first group (for review, see ref. 8). They seem to act dominantly in the sense that they can induce transformation when transferred to cultured cells. The best-known example of this group of genes is the *ras* family, in which a single point mutation can confer tumorigenicity. It is, however, unclear whether mutant *ras* genes act as simple determinants *in vivo*. For example, in tumours from human patients the normal germline *ras* allele is often lost[9].

Moreover, there is evidence for a second group of genes, the 'tumour suppressors' or 'anti-oncogenes', that may have to be deleted or functionally incapacitated before a tumour can arise. The most thoroughly analysed example of such a gene is *rb-1*, both alleles of which are lost in retinoblastoma tumour cells[10-12]. It has been suggested that the rb-*1* gene may have a normal regulatory function, possibly in the induction of an essential step in the terminal differentiation of the tissue[11,13-16]. Some other neoplasms, such as Wilms' tumour, familial renal cell carcinoma, neuroblastoma and small-cell carcinoma of the lung (SCLC), seem similarly to require the loss of both alleles of a single gene. This evidence for recessive anti-oncogenes is consistent with much earlier reports of the suppression of tumorigenic behaviour in hybrids derived from the fusion of malignant with normal cells[17-20]. Cytogenetic analysis of such hybrids and their high malignant segregants suggests the existence of different suppressor genes on different chromosomes[21-24]. There is now evidence that the transforming effect of different retroviral oncogenes can be counteracted by different suppressor genes. Recently, revertants *in vitro* of Kirsten-murine sarcoma virus-transformed fibroblasts have been found to suppress *ras*, *fes* and *src*-transformed partner cells, but not cells transformed by *mos*, *fms* and *sis*, after somatic hybridization[25]. Eventually, the suppressor genes may turn out to be as diverse as the oncogenes, if not more so.

The third group of genes that influence neoplastic behaviour may be designated modulators. They do not on their own transform normal into neoplastic cells, but they modify their spread in the organism. They probably constitute a large and heterogeneous group. For example, metastatic spread may be influenced by the genes of the major histocompatibility complex (MHC)[26-29] and invasiveness by those controlling proteolytic and homing mechanisms[30,31]; cellular resistance to immune rejection[32,33] may also play a part. Here we shall discuss the way in which some cancers may develop by a series of different steps. We have selected cases in which individual steps have been analysed in genetic and/or molecular terms.

Burkitt's lymphoma and mouse plasmacytomas

The Epstein-Barr virus (EBV)-carrying African or high endemic form of Burkitt's lymphoma (BL) provides an interesting example of a human tumour that develops in several steps. The tumours are derived from cells of the B-lymphocyte lineage that is responsible for the production of immunoglobulin. At least three different genetic systems may be involved. The first is of DNA-viral rather than of cellular origin. EBV is a highly transforming agent for human B lymphocytes. Epidemiological evidence has shown that an early EBV infection of African children at a relatively high multiplicity contributes to the causation of the tumour[34]. This is corroborated by the discovery that 97% of African Burkitt lymphomas carry multiple copies of the EBV genome in all their cells[35].

The second genetic system is of cellular origin, activated by chromosomal translocations characteristic of Burkitt lymphomas. With the exception of a single atypical BL-derived line[36], all Burkitt lymphomas so far studied have been found to carry one of three specific translocations. This applies not only to the EBV-carrying African form but also to the sporadic

Table 1 Gene interactions in the development of Burkitt's lymphoma

Oncogene or transforming gene	Documented effect	Hypothetical effect	
		In vitro	*In vivo*
EBV (Three active regions in transformed cells)	B-cell activation and immortalization *in vitro* Activation of BCGF production Synthesis of EBNA and LYDMA	Autocrine stimulation BCGF dependence, also reflected by clumping and low agarose clonability. Limited freezing of maturation; some terminal differentiation does occur or can be induced.	Creates latently infected B cells, dependent on and responsive to B-cell growth factor and susceptible to immune control.
Activated *myc*	Juxtaposition to an immunoglobulin locus by chromosomal translocation leads to constitutive *myc* expression	Ability for BCGF independent growth, with or without maintained responsiveness, also reflected by high agarose clonability and growth in single cell suspension Switched on *myc* drives cell division 'from within'?	Decreased dependence on positive growth signals from the outside Resistance to differentiation induction?
Activated *ras* and/or *Blym*?*	Transformation of NIH 3T3 fibroblasts	Changed growth factor or differentiation factor receptors?	May act in complementary or synergistic fashion with activated *myc*

* Activation of these genes has only been demonstrated in some long-established *in vitro* lines

non-endemic cases that occur all over the world. The translocations act by juxtaposing c-*myc* to one of the three immunoglobulin loci, normally active in B lymphocytes, with the result that the oncogene is constitutively activated (see ref. 37 for review). It is possible that a third oncogene system may be involved as well. it has been reported that NIH 3T3 fibroblasts can be transformed with DNA from BL-derived sequences that are related neither to EBV nor to *myc*. The identity of the transforming DNA is currently controversial. There is some evidence for activation of a *ras* gene and of *Blym*[38,39].

How do these genes interact in generating Burkitt's lymphoma? EBV is a uniquely potent immortalizing agent for human B cells. Three regions of the viral genome (LT-1, 2 and 3) are regularly transcribed in immortalized cells[40] The LT-1 region seems to be necessary for inducing the first activation step that is followed by a chain of events leading to indefinite proliferation in the normal B lymphocyte· an EBV substrain in which the LT-1 region has been deleted cannot activate and immortalize normal lymphocytes[41]. The protein product of LT-1 is a DNA-binding nuclear protein of relative molecular mass 81,000, designated EBNA-2 (refs 42-44). A second nuclear antigen, EBNA-1, which was identified earlier, is encoded by the LT-2 region[45]. Repression of the lytic cycle, a prerequisite for the continuous proliferation of the virally transformed B lymphocyte, may require the interaction of EBNA-1, EBNA-2 and some B-cell specific cellular constituent[46].

Recent experiments of Gordon *et al.*[47] suggest that at least part of the immortalizing effect of EBV on the B lymphocyte results from the activation of an autocrine loop (for a recent review of autocrine activation see ref. 48). Both the EBV-transformed B-cell lines of normal origin (LCL) and the tumorigenic BL lines release B-cell growth factor (BCGF), but although LCLs are dependent on it, BL cells are not. Some BL lines are responsive, but others have lost their responsiveness[49]. The course of events is strongly reminiscent of earlier studies on tumours in hormone-dependent tissues where a 'conditioned tumour', initially dependent on the hormonal imbalance that generated it, later becomes independent of the hormone and eventually unresponsive to it[50].

If EBV is responsible for the immortalization of BL cells, what does the *myc*/immunoglobulin translocation contribute to tumorigenesis? In the typical translocations, the *myc* oncogene breaks at its non-coding 5'-end, or at variable distances upstream of it[51-63]. The coding exons of the gene are transposed to the chromosome containing the immunoglobulin heavy-chain genes, head-to-head with the immunoglobulin gene. In contrast, the variant translocations (those involving the immunoglobulin light-chain genes) break the chromosome below the tail end of the gene[64-66]. In these cases, therefore, c-*myc* remains in its original location with the constant region of the κ or the λ light-chain attached to it, in a head-to-tail orientation. Despite these differences, all three translocations activate *myc* transcription constitutively. The importance of this activation and the intactness of the *myc* protein is stressed by the fact that the coding second and third exons of c-*myc* are always intact, despite the considerable variability of the translocation breakpoint in and around the gene. This variability and the equally large variation in the translocation breakpoints within the immunoglobulin genes also implies that no single enhancer or promoter can be responsible for *myc* activation in all or even most of the tumours. This may suggest that what is important is the active state of the chromatin, rather than single specific sites[63,67].

Early interpretations of the mechanism whereby the c-*myc*/immunoglobulin juxtaposition contributes to the tumorigenic process included claims of abnormally high transcription rates, abnormal transcript size, changed promoter use, mutations[68] and changed translational control. Each claim has been documented for some tumours, either in the BL or in the closely analogous mouse plasmacytoma (MPC) system, but none was found to apply to all or even most of the tumours. Perhaps there are several ways of activating c-*myc*, and no unifying mechanism exists[69]. Alternatively, the crucial event may be more subtle than a relatively crude quantitative or qualitative change. The current concept is *dysregulation*. The idea is that the immunoglobulin-juxtaposed *myc* gene has become subject to *cis* control by the constitutively active immunoglobulin region and must therefore behave as if it were part of the immunoglobulin locus itself. The transcription of the normal *myc* gene is associated with competence for cell division[70,71]: in terminally differentiating cells c-*myc* transcription ceases[72]. Mature plasma cells continue to produce immunoglobulins, however, and an immunoglobulin-juxtaposed *myc* may be similar. This would keep the cells in continuous division.

Although there is no direct evidence for this hypothesis, it is supported by comparative studies on the behaviour of the translocated *myc* gene and its normal allele in MPCs and BLs. It has been found repeatedly in both systems that the translocated gene is highly transcribed whereas its normal counterpart is switched off[73-78]. This is compelling evidence for the differential regulation of the two genes.

The BCGF-independence of the BL-cells may result from the locking of the immunoglobulin-juxtaposed *myc*-gene in a continuously active position. It may act as a 'pacemaker' from

Table 2 Expression of three oncogenes in three different tumours of the B-cell lineage, induced by Abelson virus, with or without mineral oil

Tumour	Inducing agent	Cell type	v-*abl* expression	c-*myb*	c-*myc*	Karyotype
ABLS	Abelson virus	Pre-B	+++	Normal	Normal	Normal diploid
ABPL	Abelson+oil	B?	–	+++ rearranged	Normal	No translocation
ABPC	Abelson+oil	Plasma cell	+++	Normal	+++ rearranged	12;15 or 6;15

(After the data of Mushinski and Potter *et al.*[84,89-92].)

within, prompting the continuous re-entry of the cells into the cycle, thereby obviating the need for exogenously supplied BCGF.

Pending further definition of the third oncogene and clarification of the important question of whether the DNA of tumours *in vivo* has the transforming activity that has been reported in the DNA of established BL lines, it may be noted that transforming *ras* genes with the characteristic point mutation have recently been isolated from certain experimental lymphomas[9,79]. The mutant *ras* gene is believed to transform fibroblasts by inducing anchorage independence; but lymphocytes are inherently anchorage independent, so if mutant *ras* genes do play a part in the aetiology of BLs, it must be by some other mechanism. The attachment of the ras protein to the inner surface of the plasma membrane prompts speculations on membrane rearrangements that may interfere with the normal receptor organization and/or the transmission of growth and differentiation controlling signals.

In the natural history of African BL, EBV-transformation of the target cell is probably the first event[80]. The environmental co-factor, perhaps chronic hyperendemic malaria[81], may act by increasing the size of the target cell population. Because the EBV-infected B cell is longer-lived than its EBV-negative counterpart, it may undergo a larger number of divisions before being eliminated by terminal differentiation or by immunological mechanisms. The probability of all genetic aberrations increases with the number of cell divisions, including the risk of the *myc*/immunoglogulin juxtaposition. Heavy malarial infection may facilitate the development of the lymphoma in a dual capacity: polyclonal B-cell activation and T-cell immunosuppression Table 1 is a schematic summary of our current view of the genesis of BL.

It is instructive to compare the course of events leading to BL with those culminating in the development of a mouse plasmacytoma. Molecular studies have confirmed our original suggestion[82] that MPC and BL carry homologous translocations that involve the activation of the same oncogene by an immunoglobulin locus. The close homology of the translocations in these two tumours is remarkable, because their natural history is very different. MPC originates from mature plasma cells, whereas BL arises from a relatively immature B cell, producing the mainly surface rather than the secreted form of immunoglobulin[83]. The human counterpart of mouse plasmacytoma is multiple myeloma, whereas the mouse equivalent of BL is B-cell lymphoma; these cells do not carry similar translocations. What then could account for their regular appearance in BL and MPC? A possible common denominator in MPC and BL is a long preneoplastic period in which the cells are chronically stimulated. Intraperitoneal granuloma induced by mineral oil is a prerequisite for MPC[84] whose development can be prevented by hydrocortisone which inhibits the formation of the granuloma In BL, a functionally analogous chronic stimulation may be provided by the combination of EBV and chronic hyperendemic malaria.

No virus is known to be involved in the development of MPC and the BCGF-mediated autocrine loop may be missing. Note, however, that the mineral oil-induced MPCs are often not transplantable unless the syngeneic recipients carry an oil granuloma similar to that in the original host. This suggests that constitutive activation of *myc* is not sufficient to produce a fully autonomous neoplasm in a single step. Therefore, it is of interest that several secondary changes have been repeatedly found in some MPCs. They include a 6;10 translocation that leads to the juxtaposition of C-κ and an unknown single-copy gene[85] or the activation of the c-*mos* oncogene by the insertion of an intracisternal A particle[86,87] and a variety of other changes (see ref. 88 for review).

These secondary changes have been found in long-propagated lines of mouse plasmacytomas and may thus reflect changes in the tumour in culture. There is, however, one interesting example of an interaction occurring at the inception of the tumour. The induction of primary plasmacytomas can be facilitated greatly by the parallel administration of Abelson virus and mineral oil.[84]. The latency period is reduced and the frequency of plasmacytomas in the susceptible BALB/c strain increased. Abelson virus carries its own oncogene (v-*abl*). In the resulting tumours, the v-*abl* gene is transcribed at high levels, in parallel with the appearance of the typical 12;15 or the variant 6;15 translocation[89]. The same treatment (combination of Abelson virus and mineral oil) also induced some plasmacytoid lymphosarcomas (ABPL), but they show a different pattern of oncogene activation. Mushinski *et al.*[90-92] have shown that these tumours carry rearranged activated *myb* genes, with a long terminal repeat (LTR) from a murine leukaemia virus inserted at the 5′ end of the oncogene. The LTR seems to be derived from the Moloney helper virus. The v-*abl* oncogene is not usually expressed, nor is there any *myc* activation by translocation in these tumours.

These findings are summarized in Table 2. They are contrasted with the Abelson virus-induced pre-B-cell lymphomas in which v-*abl* is highly transcribed, but with no anomalies of c-*myb* or c-*myc* transcription. These experiments suggest that the transformation of cells in different stages of differentiation in the same lineage is brought about preferentially by different oncogenes. A similar situation may prevail in some of the lymphomas and the leukaemias of the human B-cell series. In Burkitt lymphoma and the closely related B-cell-derived acute lymphatic leukaemia, the *myc* oncogene is activated by its juxtaposition to one of the immunoglobulin loci. In contrast, other B-cell tumours may carry 14q markers generated by the attachment of other chromosome pieces to the IgH region of chromosome 14 (ref. 93). Recently, Croce's group has analysed the relatively common 11;14 and 14,18 translocations at the molecular level. They have identified two new potential oncogenes, designated *bcl-1* and *bcl-2*, derived from chromosomes 11 and 14, respectively [94].

Small-cell lung carcinoma

The recent work of Minna's group has interesting implications for the progression of small-cell or oat-cell carcinoma (SCLC) of the lung[95,96]. SCLC exists in a typical relatively low malignant form and in a highly malignant variant form (v-SCLC). Three types of genetic change are found in this system. Most of all the tumours examined have a deletion in the short arm of chromosome 3. Shortest-region-overlap analysis has localized the consistent change to a small area (3p14). It may be significant that this region coincides with the breakpoint in two translocations associated with renal carcinoma (3;8 and 3;11)[97,98]. It also corresponds to the approximate localization of the *raf* oncogene[99]. The SCLC-associated deletion arises by a somatic

change and is not present in the normal cells of the patient. Although the deletion is visible only in one of the two homologous chromosomes, analysis by restriction-fragment-length polymorphism has revealed a deletion in the other chromosome 3 in the tumour cells of heterozygous patients (J. Minna, personal communication). This is consistent with Knudson's[13] hypothesis that some tumours develop only after the loss of both alleles of a single gene.

Activation of a *ras* oncogene by point mutation is the second type of genetic change in SCLC[100-102]. It is only found in a few cases, however, and its significance is therefore less clear

The third genetic change is the most interesting in the sense that its phenotypic consequences are the easiest to interpret. In ~20% of SCLC tumours, or cell lines derived from them, c-*myc* or N-*myc* is amplified 20–80-fold, with substantially increased transcription. This change is regularly associated with the variant v-SCLC phenotype (ref. 95, J Minna, personal communication). Such variants have increased cloning efficiency, shortened doubling times, decreased expression of differentiation markers and decreased adhesiveness compared with the typical tumour. In the patient, v-SCLC is more metastatic and has a poorer prognosis than the typical form. Occasionally, progression from the typical to the variant form can be observed in the same patient The variant appears as a morphologically different subtype in the original tumour or in one or several metastases.

The amplification of *myc* in SCLC is often associated with the appearance of double minute chromosomes, or homogeneously staining regions that do not necessarily correspond to the original location of the amplified gene and whose locations may vary between tumours. In one case, cells from different metastatic sites in the same patient all had amplifications of the same *myc* gene, but in different arrangements.

These findings strongly suggest that the amplified genes can be very labile and that amplification of either c-*myc* or N-*myc* plays an important and perhaps decisive part in the transition of low malignant SCLC to the highly malignant' variant phenotype. Moreover, the postulated double loss of one gene by deletion and the concurrent amplification of a *myc* gene strengthens the analogy with retinoblastoma in view of the recent demonstration of N-*myc* amplification in the latter system[11]. It may also be noted that Minna's findings suggest, for the first time, a functional analogy between c-*myc* and N-*myc*.

The amplification of *myc* has also been found in other types of tumour, the promyelocytic leukaemia line HL60 being a particularly well-known example[103]. This continuously growing cell line shows an extraordinarily high level of c-*myc* transcription, which, however, can be turned off very rapidly when the cell is induced to differentiate[72]. Amplification of *myc* has also been found in various solid human tumours[104] and in transplanted rat hepatomas[105]. In neuroblastoma, N-*myc* amplification appears in stage III and IV tumours and may be related to tumour progression[106]. Recently, we have found amplification of c-*myc* in a highly malignant plasma cell leukaemia, but not in the less malignant multiple myelomas[107].

It may be more than a curiosity that Bishop's group has detected amplification of c-*myc* in a 25-year old, originally polyoma-induced ascites tumour, SEWA[108]. Double minute chromosomes were first discovered in this tumour[109], they carry amplified c-*myc* sequences.

These divergent examples raise a number of intriguing questions about the roles of the *myc* genes in the oncogenic process and in tumour progression; v-*myc* is the only known virally carried oncogene that can induce tumours in all three major tissues: epithelial, mesenchymal and haemopoetic. similarly, c-*myc* activation by retroviral insertion in the chicken bursal lymphoma[110] and by chromosomal translocation in MPC and BL represent early events in the history of these tumours. By contrast, amplification is usually a late event that occurs during the progression of established tumours.

Transcription of *myc* is switched on when the cell cycle is activated in both lymphocytes and fibroblasts[70,71]. If *myc* plays a key part in this activation it is not surprising that the constitutive activation of the gene by viral or cytogenetic accidents that bring it under the *cis* control of highly active regions may keep the cell continuously in cycle. This could happen in cells of many different types. It may be the first step in the chain of events that lead to tumour development, or a late step that prompts malignant progression of established tumours. This also implies that permanent activation of a *myc* gene ought to change certain non-tumorigenic cell lines that have the appropriate genetic constitution to tumorigenic variants. This has been demonstrated experimentally by Keath *et al.*[111]. Rat fibroblast variants that became tumorigenic after transfection with appropriate *myc* constructs showed none of the usual morphological changes of transformed cells, and did not acquire high agarose clonability, but they did have a diminished requirement for serum. In these cells, the transfected gene was expressed at high levels whereas the normal c-*myc* allele was silent, just as the translocated c-*myc* gene is active and the normal allele silent in MPC and BL.

Progression never reaches an endpoint

The clonal evolution of tumour cell populations proceeds through the sequential selection of variant subpopulations from a common progenitor[5,112-114]. New variants may arise at any time; some of them have a selective growth advantage over earlier forms This process may be accelerated by the genetic instability of tumour cell populations, reflected by the high frequency of mitotic errors and variation of genetic markers This instability may become more pronounced as the neoplasm evolves (see ref. 5 for review).

This process can be particularly clearly seen in the chromosomal evolution of chronic granulomatous leukaemia (GGL) carrying a characteristic chromosomal translocation giving rise to what is known as the Philadelphia (Ph^1) chromosome[115,116] During the early, chronic and relatively benign stage of the disease the precursor pool for the entire granulocyte, erythrocyte- and thrombocyte-forming bone marrow is taken over by a single clone of cells carrying the Ph^1 chromosome[117]. Occasionally, it may involve part of the bone marrow-derived (B) lymphocyte series as well. The Ph^1 ($22q^-$) chromosome arises most frequently by a 9;22 translocation. In a variant minority, the exchange seems to involve chromosome 22 and virtually any other autosome.

When it was first discovered, the significance of the Ph^1 translocation was unknown. More recently however, as with the translocations characteristic of BL, it has become possible to look for an interpretation in terms of oncogene activation. In particular, the c-*abl* oncogene, which is located on the tip of chromosome 9, is regularly transposed to the deleted $22q^-$ chromosome in Ph^1-positive CGL[118,119]. It is particularly significant that this happens not only in the typical 9,22 but also in the variant translocations[120] where the deleted distal arm of chromosome 22 moves to another autosome, not 9. The fact that c-*abl* moves also from 9 to 22 in these cases implies that three chromosomes are involved in the variant translocations The consistency of the *abl* transposition greatly increases the probability that it is significant in tumorigenesis

The molecular analysis of this transposition is still at an early stage. On chromosome 9, the breakpoint is immediately upstream of, or within, the c-*abl* oncogene[121]. On chromosome 22, the breakpoints are clustered within a limited 5–8-kilobase (kb) region, designated *bcr*[122]. Little is known about the level of expression, except the interesting fact that a new 8-kb *abl* RNA transcript can be found in Ph^1 + CGL, whereas the 6- and 7-kb transcripts found in normal haematopoietic cells are absent[123]. The functional significance of the transposed *abl*-carrying $22q^-$ chromosome, rather than the reciprocal $9q^+$ fragment, is supported indirectly by the finding that K562, a CGL-derived, originally Ph^1 + erythroleukaemia line, has amplified the chromosome carrying the transposed *abl* gene and its immediate surroundings, but not the reciprocally translocated *sis*-oncogene, located on chromosome 22 distally of the Ph^1 breakpoint[122]

The appearance of the Ph^1 chromosome is the first clearly definable step in the development of Ph^1+CGL. Therefore, its initial action must occur during the early chronic phase of the disease, which has a median duration of 3 years. It usually precedes a second or acute phase, characterized by decreasing cell maturity and increasing blast proliferation. The transition from the chronic to the acute phase is often accociated with further nonrandom chromosomal changes[124]. The most frequent secondary events are the appearance of a second Ph^1 chromosome, or an additional chromosome 8, or an additional long arm of chromosome 17. Note that the trisomy of the c-*myc*-carrying chromosome 8 is the commonest trisomy in all human haemopoetic disorders, ranging from relatively benign myeloproliferative diseases to acute leukaemia[115]. This may be more than a coincidence, particularly if considered together with the fact that trisomy of chromosome 7 is the commonest chromosomal change in Rous sarcoma virus-induced rat tumours[125] and trisomy of chromosome 15 is the commonest and often single chromosomal anomaly in mouse leukaemia[126–128]. The c-*myc* gene has been localized to chromosome 7 in the rat[129] and to chromosome 15 in the mouse[130].

Immunology of host–tumour relationship

Widely polarized views prevail on the question of immune responses to tumours. At one extreme, they are believed to play a crucial part in protecting the host against neoplastic growth. At the other, they are regarded as virtually non-existent. The unfortunate tradition of considering this important problem in all-or-none terms often prevents a more discriminative analysis.

The probability of efficient immune rejection varies depending on the mode of tumour induction. It should be considered separately for tumours induced by DNA tumour viruses, those resulting from retrovirus insertion near a cellular oncogene, and tumours arising from oncogene activation by spontaneous or induced changes in cellular DNA. We shall consider each case in turn.

(1) Oncogenic DNA viruses carry their own transforming genes. This is why transformed cells of this category provide the best targets for host immune surveillance[131]. The evidence for such surveillance is indirect and can best be seen for man in the case of EBV. The immune mechanisms that normally suppress tumorigenesis by EBV are unknown. But it is clear that tumours develop only in special circumstances, for example in patients with relatively grave combined forms of congenital or acquired immunodeficiency. At least some of the fatal mononucleosis cases may belong in this category. The X-linked lymphoproliferative syndrome[132] arises by the proliferation of EBV-carrying lymphoid cells in patients who lack virtually all T-cell activity, have no natural or interferon-activable killer cells and little or no immunoglobin[133,134]. Organ transplant recipients, who have a more restricted immune defect, also develop EBV-carrying lymphoproliferative lesions[135,136].

An interesting but unresolved question arising in connection with these tumours is whether they carry the chromosomal translocations characteristic of Burkitt lymphomas. Such translocations have now been found in EBV-carrying Burkitt lymphomas arising in patients with acquired immune deficiency syndrome (AIDS)[136]. By contrast with Burkitt's lymphoma, which is typically a disease of childhood, Burkitt lymphomas associated with AIDS arise in adults between 20 and 40. The implication is, therefore, that EBV-positive, translocation carrying 'BL candidate' cells may arise at any time of life, but in normal immunocompetent individuals they will be eliminated by immune rejection. The immunosuppressive factor in the African childhood BLs may be chronic malaria[81]. Although T-cell mediated immune responses have been clearly demonstrated in the case of EBV-carrying BLs[138,139], there is no evidence for any host response against EBV negative BLs that carry the same translocations as the EBV positives, nor do they show the occasional spontaneous regressions or the ready curability by chemotherapy that is characteristic for the EBV-carrying and prognostically more favourable counterparts

Note in this connection that the increase in malignant disease in congenitally or iatrogenically immunosuppressed patients is limited to a small and rather unusual group of tumours, such as lymphomas and other lymphoproliferative diseases, Kaposi's sarcoma, skin papilloma and carcinoma[137,140]. It would not be surprising if most, if not all, of these tumours turned out to be DNA-virus related. EBV has already been found in most of the lymphoproliferative lesions in immunodefectives[133,135–138]. Cytomegalovirus is suspected in Kaposi's sarcoma[141] and papilloma viruses may be partly or fully responsible for the skin tumours[142].

(2) There is increasing evidence for cellular oncogene activation by retroviral promoter or enhancer insertion[110] as a mechanism of tumorigenesis. Its relevance has been demonstrated for a variety of avian and mammalian tumours. Only a small part of the retroviral genome is required for the activation of the adjacent cellular oncogene, and that region contains only the regulatory LTR: it has no coding function. Although DNA encoding some of the viral proteins (*gag* and *env*) may also be present, the proteins are not necessary and may even be disadvantageous for oncogene activation because they provide potential targets for the immunological reponse of the host. Given that the protein-coding regions of the virus may be lost while the transformed phenotype of the cell is maintained, it is not surprising that immune rejection of tumour induced by RNA tumour viruses is unpredictable.

(3) Activation of cellular oncogenes by nonviral mechanisms such as point mutations, DNA rearrangements, gene amplification and chromosomal translocations, is probably the basis for most of the spontaneous tumours. The transforming oncogene products are either normal or only slightly modified cellular proteins. It is difficult to see how they could provide a rejection target for the immune system. It is at least possible that a mutated ras protein with a changed amino acid at position 12 or 61 could be antigenic, but this has not yet been demonstrated.

Thus, it seems that specific immune recognition and rejection of antigenically foreign tumour cells is largely restricted to virally induced tumours, and in particular to those caused by DNA viruses. There is increasing evidence, however, that rejection by specifically sensitized T cells is only one of several effector mechanisms that can counteract neoplastic target cells. Another example is provided by natural cytotoxicity that can play a significant role in protecting the host against the metastatic growth of disseminated tumour cells[143,144]. Natural killer (NK) cells can kill sensitive tumours without having been immunized against specific antigens on the target cell surface. Their action is not restricted to virally or chemically induced neoplasms, although different tumours differ in their NK sensitivity. NK cells may be scavengers that can recognize and eliminate 'displaced' cells that have wandered outside their natural homing boundaries. Cells in which the expression of 'self' markers encoded in the MHC is reduced are particularly sensitive targets[26]. It is intriguing that diminished expression of MHC class I antigens can enable tumour cells to avoid rejection by specifically sensitized MHC-restricted cytotoxic T cells, but increases the probability of their elimination by NK cells. Conversely, there is evidence that an increased expression of MHC antigens in mice may increase metastasis[27–29,145] This, then, is a case in which a quantitative change in a universally occurring cell surface marker can either enhance or inhibit tumour progression.

Future of molecular oncology

In his classical paper on tumour progression[4], Foulds emphasized that most tumours develop by stepwise changes in many independent characteristics. The independent assortment of these characteristics leads to the many individual variations in the biological properties of tumours. Foulds stressed the need for a factorial analysis of the complex tumour phenotype. The advent of modern molecular biology has made such an analysis possible. We are at the threshold of a previously unknown world where oncogenes, antioncogenes and other cellular genes inter-

act within complex regulatory circuits. Thanks to the recombinational accidents of the retroviral lifestyle, we now have access to the dominantly acting oncogenes as an unexpected gift from viral oncology. So far, we have learned much at the level of the DNA sequence, something of transcriptional regulation, and a little of the mechanisms whereby the oncogene products can change cellular behaviour. At least two other worlds of genes, so far less conspicuous, remain unexplored. The suppression of tumorigenicity in hybrids between normal and malignant cells and the regular loss of both alleles of a single gene in the genesis of such tumours as retinoblastoma or Wilms' tumour suggest the existence of other, probably quite heterogeneous gene families, tentatively designated anti-oncogenes or tumour suppressor genes that may counteract the expression of the tumorigenic phenotype. There may be also special safeguards to prevent the inadvertent activation of particularly dangerous oncogenes, as illustrated by the example of c-*mos*[146], which is never transcribed in normal cells and even a low level of transcription seems to be sufficient for neoplastic transformation. A 5′ flanking sequence prevents the downstream activation of the gene in the mouse. The human c-*mos* gene shows an even more drastic change, a gap in the middle that may prevent activation altogether. These and other existing hints indicate that evolution may have fixed a whole variety of protective mechanisms that may *cis*- or *trans*-regulate the expression of activated oncogenes. A third and probably even more widely diversified world of genes is not directly involved in the initiation of neoplastic transformation or in the protection against it, but can nevertheless affect the phenotypic properties of established tumours in a prognostically important way. Proteolytic mechanisms that contribute to invasiveness, changes in 'homing markers' that can influence the neoplastic colonization of distant organs, and changes in the ability of tumour cells to induce or to succumb to an immune response fall into this category.

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Large-scale regional units in the depleted upper mantle revealed by an isotope study of the South-West Indian Ridge

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Isotope analyses of ocean island basalts have demonstrated the existence of regional geochemical provinces, the Indian Ocean being distinguishable from the North Atlantic and Pacific oceans. Equivalent analyses of basalts from the South-West Indian Ridge now indicate that the upper mantle under the Indian Ocean also has a distinct isotope composition. Thus, the upper mantle itself must be composed of several discrete provinces which may relate to the convection pattern.

INTERPRETATION of isotope variations observed in basalts from mid-ocean ridges (MORB) is used currently to infer the structure of the upper mantle and the processes acting at ridge crests[1-3]. Studies of the Mid-Atlantic Ridge (MAR)[4-8], the East Pacific Rise (EPR)[9] and Juan de Fuca and Gorda ridges[10] have shown that the isotope results for MORB from these three ridges define common linear correlations in the Pb/Pb, Pb/Sr and Sr/Nd diagrams. These 'MORB' alignments are often interpreted as evidence of mixing between two types of mantle sources under the ridges. The first source is convecting asthenospheric material, corresponding to the 'depleted values', less radiogenic for Pb and Sr and more radiogenic for Nd, whereas the second is composed of blobs thought to be the source material of ocean island basalts and is characterized by more radiogenic Pb and Sr and less radiogenic Nd compositions. This model is based partly on the fact that isotope results for islands of the North Atlantic and East Pacific oceans extend the MORB alignments for these oceans in all the diagrams[5,11-13].

The study of the Indian Ocean allows an interesting test of this model, because it has been demonstrated[11,14,15] that Indian Ocean islands have isotope compositions which are not on the extension of the Atlantic and Pacific MORB alignments in the Pb/Sr diagram. Dupré and Allègre[15] have given preliminary results for MORB samples of the Carlsberg Ridge which suggest that such a regional characteristic of the Indian Ocean is also found in ridge products; the present study of the South-West Indian Ridge (SWIR) further documents this.

The SWIR is interesting because two hotspots, the isotope compositions of which have already been determined, are very close to it: Bouvet[11,16], on the south-west extremity, and Marion–Prince Edward (S. R Hart and A. J. Erlank, manuscript in preparation), on the centre of the ridge. Le Roex *et al.*[17] have measured Nd and Sr isotope compositions of samples from the south-west part of the SWIR, whereas we report here the Pb, Sr and Nd isotope results for the north-east part.

Samples

The samples from the SWIR analysed here were collected during the MD34 cruise of the RV *Marion Dufresnes* in February 1983. Eight dredging locations were spaced regularly from the Atlantis fracture zone (57 °E) (at 13° longitude from the Rodriguez triple junction) to the Prince Edward fracture zone (35 °E). The ridge rises continuously from depths of ~4,000 m at the triple junction up to the swelling where the islands of Marion and Prince Edward emerge near the ridge. This bathymetric swelling also corresponds to the convergence of the Madagascar plateau to the north and the Crozet plateau to the south-west (Crozet Island itself being 770 km from the ridge at this point).

The dredges were all performed in the axial valley, clearly identifiable by its magnetic signature. The centre of each ridge section delimited by major fracture zones was chosen for sampling (Fig. 1). The dredge D8 (62 °E) yielded only serpentinites, but in all other cases variable amounts of basaltic glasses were obtained

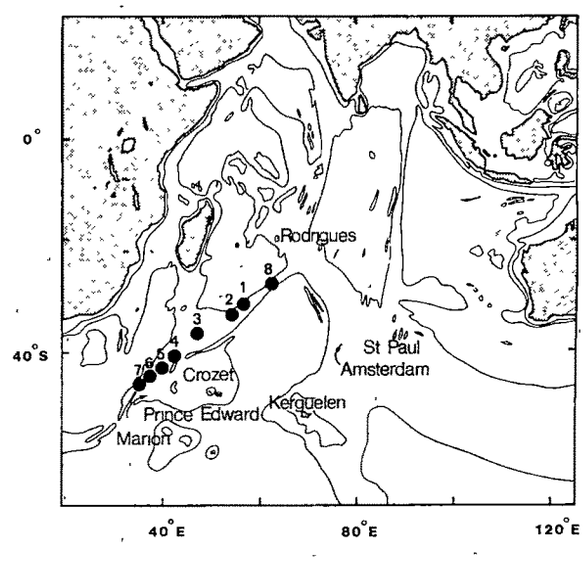


Fig. 1 Bathymetric chart of the Indian Ocean showing the location of the height dredges of the MD34 *Marion Dufresnes* cruise.

Two samples from the MAR have been added to this set of samples, to assess the differences between the two oceans. Both samples are from the Kane fracture zone region, which has given some of the least radiogenic Pb and Sr isotope results[9,18]. One of these samples (AII 96 18-1) had already been analysed for Sr and Nd by Machado *et al.*[18].

Analytical procedures

Between 100 mg and 700 mg of glass was chosen, using a binocular microscope to eliminate any pieces of basalt with visible signs of alteration, encrusted sediments or plagioclase phenocrysts. Pb, Sr and Nd separations were performed using the standard procedures of Manhes *et al.*[19], Birck and Allègre[20] and Richard *et al.*[21]. Powders obtained by crushing millimetre-sized pieces of glass in a boron carbide mortar were leached in 6 M HCl before dissolution, to remove marine Sr contamination.

Pb, Sr and Nd isotope compositions have been determined sometimes on the same powder sample. Pb was first separated in 0.5 M HBr solution after dissolving the powder in HF and HBr; fluoride residues were next eliminated by dissolving in $HClO_4$ and evaporation before Sr and Nd separations. Blank levels and standard calibrations are shown in Table 1.

Table 1 Pb, Sr and Nd isotope data for SWIR glasses

Sample	Location	Depth (km)	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{87}Sr/^{86}Sr$	$^{143}Nd/^{144}Nd$
D1	31°41.6′ S	4,050	17 554±12	15.433±15	37 289±46	0 70275±2	0 513081±20
	57°50.5′ E		17.497±18	15.399±16	37.181±51	0.70276±2*	
D2	33°45.3′ S	3,800	18.114±18	15.505±26	37.839±65	0.70269±1*	0.512981±22
	56°16.4′ E					0.70269±2	0.513001±28
D3	37°42 6′ S	3,260	18.137±11	15 491±15	37.894±48	0.70269±3	
	49°52.9′ E						
D4	40°59 0′ S	3,700	17.767±12	15.467±16	37.641±49	0.70293±2	0 513068±34
	43°42.0′ E		17.761±15	15.468±18	37.624±60	0.70299±3	
D5	43°53.6′ S	2,550	16.959±17	15.490±20	37.312±65	0.70475±3*	0 512448±60
	40°39.2′ E		16 927±13	15.504±16	37 320±48	0.70470±3	
D6	44°10.8′ S	2,190	18.373±11	15.509±14	38 096±46	0.70280±3*	0.513071±54
	38°47.7′ E					0.70281±2	
D7	44°48.4′ S	1,500	18.237±13	15.499±15	37 976±47	0.70280±3	0.513041±26
	36°18.1′ E						
D8	28°44.4′ S	5,250	18 362±17	15 585±17	38.050±55	0 70781±2	
	62°02.0′ E		18.330±13	15.612±16	38.107±53	0.70699±5	
Residue leaching			18 327	15.561	37.835		
			18.302	15.597	38.195		
CH77	23°42′ N	4,200	18 141±13	15.456±16	37.514±49	0.70230±3*	0.513203±36
DR04 106	45°26′ W						
AII 96	23°31 5′ N		18.015±15	15.463±18	37 438±51	0.70233±3*†	
	44°49 1′ W						

Pb isotope results are calibrated against SRM 981 measurements, with a discrimination factor of 0.1% per mass unit Errors are 1σ uncertainties taking into account the uncertainty of this discrimination correction[9] Pb concentrations have been determined in most cases using a ^{205}Pb spike, and range from 290 to 700 parts per 10^9 (except for the serpentinite D8, 50 parts per 10^9). Sr isotopes are measured with a CAMECA double collector The results are calibrated against the NBS 987 standard, whose measured value is 0 710254±2 (23 measurements) The results are then corrected to the 0.71015 value previously measured with a single collector spectrometer. Errors are 2σ on the mean uncertainties. Duplicates are separate determinations on different glasses from the same dredge. Nd isotopes are measured with double filament technique with H_3PO_4 loading. The results are calibrated against a Johnson Mattey 321 standard, whose measured value is 0.511959±8 (11 runs). Pb blanks are controlled routinely and are always <0.7 ng (the blank correction is then much lower than uncertainties within the run). Sr blanks are <1 ng; Nd blanks are <0.1 ng.

* Pb/Sr/Nd analysis on the same dissolution

† Value from Machado *et al.*[18]: 0.70233±3

Results

Pb/Pb diagrams. As already found for other ridges, the Pb isotope results are correlated positively in both $^{207}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$ diagrams (Fig. 2). The dredge D5 is an exception and will be discussed later.

The most salient feature of these results is that the least radiogenic extremity of the Pb/Pb correlations of the SWIR is clearly different from that obtained for the Pacific and Atlantic oceans: these values are less radiogenic for $^{206}Pb/^{204}Pb$ (17.5) but comparable for $^{207}Pb/^{204}Pb$ (15.43) and $^{208}Pb/^{204}Pb$ (37.3). This can be verified in Fig. 2 where the isotope domains obtained for other oceans[1,7–9] have been plotted for comparison. The two Atlantic samples analysed during the present study (Table 1) confirm that this distinction is indeed real and cannot result from analytical bias.

At the other extremity of the SWIR Pb/Pb correlations, the values obtained are equivalent to the results for common MORB in the Atlantic and Pacific oceans. As a consequence, the Pb/Pb slopes calculated in both diagrams are lower for the SWIR than for the MAR and EPR. Note that these results are consistent with those of Dupré and Allègre[15] on the Carlsberg Ridge, which plot exactly on the same correlation in the $^{208}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$ diagram.

The dredge D5 sample displays a very different lead isotope composition. It has the smallest radiogenic $^{206}Pb/^{204}Pb$ (16.96) so far measured for an oceanic sample, and its $^{207}Pb/^{204}Pb$ (15.49) and $^{208}Pb/^{204}Pb$ (37.31) are distinctly above the MORB Pb/Pb correlations. Such values are exceptional for zero age mantle samples. Samples from islands such as Kerguelen[14], Tristan da Cunha and Gough[11] or from aseismic ridges such as Walvis[22] and Ninety-East[15] also have $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios above the MORB correlation, but their $^{206}Pb/^{204}Pb$ are more radiogenic than the D5 value.

Finally, the peridotite D8 (not plotted) has a $^{207}Pb/^{204}Pb$ (15.58) slightly higher than the basalts of similar $^{206}Pb/^{204}Pb$ (18.36).

Pb/Sr diagram. The range of $^{87}Sr/^{86}Sr$ variation is from 0.7027 to 0.7030, again with the exception of D5 (0.7047; Fig. 3) These Sr values are higher than those obtained on the EPR and MAR for comparable $^{206}Pb/^{204}Pb$, and again this difference is confirmed by the two new results on Atlantic samples. The data for the SWIR are then above the Pb/Sr correlation discovered by Dupré and Allègre[7] and Cohen *et al*[8] for the Atlantic and confirmed by Hamelin *et al.*[9] for the Pacific. Again, this feature is also found for the Carlsberg samples[8,15,23] and seems to be a regional characteristic of Indian MORB

The unusual dredge D5 is far removed from the usual depleted mantle domain, with a $^{87}Sr/^{86}Sr$ of 0.70472. Again, this value is similar to those obtained on Kerguelen Island[14] or the Walvis Ridge[22].

The peridotite D8 gave a $^{87}Sr/^{86}Sr$ of 0.7071. Such high values, described in ophiolite peridotites[24,25], have been interpreted as evidence of interaction with sea water in oceanic layer 3.

Nd/Sr diagram. As for Sr, the range of Nd variation is restricted (0.51307–0.51291). Note that these results display less variation than those near the Bouvet hotspot of Le Roex *et al.*[17]. The data are in the usual Sr/Nd correlation for MORB. The ε_{Nd} values (5.7–8.8) are lower than those of the most depleted MORB of the Atlantic[8,18] and Pacific[26], and comparable to intermediate

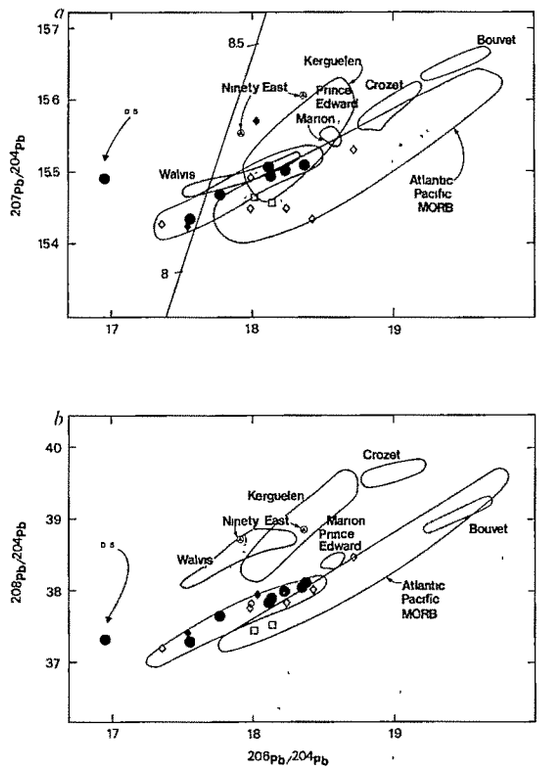


Fig. 2 *a*, Pb/Pb (α, β) diagram; *b*, Pb/Pb (α, γ) diagram ●, SWIR (this study); ◆, SWIR data[15]; ◇, Carlsberg ridge data[15,23]. □, Kane fracture zone (this study). References of the islands and Atlantic and Pacific MORB are given in the text, except for the Crozet field (W M White and B Dupré, unpublished data) The geochron is calculated for an age of the Earth $T_0 = 4.55 \times 10^9$ yr Two values of μ ($^{238}U/^{204}Pb$) are given as reference

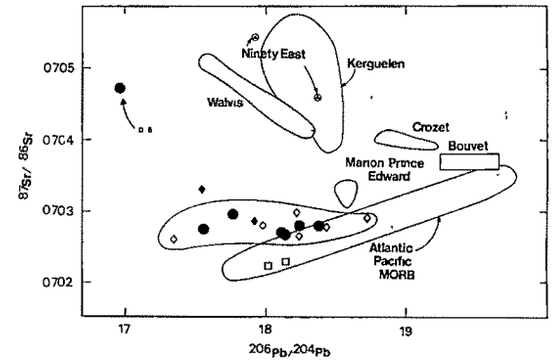


Fig. 3 Pb/Sr diagram. Symbols as described in Fig. 2 legend.

values such as those of the Reykjanes Ridge near Iceland[27] Their Sr/Nd compositions would then seem to be somewhat more 'enriched' than Atlantic MORB whereas the Pb/Pb results are more depleted

The D5 dredge is again exceptional, with a $^{143}Nd/^{144}Nd$ of 0.512448 ($\varepsilon_{No} = -3.3$), comparable with those of Kerguelen[28] or Walvis Ridge[22] samples.

Geographical distribution of the isotope variations. The least radiogenic Pb and Sr values of the SWIR are found on the eastern part of the ridge, which corresponds to the deepest portion and to the triple junction zone. Dupré and Allègre[15] reported a similar nonradiogenic result for a sample dredged at the triple junction.

At the opposite extreme, the more radiogenic values (D6 and D7) correspond to the shallowest part of the ridge, near the Prince Edward and Marion islands, and the isotope values approach those obtained by Hart and Erlank (manuscript in preparation) for basalts from these islands.

Discussion

Present-day source mixing under the SWIR. Isotope variations along ridges are thought to result from mixing between asthenospheric material and blobs of more radiogenic material. This has been well documented near Iceland[4,5], the Azores[6,7,9] and Bouvet[17] Island for the MAR and for the Galapagos spreading centre[30] near the Galapagos archipelago. In all these areas, hotspot influence on the ridges is well known from geochemical and bathymetric evidence[29].

A more complete sampling of the SWIR will be needed to investigate fully the isotope gradient away from Marion and Prince Edward islands, but it is clear that dredges located on the bathymetric swell of the ridge (D6, D7) display Pb and Sr isotope results analogous to those of the islands (S. R. Hart and A. J. Erlank, in preparation) and that these results are thus in agreement with the model of present-day mixing for ridges.

Note that Pb seems to be the most sensitive tracer for this mixing process, because the Nd and Sr ranges of isotope variation remain smaller than for Pb. This suggests a larger difference in isotope composition between the asthenosphere and blob material for Pb than for Nd and Sr.

The question of the identification of the exact isotope composition of the enriched end-member cannot be solved only from isotope arguments, because the results measured on the islands are themselves representative of mixtures between blob and asthenospheric compositions[1,11,30]. For instance, in this geographical area, the isotope difference between Crozet[15] and Marion–Prince Edward (S. R. Hart and A. J. Erlank, in preparation) could be interpreted either as resulting from completely different sources for these blobs or, conversely, as variable proportions of a common blob in the material erupted at the surface. More detailed studies of the Crozet swell are needed to elucidate this question

Origin of the depleted end member. At the other extreme of the SWIR mixing trend, the depleted end member, considered as representative of the asthenosphere, is characterized by particularly nonradiogenic $^{206}Pb/^{204}Pb$ values, but higher $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$ and $^{87}Sr/^{86}Sr$ and lower $^{143}Nd/^{144}Nd$ ratios, compared with the lower end-member values of the MAR and EPR. It must be emphasized that, whereas several studies have already suggested some unexpected enrichment of Indian MORB[11,15,31], we present here the first evidence that this difference is indeed a regional characteristic of the most depleted end-member in this ocean.

The present results for the SWIR, the Dupré and Allègre[15] data for the Carlsberg Ridge and our unpublished results on the East Indian Ridge now demonstrate fully that the whole Indian Ocean ridge system displays a characteristic isotope signature. Moreover, analogous Pb/Pb results have been found recently in the Xigaze ophiolites of Tibet[32], which originated in the same geographical area 100-Myr ago. A geographical mapping of isotope compositions has already been discussed for oceanic island results[15,33]. Our results described here demonstrate that similar regional variations also exist for the depleted upper-mantle value. Therefore, we conclude that the upper mantle itself must be divided in different provinces, which are probably related to different convective units. This raises the obvious question of the physical meaning of the different units and of the boundaries between them (for example, subduction planes, continents, transform faults).

Two issues are then to be discussed: first, to determine possible mechanisms for creating such an isotope composition, and second, to evaluate the consequences of such regional isotope units in asthenospheric reservoirs, all within the overall scheme of mantle convection.

Dupré and Allègre[15] have argued that the specific isotope signature of Carlsberg MORB is a further demonstration of mixing between asthenosphere and blobs under ridges, and that the deviation of these samples from the main Atlantic–Pacific

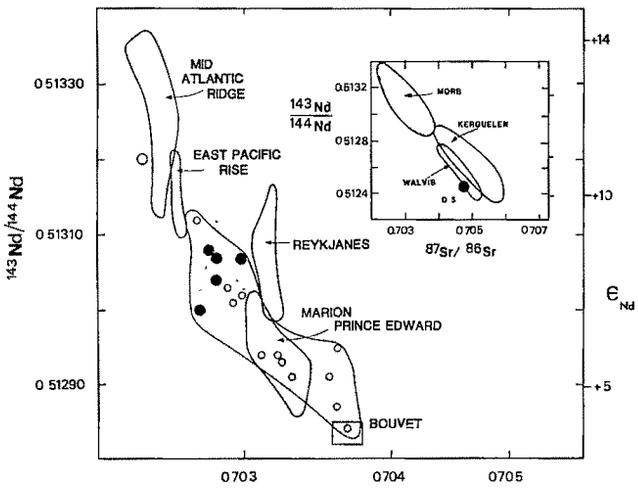


Fig. 4 Sr/Nd diagram. Open circles, data of Le Roex *et al.*[17] for SWIR basalts in the region of Bouvet Island.

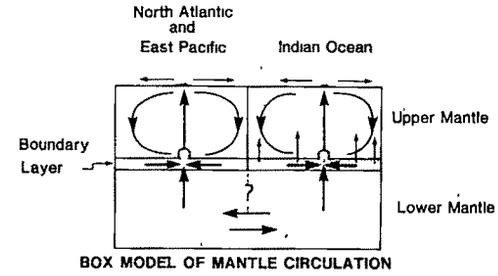


Fig. 5 Possible scheme of mantle dynamics. The upper mantle is divided in different discontinuous boxes, according to the SWIR MORB isotope results. The model where ocean island basalts originate in the boundary layer between upper and lower mantle[38] is illustrated as an example. Since the Indian Ocean island basalt source material (dotted area) is different from other parts of the world, the sub-Indian upper mantle is contaminated specifically by these Indian blobs, some of which reach the surface and others becoming progressively mixed within the upper mantle convection cell.

isotope trend results from the fact that the Indian Ocean islands have isotope characteristics removed from this trend. However, this model cannot explain the isotope composition of the depleted end-member itself, as there is no evidence from available data of any present-day mixing trend between the Atlantic-Pacific depleted end-member and an Indian Ocean island basalt contaminant. At the opposite extreme, the Indian depleted end-member seems to be fairly homogeneous. An additional mechanism, different from the present-day contamination of ridges by blobs discussed in the previous section, must then be proposed. This mechanism could involve a contamination of the whole upper mantle convection cell beneath the Indian Ocean by material with Kerguelen-type isotope values, if enough time has elapsed to allow the isotope homogenization of the contaminated reservoir.

For example, one could first advocate contamination of the upper mantle through reinjection of subducted oceanic lithosphere. The latter is first 'polluted' in accretion zones by blobs with Kerguelen-type signature, then later contaminates the whole upper mantle beneath the Indian Ocean, when it is mixed back after being subducted. This implies that the sub-Indian Ocean mantle has been isolated for several hundred Myr, and, moreover, that subduction-related material has been recycled continuously within the same convection cell. This model then seems unrealistic, especially taking into account the small number of subduction zones in the Indian Ocean. An alternative hypothesis could be that the upper mantle is contaminated by an ocean island basalt reservoir with Kerguelen signature through a 'diffusion-type' process; this contamination operates continuously and uniformly in solid state at depth in the mantle, it allows isotopic homogenization within the upper mantle convection cell, but does not appear any more at the surface as ocean island volcanism, contrary to the present-day occurrence at ridges, for example, near Marion and Prince Edward islands. These different processes can operate simultaneously (Fig. 5); their relative importance depends on the respective scales and rates of mixing, reinjection and homogenization.

The final problem is the interpretation of the unusual values of the D5 dredge. At present, we can speculate using the duplicated analyses of the one sample recovered in that dredge; it will be of great interest to study the lateral extension of this anomaly. Similar results have not been found before on active ridges. On the other hand, the D5 composition is close to the lowest end-member of the Walvis Ridge, as discussed by Richardson *et al.*[22], or to the nonradiogenic lherzolite xenolith from Tanzania analysed by Cohen *et al.*[34]. These results are slightly outside the oceanic mantle array[35] and are not easily interpretable as a mixing between blob-type material and typical depleted mantle values. Possible involvement in the genesis of these structures of material with continental isotope characteristics, through reinjection of sediments[36], continental lithosphere reassimilation[34,37] or percolation of metasomatizing fluids[22] should then be considered.

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A short conserved sequence is involved in the light-inducibility of a gene encoding ribulose 1,5-bisphosphate carboxylase small subunit of pea

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Light induction of chloroplast maturation in plant seedlings ('greening') is associated with the induction in expression of the enzyme ribulose 1,5-bisphosphate carboxylase. To define the regions of a gene encoding the small subunit of ribulose 1,5-bisphosphate carboxylase involved in light inducibility of pea plants, we have introduced derivatives of the gene into petunia cells using a tumour-inducing plasmid vector. A conserved 33-base pair sequence close to the 'TATA' box of the gene is sufficient to confer light inducibility of the small subunit gene.

LIGHT has profound morphogenetic effects on chloroplast development. Seedlings germinated in the dark are arrested in plastid development at the etioplast stage that can then be converted to chloroplasts on illumination. During etioplast–chloroplast transformation there is a rapid synthesis of chlorophylls and a dramatic increase in thylakoid membrane polypeptides and stromal proteins[1]. Although the molecular mechanisms by which these biosynthetic events are turned on by light are still unknown, an important regulatory stage is probably the transcription control of genes encoding chloroplast proteins.

The most abundant protein that increases in concentration during greening is the stromal enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase. This enzyme complex, which accounts for ≤50% of soluble protein in plants, is composed of eight copies each of two non-identical subunits[2]. The large subunit (rbcL) is encoded in the chloroplast[3], whereas the small subunit (rbcS) is encoded in the nucleus by a small multigene family[4–8]. In peas, the amount of rbcS polypeptide in green leaves is 100 times that in etiolated leaves. The increase in rbcS polypeptide during greening is followed by a concomitant increase in the steady-state messenger RNA level[9–11] so that in fully greened leaves, the *rbcS* transcript becomes the most abundant mRNA in mesophyll cells[12]. The light-stimulated increase in *rbcS* mRNA level in peas, as well as other higher plants, has been shown to be mediated by phytochrome[13–15] There is evidence that the control of *rbcS* gene expression by light[16] or phytochrome[17] is at the transcriptional level.

We are interested in defining the molecular events surrounding light-regulated expression of plant nuclear genes. For our investigations we have selected one member (*rbcS-E9*) of the pea *rbcS* gene family as a model system, because the nucleotide sequence of this gene is known and its expression in different tissues of green and etiolated pea plants has been well characterized[11]. Our first objective is to define DNA sequence elements needed for its light/dark regulation and efficient transcription. Previously, we transferred the pea *rbcS-E9* gene into petunia via a tumour-inducing (Ti)-mediated gene transfer vector[18] and demonstrated that this gene is transcribed accurately using its own promoter[19]. Moreover, the *rbcS-E9* mRNA level is regulated by light in petunia calli as it is in pea leaves. These results provide evidence that the petunia expression system can be used to characterize the control sequences of the *rbcS-E9* gene. Our confidence in the fidelity of heterologous expression systems is reinforced by the finding that the 5′ flanking region of another pea *rbcS* gene (*ss3.6*) also shows light-regulated expression after transfer into green tobacco calli[20].

Here we have examined the expression of 5′ deletion mutants of *rbcS-E9* and chimaeric genes in petunia calli We have identified an upstream region needed for maximal expression and delimited a light-responsive sequence to a 33-base pair (bp) region around the TATA box.

Internal deletion and 5′ mutants

With a few exceptions[21,22], most of the animal genes transcribed by RNA polymerase II contain regulatory elements upstream of their transcription start sites[23]. Therefore, we investigated first the role of 5′ upsteam sequences in the regulation of *rbcS-E9* gene expression. Previously[19], we demonstrated that a *rbcS-E9* gene (*rbcS-E9 5′Δ-1052*) containing 1,052 bp of 5′ upstream sequence was transcribed accurately in petunia calli in a light-regulated manner. This gene, therefore, was chosen as the wild type and from this a series of 5′ deletion mutants were constructed by *Bal*31 nuclease digestion (Fig. 1). The deletion mutants were first screened by gel electrophoresis and those in the desired size range were characterized further by sequence analysis to determine their end points. A total of seven 5′ deletion mutants with end points at strategic locations in the 5′ region were chosen for the present study. The first mutant (*rbcS-E9 5′Δ-437*) contains a deletion of ~600 bp; the next three (*rbcS-E9 5′Δ-352*; *rbcS-E9 5′Δ-255*; *rbcS-E9 5′Δ-96*) have end points spaced ~100 bp apart; the next (*rbcS-E9 5′Δ-87*) has its end point just 5′ of the CAAT box; one (*rbcS-E9 Δ-35*) has its end point between the CAAT box and the TATA box, and the remaining mutant (*rbcS-E9 5′Δ-13*) contains neither the CAAT box nor the TATA box and retains only 13 bp upstream of the transcription start site. In addition, we have exploited two convenient *Bst*XI sites flanking the CAAT box to construct a mutant with an internal deletion of 51 bp from −107 to −56.

The internal deletion mutant (*rbcS-E9 5′Δ-107/-56*) and the 5′ deletion mutants were transferred into petunia cells via the Ti-plasmid of *Agrobacterium tumefaciens*, using the pMON145 vector for plant gene transfer. This vector contains the nopaline synthase (*nos*)-neomycin phosphotransferase (*npt*)-II chimaeric gene that confers resistance to kanamycin[18]. Transformed calli were selected and maintained on hormone-free medium containing kanamycin (100 μg ml^{-1}) in continuous light or kept in complete darkness for 5 days before RNA extraction and analysis. To eliminate any possible variations in the physiological state of the calli or in the purity of poly(A)$^+$ RNA among samples, we have used the level of the *npt*-II mRNA transcribed from the *nos* promoter as an internal standard. The use of an internal control also enabled us to reduce the variation resulting from different copy numbers of integrated tumour(T)-DNA, which contained the two genes (*rbcS-E9* and *nos–npt*-II) linked together. Furthermore, calli from different transformation events were pooled to minimize any possible position effects on expression. Transcriptional activities of the mutants were

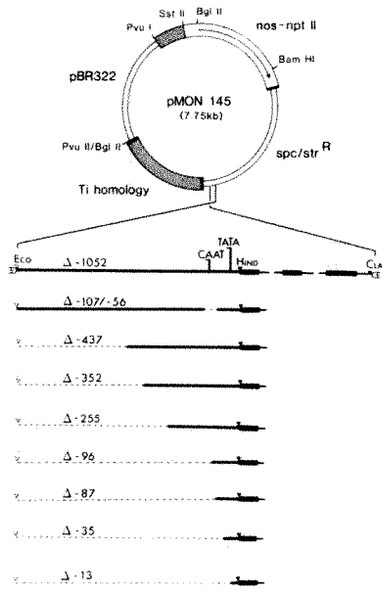


Fig. 1 Construction of *rbcS-E9* deletion mutants. The pea *rbcS-E9* gene is encoded by a 2.4-kb *Eco*RI/*Cla*I fragment that has 1,052 bp of DNA sequence upstream from the transcriptional start site[11]. This fragment was cloned between the *Eco*RI and *Cla*I site of pBR325 to give the parental plasmid, pE9. (A restriction map of the 2.4-kb *Eco*RI/*Cla*I fragment is shown in Fig. 3.) To generate 5′ deletion mutants, pE9 was linearized at the unique *Eco*RI site and digested with *Bal*31 (ref. 36); the digested DNA molecules were ligated to *Eco*RI linkers (CGGAATTCCG; New England Biolabs), treated with *Eco*RI and then recircularized by T_4 ligase. The resulting series of 5′ deletion mutants was analysed by agarose gel electrophoresis to determine the extent of deletion and selected mutants were further characterized by sequence analysis according to Maxam and Gilbert[37]. Mutants with end points at −437, −352, −255, −96, −87, −35 and −13 were selected for subsequent experiments. To generate the internal deletion mutants (*5′Δ-107/-56*), pE9 was digested with *Bst*XI (CAANNNNNNTTG), which cuts the promoter region of the *rbcS-E9* gene at −56 and −107 (ref. 11). The staggered ends of the deleted plasmids were repaired with T_4 DNA polymerase and the DNA was then recircularized by T_4 DNA ligase. The promoter fragment (*Eco*RI/*Hin*dIII) of the internal deletion mutant (*rbcS 5′Δ-107/-56*) was subcloned with the *Eco*RI and *Hin*dIII sites of M13 mp8 and the break points determined by dideoxy sequence analysis[38]. The parental gene pE9(*5′Δ-1052*), the 5′ deleted mutants (*5′Δ-437*, and so on) and the internal deletion mutants (*5′Δ-107/-56*) were subcloned into the unique *Eco*RI and *Cla*I restriction sites of the intermediate vector, pMON145 (ref. 19). The intermediate vector and its derivatives containing the different *rbcS-E9* deletion mutants in *Escherichia coli* (LE392) were mobilized into an octopine strain of *A. tumefaciens* (GV3111) by triparental mating and the co-integrates selected according to Fraley *et al.*[18].

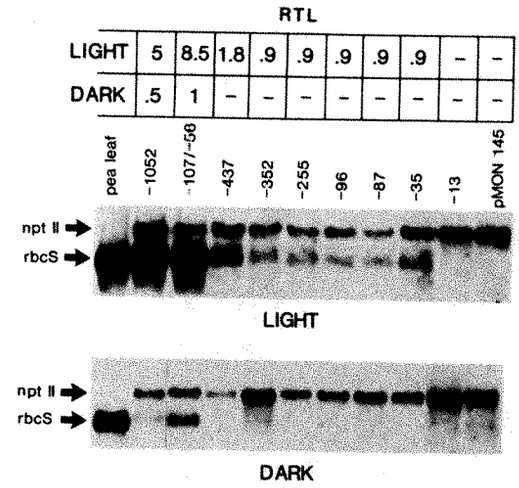


Fig. 2 Northern blot analysis of deletion mutants. The extreme right lane contains 1 μg poly(A)$^+$ RNA from pMON145 calli, whereas the extreme left lane contains 60 ng poly(A)$^+$ RNA from pea leaves. The remaining lanes, indicated by numbers, contain 1 μg poly(A)$^+$ RNA from each of the deletion mutants. The relative transcript level (RTL) refers to the ratio of the *rbcS-E9* transcript to the *nos-npt*-II transcript (upper panel).
Methods. Petunia cells were transformed with *A. tumefaciens* by co-cultivation[18]. Calli obtained from transformation with the pMON145 vector alone or the vector containing the various deletion mutants were propagated on 1% agar plates containing Murashige and Skoog medium and 100 μg ml^{-1} kanamycin[19]. Tissues were maintained in continuous light or transferred to total darkness for 5 days before collecting. Total RNA was isolated with guanidinium thiocyanate as a protein denaturant[39] and poly(A)$^+$ RNA was purified by chromatography on poly(U)-Sepharose[40]. Aliquots of RNA were denatured in glyoxal at 50 °C[41], electrophoresed on 1% agarose gels and transferred onto nitrocellulose filters[42]. Filters were hybridized to ^{32}P-labelled probes containing the coding sequence of the *nos-npt*-II chimaeric gene and of the *rbcS-E9* gene using the hybridization and washing conditions described previously[11,19]. Radioactive bands on Northern blots were visualized by autoradiography and quantified according to Suissa[43].

assessed by their relative transcript level, which is the ratio of *rbcS-E9* mRNA to the *nos-npt*-II mRNA. Poly(A)$^+$ RNAs from pea leaves and from pMON145 calli were analysed along with the mutant samples to localize the positions of the *nos-npt*-II and pea *rbcS-E9* transcripts.

Figure 2 shows that in the hybridization and washing conditions used, poly(A)$^+$ RNA from the gene transfer vector, pMON145, gives only one radioactive band on Northern blots, corresponding in size to the *nos-npt*-II transcript. No cross-hybridization of the pea *rbcS-E9* probe to the petunia *rbcS* mRNA, which is identical in size to the *rbcS-E9* mRNA, was detected. In agreement with previous results[19], we found that the wild-type construct (*rbc-E9 5′ Δ-1052*) produces about five times as much *rbcS-E9* mRNA compared with *nos-npt*-II mRNA (Fig. 2). Deletion of 615 bp from the 5′ upstream region of *rbcS-E9 5′ Δ-1052* decreases the relative transcript level by about three times (mutant *rbcS-E9 5′ Δ-437*) and an additional deletion of 85 bp reduces the relative transcript level further by about twofold (mutant *rbcS-E9 5′ Δ-352*). Sequences between −352 and −35, which include the CCAAT box, have little effect on the relative transcript level, whereas no *rbcS-E9* mRNA was detected in the mutant *rbcS-E9 5′ Δ-13* that has the entire 5′ upstream region, including the TATA box, deleted. From these results, three conclusions can be drawn: (1) a 700-bp sequence between −1,052 and −352 of the *rbcS-E9* upstream region modulates the relative transcript level about six times; (2) The CAAT box centred around −85 does not change the level; and (3) The TATA box is needed for transcritpion of the *rbcS-E9* gene.

To collect further evidence on the function of the CAAT box, we made use of two convenient *Bst*XI sites to generate a mutant (*rbcS-E9 5′ Δ-107/-56*) with an internal deletion from −107 to −56. The deleted segment contains the CAAT box with ~50 bp of surrounding sequences. Figure 2 (upper panel) shows that the mutant *rbcS-E9 5′Δ-107/-56* is expressed in light-grown calli with a relative transcript level of about twice that of the wild type. This result provides unequivocal evidence that the CAAT box is not required for the expression of the *rbcS-E9* gene in petunia calli.

In an attempt to localize DNA sequences required for light-regulated expression of the *rbcS-E9* gene, transformed petunia calli carrying the different deletion mutants were transferred to the dark for 5 days before their RNAs were extracted for Northern blot analysis. The wild-type construct produces a reduced level of *rbcS-E9* mRNA in the dark compared with the light (Fig. 2, lower panel), confirming previous observations[19]. We

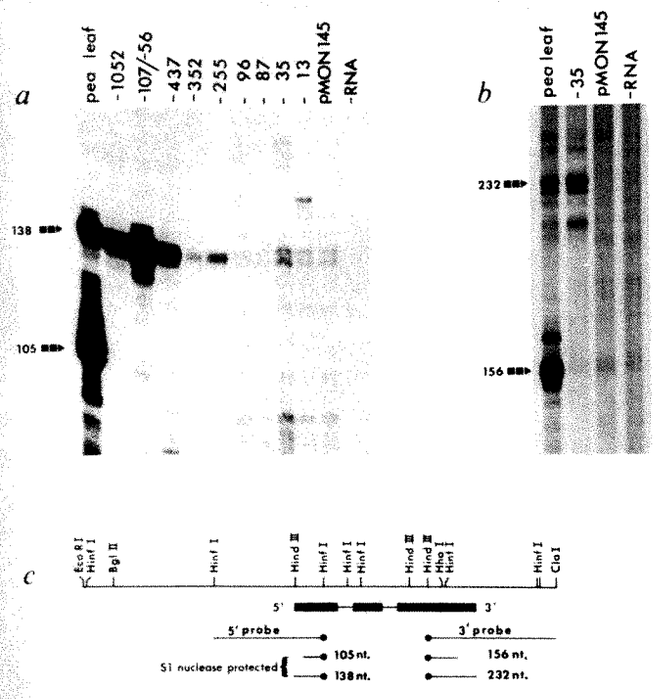


Fig. 3 S_1 nuclease protection assays of pea *rbcS* transcripts from the deletion mutants. Poly(A)$^+$ RNAs from both pea leaf and the deletion mutants were probed with the 541-nucleotide *Hin*fI/*Hin*fI fragment (5′ probe; *c*, left) or with the 690-nucleotide *Hin*dIII/*Cla*I (3′ probe; *c*, right). *a*, Pea-leaf poly(A)$^+$ RNA, 60 ng; poly(A)$^+$ RNA from *rbcS-E9 5′Δ-1052*, 40 ng; poly(A)$^+$ RNA from the other deletion mutants, 1 μg. Arrow 138 indicates the end and the length of the 5′ protected fragment. *b*, Pea-leaf poly(A) RNA, 60 ng; *rbcS-E9 5′Δ-35* poly(A)$^+$RNA, 10 μg; pMON145 poly(A)$^+$ RNA, 10 μg. Arrow 232 indicates the end and the length of the 3′ protected fragment. Pea-leaf RNAs give two additional S_1 signals at the 5′ (*a*, arrow 105) and 3′ (*b*, arrow 156) ends. These result from limited hybridization of other pea *rbcS* transcripts to the probe[11,19].

Methods. S_1 nuclease protection assays were performed according to Berk and Sharp[44] as modified by Weaver and Weissman[45]. The 5′ probe is the 541-nucleotide *Hin*fI/*Hin*fI fragment, whereas the 3′ probe is the 690-nucleotide *Hin*dIII/*Cla*I fragment (lower panel). The ^{32}P-DNA fragment labelled at either their 5′ or 3′ ends were denatured and the dissociated strands separated by polyacrylamide gel electrophoresis. The isolated strands were hybridized with poly(A)$^+$ mRNA in 10 μl of a solution containing 50% formamide, 0.4 NaCl, 2 mM EDTA and 20 mM PIPES (*p*H 6.8) for 12 h at 42 °C. After hybridization, the reaction mixture was diluted to 300 μl with a solution containing 0.3 M NaCl, 30 mM NaOAc (*p*H 4.6), 1 mM $ZnSO_4$, 20 μg ml^{-1} denatured salmon sperm DNA, 300 U ml^{-1} S_1 nuclease and incubated at 37 °C for 45 min. DNA fragments protected from S_1 nuclease digestion were sized on 6% sequencing gels and visualized by autoradiography.

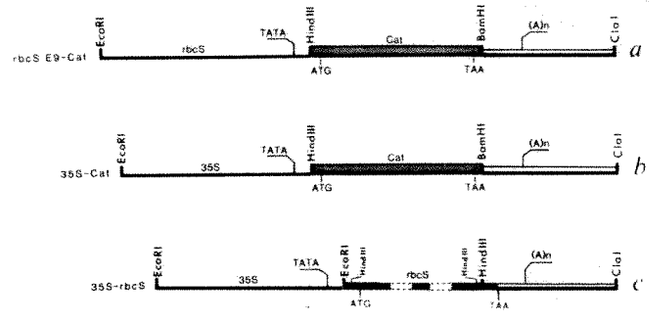


Fig. 4 Construction of chimaeric genes. *a*, *rbcS-E9-cat.* The *Hin*dIII/*Sau*3A fragment coding for CAT in pSV2-cat(24) was subcloned into the *Hin*dIII/*Bam*HI sites of pUC12 to give pUC-cat. The 699-bp *Hin*dIII/*Hin*dIII fragment containing the 3′ end of *rbcS-E9* (ref. 11) was then subcloned into the *Bam*HI site of pUC-cat giving pUC-cat 3′ rbcS. The *rbcS-E9* gene was subcloned in between the *Eco*RI and *Cla*I site of a *Hin*dIII minus a derivative of pMON145 (*rbcS-E9 5′ Δ-1052). The rbcS-E9* coding sequence from *Hin*dIII (−2) to *Cla*I (+1,349) was replaced by the 1,549-bp *Hin*dIII/*Cla*I fragment from pUC-cat 3′ rbcS. The resulting chimaeric genes were designated *rbcS-E9-cat.* The 1,052-bp *Eco*RI/*Hin*dIII fragment of *rbcS-E9-cat*, containing the *rbcS-E9* 5′ flanking sequence, was replaced by a 950-bp *Eco*RI/*Hin*dIII fragment containing the 35S promoter of CaMV[25]. *c*, ^{35}S-promoter-*rbcS.* The 950-bp *Eco*RI/*Hin*dIII containing the 35S promoter was cloned into the unique *Eco*RI site of *rcbS-E9 5′ Δ-13*, using *Eco*RI linkers. The thick line between *Hin*dIII and *Bam*HI indicates the *cat* coding sequence (*a*, *b*). The white line between *Bam*HI and *Cla*I (*a*, *b*) or *Hin*dIII/*Cla*I (*c*) indicates the 3′ end of *rbcS-E9.* The marked line between *Eco*RI and *Hin*dIII indicates the *rbcS* coding sequence.

estimated that the relative transcript level of the dark sample is ~0.5, giving a light-induction factor of ~10 for the *rbcS-E9* mRNA. None of the 5′ deletion mutants (*rbcS-E9 5′Δ-437, Δ-352, Δ-255, Δ-96, Δ-87* and *Δ-35*) active in the light produce detectable amounts of *rbcS-E9* mRNA in the dark. The extremely low level of *rbcS-E9* mRNA in the dark has prevented the estimation of the light-induction factor for the deletion mutants. Nevertheless, it is clear from our results that the mutant, which contains only 35 bp of sequences 5′ to the transcription start site, is still capable of light-regulated expression.

The internal deletion mutant (*rbcS-E9 5′Δ-107/-56*) produces a higher level of *rbcS-E9* mRNA in the dark compared with the wild type (Fig. 1, lower panel), with a dark relative transcript level ~1. Because this mutant is also more active in the light, its light-induction factor is about the same as that of wild type. The light-regulated expression of this mutant provides direct evidence that the 5′ sequence region from −107 to −56, including the CAAT box, is not required for light responsiveness of the *rbcS-E9* gene.

5′ and 3′ S_1 analyses

We have reported previously that a *rbcS-E9* gene containing 1,052 bp of 5′ upstream sequence is transcribed with the correct start site in petunia calli[19]. To examine the effects of upstream sequences on transcriptional initiation, we subjected poly(A)$^+$ RNAs from the different deletion mutants to 5′ S_1 nuclease protection assays. Figure 3*a* shows that pea leaf poly(A)$^+$ RNA gives two S_1-protected fragments, using a probe that covers the 5′ end of *rbcS-E9.* The longer fragment is produced by hybridization of the *rbcS-E9* probe with its cognate mRNA, whereas the shorter fragment is the result of hybridization by other pea *rbcS* transcripts that show sequence homology to the probe up to the first AUG[11]. Consistent with this interpretation, RNA from petunia calli transformed with *rcbS-E9 5′Δ-1052* gives only the longer fragment, indicating that the correct transcription start site is used. Deletion of the 5′ upstream sequence from −1,052 to −255 does not change the S_1 start site. We were unable to obtain reproducible S_1 signals for the remaining 5′ deletion mutants because of the low amount of *rbcS-E9* mRNA, but an internal deletion between −107 to −56 also has no effect. Taken together, these results demonstrate that the sequence elements surrounding the CAAT box as well as DNA sequences upstream of −255 do not affect the accuracy of transcriptional initiation.

The polyadenylation site of the pea *rbcS-E9* gene has been mapped previously to 150 bp downstream from the termination codon[11]. To see whether the same site on the *rbcS-E9* gene of pea is also recognized in petunia calli, poly(A)$^+$ RNAs from *rcbS-E9 5′Δ-35* were subjected to S_1 analysis using a probe that covers the 3′ region of *rbcS-E9.* Figure 3 shows that identical polyadenylation sites are used in petunia and pea, indicating conservation of this processing signal in both plant species.

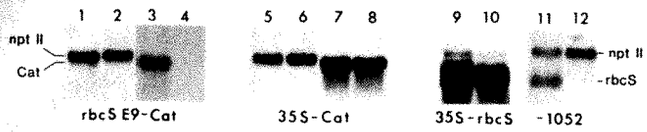


Fig. 5 Northern blot analysis of chimaeric genes. All lanes contain 1 μg poly(A)$^+$ RNA from: *rbcS-E9-cat* light-grown (lanes 1, 3) and dark-grown calli (lanes 2, 4); from 35S promoter-*cat* light-grown (lanes 5, 7) and dark-grown calli (lanes 6, 8); from 35S promoter-*rbcS* light-grown (lane 9) and dark-grown calli (lane 10); from *rbcS-E9 5′ Δ-1052* light-grown (lane 11) and dark-grown calli (lane 12). The filters were hybridized to the following ^{32}P-nick translated probe: *npt*-II coding sequence (lanes 1, 2, 5, 6); *cat* coding sequence (lanes 3, 4, 7, 8); *npt*-II coding sequence plus 3′ end of *rbcS-E9* (lanes 9–12). (Conditions for transformation and growth of the transformed calli and isolation and analysis of the RNAs are described in Fig. 2.)

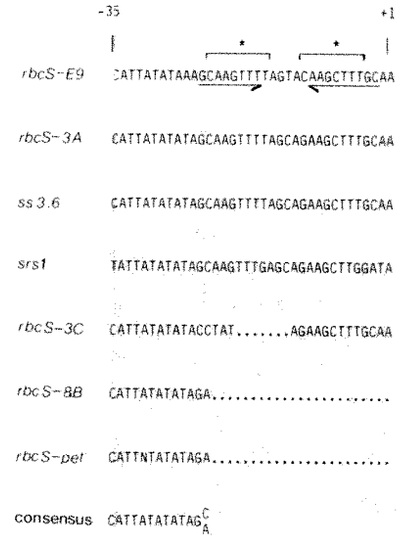


Fig. 6 Comparison of sequences between the TATA box and the transcriptional start site of several *rcbS* genes. Dicotyledonous gene sequences were obtained from the following authors: *Pisum sativum rbcS-E9*[11]; *P. sativum rbcS-3A* (P. Moses, personal communication); *P. sativum ss3.6*[46]; *Glycine max srs1 max*[4]; *P. sativum rbcS-3C* (P. Moses, personal communication); *Nicotiana plumbaginifolia rbcS-8B* (C. Poulsen, personal communication); *Petunia hybrida rbcS-pet* (Monsanto Company, personal communication). Parentheses, direct repeats found in *rbcS-E9*; asterisks, mismatch in the repeat; arrows underline the imperfect palindromic sequence. Sequence downstream of the *rbcS-3C* TATA box are aligned with those of *rbcS-E9* to maximize homology. Similar sequences are not shown for *rbcS-8B* and *rbcS-pet* because of the lack of obvious homology. A consensus sequence derived from the TATA box for the various *rbcS* genes is given.

Expression of chimaeric genes

Our results with the 5′ deletion mutants have allowed us to place the 5′ boundary of the light-responsive sequence of *rbcS-E9* at −35. To determine the 3′ limit of this sequence, we used a convenient *Hin*dIII site near the S_1 start site of *rbcS-E9*. An *Eco*RI/*Hin*dIII fragment containing the 5′ region (from −1,052 to −2) of *rbcS-E9* was fused to the coding sequence of bacterial chloramphenicol acetyltransferase (*cat*)[24] followed by the 3′ polyadenylation sequence of *rbcS-E9* (Fig. 4). We found that this chimaeric gene is expressed in petunia calli; moreover, its activity is regulated by light (Fig. 5, lanes 3, 4). Control experiments showed that the *nos-npt*-II mRNA is expressed equally in the light and dark (Fig. 5, lanes 1, 2). The light/dark regulation of the *cat* mRNA is abolished when the *rbcS-E9* promoter fragment is replaced with a 35S promoter fragment (from −941 to +9) of cauliflower mosaic virus (CaMV) (Fig. 5, lanes 7, 8), which is known to be expressed constitutively[25]. Because the second chimaeric gene contains the 3′ polyadenylation sequence of *rbcS-E9* (Fig. 4), these results indicate that the sequence, by itself, does not have a direct role in light response. In addition, the results also establish that the 5′ upstream sequence of *rbcS-E9*, from −1,052 to −2, is sufficient to confer light-regulated transcription, thus placing the 3′ boundary of the light-responsive sequence at position −2. Our results with the *rbcS-E9* promoter fragment are in agreement with the previous report of Herrera-Estrella *et al.*[20] who used a similar 5′ flanking fragment of another pea *rbcS* gene (*ss3.6*).

In the experiments described above, the *npt*-II and the *cat* mRNA could not be resolved on the same Northern blot because of their size similarity. Therefore, the two transcripts were probed separately. The *npt*-II hybridization signal was obtained after 24 h exposure of the X-ray film (Fig. 5, lanes 1, 2) whereas that for the *cat* mRNA required an exposure time of 7 days (Fig. 5, lanes 3, 4). These results, compared with those in Fig. 2, strongly suggest that the *cat* mRNA level is much lower than that for the *rbcS-E9* mRNA when driven by the same promoter of *rbcS-E9*. The poor expression of the *rbcS-E9* 5′ flanking sequence when joined to a heterologous coding sequence suggests a role for the *rbcS-E9* intragenic region in transcription.

Although we have shown that the *rbcS-E9* 5′ flanking sequence (−1,052 to −2) of pea is able to drive the light-regulated transcription of a test gene, it is possible that differential stability of the *rbcS-E9* mRNA may also contribute to the observed differences in *rbcS-E9* mRNA accumulation in the light and dark. To examine this possibility we used the CaMV 35S promoter fragment[24] to drive the expression of the *rbcS-E9* coding sequence. Figure 5 (lanes 9, 10) shows that the CaMV 35S promoter-*rbcS* hybrid gene is expressed equally in the light and dark, as is the case with *nos-npt*-II, which has been shown previously to be expressed constitutively[25]. Control experiments confirm the expected difference in the *rbcS-E9* mRNA levels between light and dark when the coding sequence is driven by its cognate promoter (Fig. 5, lanes 11, 12). These results provide strong evidence that light has no effect on the *rbcS-E9* transcript processing or mRNA stability. The light-regulated expression of the *rbcS-E9* gene and its mutant derivatives in pea, therefore, mainly reflect control at the transcriptional level.

The experiments described above provide information on the relative strength of the CaMV 35S promoter and the *rbcS-E9* promoter. In these experiments, the Northern blots were hybridized with a probe containing a 1.2-kilobase (kb) coding sequence of *npt*-II joined to a 0.69-kb 3′ sequence from the *Hin*dIII (+660) to the *Cla*I (+1,350) of the *rbcS-E9* gene[11]. The *rbcS-E9* 3′ fragment contains only 232 bases in the transcribed region that could hybridize to the *rbcS-E9* mRNA. In these conditions, the *nos-npt*-II and *rbcS-E9* mRNA show the same hybridization signal (Fig. 5, lane 11). However, the hybridization intensity of the *rbcS-E9* transcript produced by the CaMV 35S promoter relative to that of *npt*-II produced by the *nos* promoter is ~10–15 times higher (Fig. 5, lane 9). These results indicate that the CaMV 35S promoter is ~10–15 times stronger than the *rbcS-E9* promoter in petunia calli.

Discussion

Here we describe the first functional analysis of the control sequences of a major regulated plant gene, the *rbcS-E9* gene of pea. In pea leaves[11] and in transformed petunia calli[19] the accumulation of the *rbcs-E9* mRNA is increased by light. Gallagher and Ellis[16] have shown that an effect of light is to stimulate the rate of transcription initiation of the pea *rbcS* genes. However, the extent of light-stimulated transcription obtained by these authors[16] is twofold less than the *rbcS* mRNA ratio between green and etiolated pea leaves[9,11]. To determine whether light also exerts a post-transcriptional effect on *rbcS* gene

expression, we placed the *rbcS-E9* coding sequence under the control of the 35S promoter of CaMV[25] that is insensitive to light (F.N., unpublished results). The level of the *rbcS-E9* transcript produced by the 35S promoter is the same either in the light or the dark. These results clearly exclude any pronounced light effect on post-transcriptional processing of the *rbcS-E9* transcript or its stability. In addition, they provide evidence that light-induced accumulation of the *rbcS-E9* mRNA in petunia calli reflects largely transcriptional control of its cognate promoter by light.

To map the *rbcS-E9* gene control sequences, the activities of several 5′ deletion mutants and chimaeric gene constructs were examined. Our analyses reveal that the *rbcS-E9* 5′ flanking sequence can be divided roughly into three regulatory regions. An upstream region of ~700 bp (−1,052 to −352) is needed for maximal transcription but not light–dark regulation. Mutants lacking this region show 15–20% of the wild-type activity but still retain light responsiveness. Whether this region alone may also provide a light-regulated function when joined to a heterologous promoter is being tested.

The sequence element, bordered by positions −107 to −56 and including the CAAT box at position −85, is required neither for accurate transcription nor for light induction. In fact, this element seems to have a negative regulatory function, because in its absence transcription is increased by approximately two times with no significant effect on the light-induction factor. This finding is in contrast to the results obtained with the nopaline synthase promoter[26] and the CaMV 35S promoter[25], both of which require sequences in and around the CAAT box for efficient transcription. On the other hand, there are examples of animal genes that either do not have recognizable CAAT sequence[27–30] or still retain full transcriptional activities after deletion of the CAAT sequence[31,32]. We note that in the internal deletion mutant *5′ Δ-107/-56*, a new sequence, AATCCATT, is reconstituted at ~position 60 on re-ligation of the *Bst*XI-cut sites. Although unlikely, we cannot exclude the possibility that the function of the CAAT box at position −85 may be supplanted by this new sequence at position −60 in the deletion mutant.

We have identified a 33-bp sequence fragment surrounding the TATA box involved in light control of *rbcS-E9* gene transcription based on analyses of the *rbcS-E9-cat* chimaeric gene and the *5′ Δ-35* mutant, both of which show light-induced transcription. The chimaeric gene contains the *rbcS-E9* 5′ region from −1,052 to −2. On the other hand, results from the *5′ Δ-35* mutant clearly demonstrate that sequences upstream of −35 are not needed for light-induced transcription. Taken together, we conclude that the sequence from −35 to −2, which includes the TATA box, contains the necessary information for responsiveness to light.

Comparison of the 10-bp sequence (−35 to −24) containing the *rbcS-E9* TATA box with similar regions of all the *rbcS* genes of dicotyledonous plants sequenced so far reveals a striking degree of homology (Fig. 6). Point mutations or deletions in the TATA box are known to reduce the efficiency and accuracy of transcription but have no effect on gene regulation[31,33]. In heat-shock[34], metallothionein[30] and His [35]genes, sequence elements that confer regulated transcription are found as short direct or inverted repeats 5′ upstream of the TATA box. Examination of the *rbcS-E9* light-responsive sequence reveals an 8-bp direct repeat (from −23 to −16 and from −12 to −5) with only one mismatch (at positions −19 and −8) located just downstream of the TATA box. In addition, this region also contains an imperfect palindrome (Fig. 6). Although the functional significance of these structural features remains to be elucidated, we note that the sequence between the *rbcS-E9* TATA box and its mRNA cap site is conserved in two other pea *rbcS* genes (*rbcS-3A* and *ss3.6*) and in a soybean *rbcS* gene (*srs1*). A fourth pea *rcbS* gene, *rbcS-3C*, contains only the second repeat sequence of *rbcS-E9* (Table 1). The CaMV 35S promoter–*rbcS* chimaeric gene contains one (from −13 to −2) of the repeat sequences; nevertheless, it is not light-regulated (Fig. 5), suggesting that one of the repeat sequences by itself is not sufficient to confer light responsiveness. 3′ deletion and site-specific mutagenesis in this region will allow us to investigate the relative importance of the TATA box and the direct repeats, and to define more precisely the critical sequences for light-controlled transcription.

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First observation of ultra-high-energy γ rays from LMC X-4

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Ultra-high-energy γ rays ($>10^{15}$ eV) have recently been observed from Cygnus X-3 (refs 1, 2) and from Vela X-1 (ref. 3). The discovery that some neutron star binary X-ray sources are emitting ultra-high-energy (UHE) γ rays has led to considerable interest and speculation as to the nature of the cosmic particle accelerators responsible for the γ rays[4–7] and to the notion that much of the galactic cosmic radiation above 10^{16} eV may originate from such objects[7–9]. Data from the University of Adelaide air-shower array at Buckland Park taken over a 3-year period have been analysed to search for evidence of UHE γ-ray emission from additional neutron-star binary X-ray sources having known orbital periods. We present here the result of this search and report the detection of UHE γ rays from LMC X-4 above 10^{16} eV with an integral flux of $(4.6\pm1.7)\times10^{-11}$ photons m^{-2} s^{-1}. This is the first extragalactic object to be positively detected at these energies.

Observations of air showers were made continuously (except for maintenance) during 1979, 1980 and 1981 at Buckland Park (sea level, latitude 35° S). During this time, the array[10] consisted of eleven 1-m^2 plastic scintillator detectors, five of which had fast-timing capability and were used for shower arrival direction analysis. Over the 3-year period some 1.3×10^5 air showers were recorded[11,12] with primary energies $\geq3\times10^{15}$ eV Following our detection[3] of UHE γ rays from Vela X-1, we examined the Buckland Park data to test for an excess of air showers from regions around the galactic plane and galactic centre[13], and from the directions of known sources of γ rays at 100-MeV energies[14]. Here we describe our continued search for evidence of periodic emission of UHE γ rays from neutron-star binary X-ray sources. In addition to Vela X-1, there are 14 such objects in the southern celestial hemisphere which have known orbital periods These objects are listed in Table 1 together with their type, estimated distance and orbital period. Of the neutron-star binary systems, 11 are massive and three are of low mass. Five (all massive systems) are in the Magellanic Clouds.

For each object, we examined the air showers that had apparent arrival directions within angle θ_c of the source direction. This 'resolution angle' depends on the declination[14] and has been chosen so that 72% of any primary particles from the source direction would produce air showers that appear to arrive from within θ_c of the source direction. This procedure optimizes the signal-to-noise ratio. The observed number of events, n, is given in Table 1 together with the number of background cosmic-ray events expected, $\hat{B}$, based on air showers arriving from the same declination but from different right ascensions (the exposure ratio, α, is the ratio of the solid angle contained within θ_c of the source direction to that used in determining the background). All air showers with apparent arrival directions within θ_c were included in the analysis. Because the rate of detecting air showers at fixed shower electron number drops off very steeply with zenith angle[14], the expected number of background events within θ_c of a source direction depends strongly on its declination (this is evident from the $\hat{B}$ values given in Table 1). Another consequence of this is that almost all the air showers analysed had zenith angles <45°. This is in contrast to arrays comprising detectors sensitive to the (more penetrating) muon component, such as water Cerenkov detectors, which can readily detect showers to larger zenith angles.

In our observation[3] of Vela X-1, and in the observation of Cyg X-3 by the Kiel group[1], it was assumed that the shower lateral-distribution age parameter, s, could be used to reduce the cosmic-ray background events by using only showers with large s. In both cases, the air showers containing an excess attributed to γ-ray initiated showers had only high s values. Furthermore, in observations[15] of 'muon-depleted' showers, attributed to γ rays, the mean age parameter was found to be 1.4, whereas for 'ordinary' air showers it was 1.2. This seems to justify the use of an age cut. However, no age cut was used by the Haverah Park group[2] in their successful search for UHE γ-ray emission from Cyg X-3. The distribution in shower age depends on zenith angle and hence on declination. We find that the age distribution of cosmic-ray background events is quite broad if the declination of the suspected source differs greatly from the latitude of the air-shower array. The age distribution of γ-ray showers is also expected to be broad in this case. For such sources, the use of an age cut would not be helpful because of the greater overlap in age distributions of cosmic-ray and γ-ray showers. Of the sources listed in Table 1, only 1700−377 passes nearly overhead at Adelaide and only for this case was an age cut used to reduce the background of cosmic-ray events. For all the other sources, the source declination differs from the latitude of the air shower array by more than 17° and no age cut was used.

Table 1 Search for periodic emission of UHE γ rays from neutron star binaries

Source	Name	Type*	Distance† (kpc)	Period (day)	Ref	θ_c (°)	α	$\hat{B}$	n	Y_n	$Y_{n,5\%}$	$Y_{n,1\%}$	E_{thr} (10^{15} eV)	95% upper limits‡ n_γ	F_γ (m^{-2} s^{-1})	L_γ (erg s^{-1})	L_γ/L_X
0115−737	SMC X-1	1	65	3 892387	20	2 7	0 044	25 1	23	5.07	5 53	5 85	15	10	4 4 (−11)	1 8 (+38)	0 3
0532−664	LMC X-4	1	50	1 40832	27	2 5	0 027	53 0	63	7 55	7 32	7 54	8	—§	—	—	—
0535−668	LMC Trans	1	50	16 6515	29	2 5	0 028	50 7	56	6 61	7 11	7 34	8	20	1 3 (−10)	1 6 (+38)	0 13
0538−641	LMC X-3	1	50	1 70480	30	2 4	0 025	63 6	73	7 40	7 59	7 80	7	26	1 6 (−10)	2 3 (+38)	0 8
0540−697	LMC X-1	1	50	3 909 4 039	31	2 6	0 033	39 8	44	6 45 6 26	6 67	6 92	10	18	9 9 (−11)	1 8 (+38)	0 9
0921−630	—	2	8	8 987	32	2 4	0 024	68 3	72	7 10	7 56	7 77	6	21	1 8 (−10)	4 4 (+36)	10 0
1119−603	Cen X-3	1	10	2 0871061	33	2 3	0 021	83 7	73	7 18	7 59	7 80	5	14	1 2 (−10)	6 1 (+36)	0.08
1223−624	GX301-2	1	2	41 50	34	2 4	0 023	72 6	69	7 41	7 48	7 70	6	16	1 2 (−10)	1 5 (+35)	0 015
1258−613	GX304-1	1	2 4	132 5	34	2 4	0 022	79 6	63	7 17	7 32	7 54	6	11	7 0 (−11)	1 8 (+35)	0 06
1516−569	Cir X-1	1	10	16 594	35	2 3	0 019	97 2	114	8 40	8 40	8 57	5	36	2 6 (−10)	1 2 (+37)	0 015
1538−522	—	1	7	3 730	36	2 2	0 016	118 0	109	7 76	8 32	8 50	4	18	1 6 (−10)	2 3 (+36)	0 6
1617−155	Sco X-1	2	0 7	0 787313	37	2 3	0 010	88 9	76	7 32	7 66	7 87	4	13	1 6 (−10)	1 4 (+34)	0 0005
1627−673	—	2	—	0 02883171 0 02884630	38	2 5	0 029	48 2	55	6 77 6 59	7 07	7 31	8	22	1 4 (−10)	—	—
1700−377‖	—	1	1 7	3 41168	39	2 1	0 011	59 7	63	7 25	7 32	7 54	3	20	2 3 (−10)	9 0 (+34)	0 03

* 1, Massive system, 2, low-mass system

† From ref. 23.

‡ γ-Ray flux is above the effective energy threshold E_{thr}, luminosity is per decade at 10^{16} eV assuming an E^{-2} differential photon spectrum at the source, X-ray luminosity is in the 2–11 keV range and is taken from ref 23

§ Positive detection, flux and luminosity given in text

‖ For this source, only showers with age parameters >1 3 were included in the analysis (see text)

When the observations were made, the Buckland Park array was sensitive to air showers with sea-level sizes $\geqslant 3\times10^5$ particles corresponding approximately to a primary energy of 3×10^{15} eV for nearly vertical showers. For large zenith angles, the threshold energies are higher. We have estimated the effective energy threshold of the array, $E_{\rm thr}$, for showers incident from the declination of each object considered. This was based on the average atmospheric depth of the array as seen by showers from that declination and on an attenuation length[12] of 185 g cm^{-2} appropriate to showers of sizes about 10^6 particles. The effective threshold energies are given in Table 1.

To test for evidence of periodic emission of UHE γ rays in phase with the orbital motion of each binary system, the arrival time of each air shower was converted to an orbital phase, ϕ $(0<\phi<1)$, after applying a heliocentric correction. Cosmic-ray events would be expected to have a uniform phase distribution and the hypothesis of uniformity was tested using the test statistic Y_n proposed by Protheroe[16]

$$Y_n = 2[n(n-1)]^{-1}\sum_{i=1}^{n-1}\sum_{j=i+1}^{n}(\Delta_{ij}+1/n)^{-1} \qquad (1)$$

where,

$$\Delta_{ij} = 0.5 - |[|(\phi_i-\phi_j)|-0.5]| \qquad (2)$$

This statistic was designed specifically for testing circular data for uniformity while being powerful against alternatives in the form of a uniform distribution plus a small narrow distribution at an arbitrary phase. The statistic Y_n representing the data is given in Table 1 together with the 5% and 1% critical value[16] for each object. Y_n exceeded the 5% critical value for LMC X-4 and Cir X-1 but, because 14 objects were examined, only LMC X-4 for which Y_n exceeded the 1% critical value is considered to be significant.

For the 13 other objects, 95% upper limits[17] to the expectation of the number of γ rays contributing air showers which arrive within θ_c are given in Table 1. Also given are 95% upper limits to the γ ray flux and luminosity. The latter takes into account a correction for attenuation by electron-photon cascading[18,19] involving photon-photon pair production and inverse Compton interactions with photons of the 2.7-K microwave background assuming an E^{-2} photon spectrum at the source. In the case of galactic objects, the presence of the interstellar magnetic field rapidly removes energy from the cascade through synchrotron emission and so the photon-photon interactions were treated purely as an absorption process.

Figure 1 plots Y_n against period for the 63 events observed from within 2.5° of LMC X-4. Previous measurements of the orbital period[20–22], which are also indicated, are consistent with the range of periods for which Y_n is large.

The LMC X-4 observation may actually be more significant than suggested by the 1% significance level obtained using the Y_n statistic if the peak in the phase distribution is broader than the effective window width implied by equation (1), that is about n^{-1} of the period. The phase distribution of events from within 2.5° of the direction of LMC X-4 is plotted in Fig. 2. There is a significant excess of events between phases 0.90 and 0.95 where we observe 12 events when we expect 2.65 events. The probability, p, of this occurring by chance in a particular bin, obtained using the Poisson distribution, is 2.12×10^{-5}. The phase distribution is shown binned in 20 bins in Fig. 2, but was previously binned in 10 bins and this binning and rebinning must be taken into account when assigning a significance level for the observation. For LMC X-4 alone, the probability must then be increased to $1-(1-p)^{(20+10)} = 6.36\times10^{-4}$ corresponding to a 3.2-standard-deviation observation. Because 14 sources were examined for UHE γ-ray emission, the probability that the observed phase distribution for LMC X-4 resulted from a chance fluctuation of cosmic-ray background events is $1-(1-p)^{30\times14} = 0.009$. It is, therefore, probable (99.1% confidence) that the phase distribution is not the result of a background fluctuation.

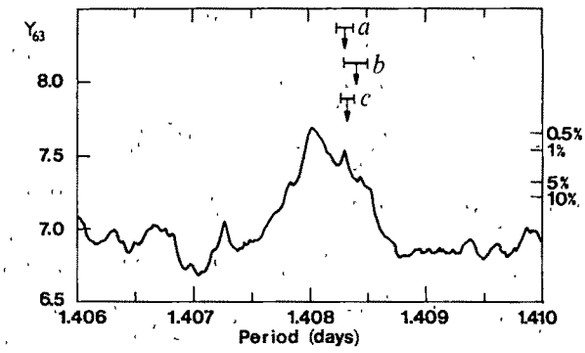


Fig. 1 The statistic Y_n derived from the phase distribution of the 63 air showers arriving from within 2.5° of LMC X-4 plotted as a function of period. Orbital periods derived by: *a*, Huchings *et al.*[21]; *b*, Lang *et al.*[22]; and *c*, Kelley *et al.*[27] are indicated.

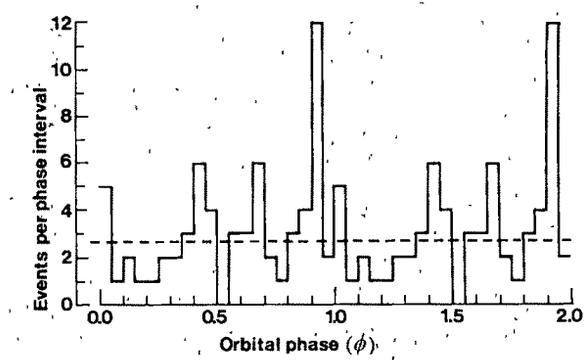


Fig. 2 Phase distribution of the 63 air showers observed within 2.5° of LMC X-4. The ephemeris of Kelley *et al.*[27] was used. The dashed line shows the expected phase distribution of the cosmic-ray background events.

We attribute the excess between phases 0.90 and 0.95, that is 9.4 ± 3.5 events, to γ rays. This corresponds to a time-averaged integral flux of $(4.6\pm1.7)\times10^{-11}$ photons m^{-2} s^{-1} above 10^{16} eV. Assuming an E^{-2} photon spectrum at the source and taking account of electron-photon cascading in the 2.7-K microwave background, we obtain a luminosity of about 10^{38} erg s^{-1} per decade, which is of the same order of magnitude as that emitted at 2–11 keV energies[23]. At ultra-high energies then, LMC X-4 has a luminosity ~20 times[2] that of Cyg X-3 and ~5,000 times[3] that of Vela X-1, making it the most luminous object known at these energies. If the magnetic field between our Galaxy and the Large Magellanic Cloud exceeds $\sim10^{-7}$ G, then the electron-photon cascade in the microwave background radiation field would be reduced by removing energy from it by synchrotron radiation[18]. In this case, to give the same flux of UHE γ rays the luminosity of LMC X-4 would need to be correspondingly higher by up to a factor of three.

Because LMC X-4 is in the Large Magellanic Cloud, some 50 kpc away, we would expect γ rays below $\sim10^{16}$ eV to be severely attenuated in traversing the 2.7-K microwave background. This should be reflected in the energy spectrum of the γ rays. Unfortunately, the effective energy threshold of the array at the time of the observations was close to 10^{16} eV for showers incident from the declination of LMC X-4 and hence no test can be made with the current data for this effect. Recent extensions[24] to the Buckland Park array have increased its sensitivity to small air showers and future observations down to $\sim10^{15}$ eV (for declination −67°) should reveal the presence of the absorption feature. Ideally, a new array at a more southerly latitude should be used to map out fully the absorption feature between below 10^{14} and 10^{16} eV. Such measurements have the potential to test the universality of the 2.7-K microwave background, measure the magnetic field between our Galaxy and the Large Magellanic Cloud, or to provide an independent measurement of the distance[25] to the Large Magellanic Cloud.

LMC X-4 is a massive binary system comprising an 07III – V or 08III – V star[21,26] with a mass of ~17 $M_\odot$ together with a ~1.6 $M_\odot$ neutron star[27] in orbit with a 1.408-day period. The X-ray source has occasional flaring episodes during which ~30% of the X rays are pulsed[27] with a period of 13.5 s, presumably the spin period of the neutron star. The inclination[27] of the system is ~66° and the X-ray source is eclipsed between phase 0.92 and 0.08. If UHE protons are accelerated in the region of the compact object UHE γ rays could be produced by nuclear interactions in the atmosphere of the companion star. In this case, we would expect γ-ray emission at orbital phases ~0.92 and 0.08 when our line-of-sight to the neutron star just grazes the star's surface. The observation of UHE γ rays at a phase of 0.90–0.95 is consistent with this picture, although the absence of UHE emission at phases 0.05–0.10 is puzzling (a similar situation exists for Cyg X-3 and Vela X-1 where only one burst of UHE γ rays is observed per orbit). The X-ray source has high and low states associated with a 30.5-day period[22] attributed to precession of an accretion disk. This precessing disk picture is supported by ultraviolet observations[28] which indicate an almost constant X-ray heating of the stellar atmosphere.

The star does not appear to fill its Roche lobe[21] and seems to have a rather low stellar wind[21,28]. Mass transfer may occur through a trailing accretion stream[21] which may feed the accretion disk. Evidence for this comes from variable obscuration of the companion star[21] between orbital phases 0.6 and 0.9 corresponding to matter trailing behind the neutron star by between 0.2 and 0.3 of an orbit. If this matter is of considerable extent above the orbital plane it could be the target material for interactions of UHE protons produced near the neutron star. Such a scenario would produce γ rays only at a phase of ~0.9 as observed.

We conclude that we have found evidence for UHE γ-ray emission by the LMC X-4 system modulated with the 1.408-day orbital period. The emission occurs when the neutron star is just entering its eclipse by the companion star and could result from nuclear interactions in the atmosphere of the companion by UHE particles produced near the neutron star. Alternatively, a trailing accretion stream could provide target material and explain the absence of UHE γ rays on leaving eclipse. Further observations in progess above 10^{15} and 10^{16} eV coupled with observations from a more southerly site at about 10^{14} eV could provide a test for the universality of the microwave background, be used as an indirect measurement of the magnetic field between our Galaxy and the Large Magellanic Cloud, or provide an independent measurement of the distance to the Large Magellanic Cloud.

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Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction

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Recent attempts[1,2] to consolidate assessments of the effect of human activities on stratospheric ozone (O_3) using one-dimensional models for 30° N have suggested that perturbations of total O_3 will remain small for at least the next decade. Results from such models are often accepted by default as global estimates[3]. The inadequacy of this approach is here made evident by observations that the spring values of total O_3 in Antarctica have now fallen considerably. The circulation in the lower stratosphere is apparently unchanged, and possible chemical causes must be considered. We suggest that the very low temperatures which prevail from midwinter until several weeks after the spring equinox make the Antarctic stratosphere uniquely sensitive to growth of inorganic chlorine, ClX, primarily by the effect of this growth on the NO_2/NO ratio. This, with the height distribution of UV irradiation peculiar to the polar stratosphere, could account for the O_3 losses observed.

Total O_3 has been measured at the British Antarctic Survey stations, Argentine Islands 65° S 64° W and Halley Bay 76° S 27° W, since 1957. Figure 1*a* shows data from Halley Bay. The mean and extreme daily values from October 1957 to March 1973 and the supporting calibrations have been discussed elsewhere[4,5]. The mean daily value for the four latest complete observing seasons (October 1980–March 1984) and the individual daily values for the current observing season are detailed in Fig. 1. The more recent data are provisional values. Very generous bounds for possible corrections would be ±30 matm cm. There was a changeover of spectrophotometers at the station in January 1982; the replacement instrument had been calibrated against the UK Meteorological Office standard in June 1981. Thus, two spectrophotometers have shown October values of total O_3 to be much lower than March values, a feature entirely lacking in the 1957–73 data set. To interpret this difference as a seasonal instrumental effect would be inconsistent with the results of routine checks using standard lamps. Instrument temperatures (recorded for each observation) show that the March and October operating conditions were practically identical. Whatever the absolute error of the recent values may be, within the bounds quoted, the annual variation of total O_3 at Halley Bay has undergone a dramatic change.

Figure 1*b* shows data from Argentine Islands in a similar form, except that for clarity the extreme values for 1957–73 have been omitted. The values for 1980 to the present are provisional, the extreme error bounds again being ±30 matm cm. The

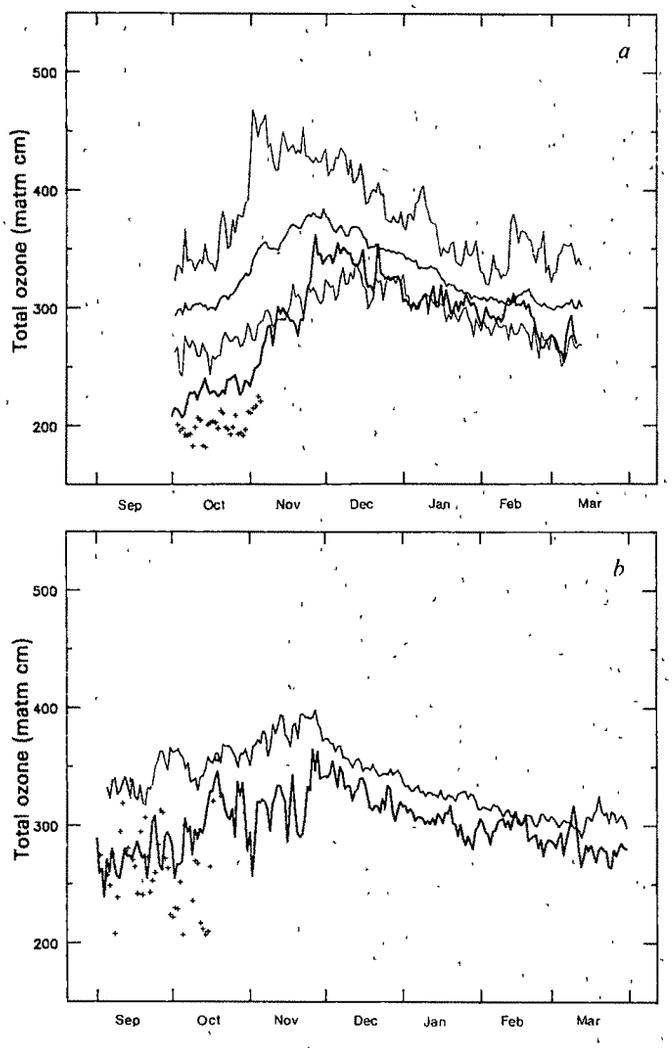


Fig. 1 Daily values of total O_3. *a*, Halley Bay: thin lines, mean and extreme values for 16 seasons, 1957–73; thick line, mean values for four seasons, 1980–84; +, values for October 1984. Observing season: 1 October to 13 March. *b*, Argentine Islands: as for Halley Bay, but extreme values for 1957–73 omitted. Observing season: 1 September to 31 March.

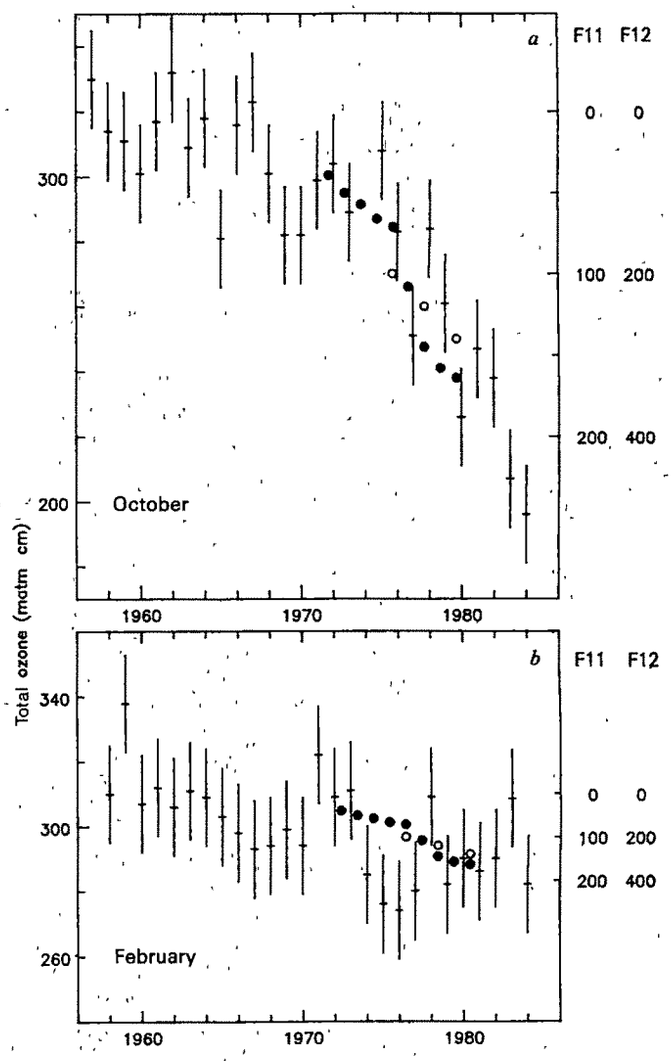


Fig. 2 Monthly means of total O_3 at Halley Bay, and Southern Hemisphere measurements of F-11 (●, p.p.t.v. (parts per thousand by volume) $CFCl_3$) and F-12 (○, p.p.t.v. CF_2Cl_2). *a*, October, 1957–84. *b*, February, 1958–84. Note that F-11 and F-12 amounts increase down the figure.

changes are similar to those seen at Halley Bay, but are much smaller in magnitude.

Upper-air temperatures and winds are available for these stations from 1956. There are no indications of recent departures from established mean values sufficient to attribute the changes in total O_3 to changes in the circulation. The present-day atmosphere differs most prominently from that of previous decades in the higher concentrations of halocarbons. Figure 2*a* shows the monthly mean total O_3 in October at Halley Bay, for 1957–84, and Fig. 2*b* that in February, 1958–84. Tropospheric concentrations of the halocarbons F-11 ($CFCl_3$) and F-12 (CF_2Cl_2) in the Southern Hemisphere[3] are also shown, plotted to give greatest emphasis to a possible relationship. Their growth, from which increase of stratospheric ClX is inferred, is not evidently dependent on season. The contrast between spring and autumn O_3 losses and the striking enhancement of spring loss at Halley Bay need to be explained. In Antarctica, the lower stratosphere is ~40 K colder in October than in February. The stratosphere over Halley Bay experiences a polar night and a polar day (many weeks of darkness, and of continuous photolysis, respectively); that over Argentine Islands does not. Figure 3 shows calculated amounts of NO_x in the polar night and the partitioning between the species[6] Of these, only NO_3 and NO_2 are dissociated rapidly by visible light. The major reservoir, N_2O_5, which only absorbs strongly below 280 nm, should be relatively long-lived. Daytime levels of NO and NO_2 should be much less in early spring, following the polar night, than in autumn, following the polar day. Recent measurements[7] support these inferences. The effect of these seasonal variations on the strongly interdependent ClO_x and NO_x cycles is examined below.

The O_3 loss rate resulting from NO_x and ClO_x may be written[8]

$$L = N + C = 2\,k_2[O][NO_2] + 2\,k_6[O][ClO] \qquad (1)$$

L accounts for over 85% of O_3 destruction in the altitude range 20–40 km. At 40 km, N and C are roughly equal. Lower down, C decreases rapidly to 10% of L at 30 km, 3% at 20 km (refs 6, 8). Equation (1) is based on two steady-state approximations, (see Table 1*a* for the reactions involved)

$$\psi = \frac{[NO_2]}{[NO]} \sim \frac{k_1[O_3] + k_4[ClO]}{k_2[O] + j_3} \qquad (2)$$

and

$$\chi = \frac{[Cl]}{[ClO]} \sim \frac{k_6[O] + k_4[NO]}{k_5[O_3]} \qquad (3)$$

valid in daytime, with [O] in steady state with $[O_3]$. Reaction (4) has a negative temperature coefficient, whereas reaction (1)

Table 1 Reaction list

a	Governing ψ and χ (see text)	
	$NO+O_3 \rightarrow NO_2+O_2$	(1)
	$NO_2+O \rightarrow NO+O_2$	(2)
	$NO_2+h\nu \rightarrow NO+O$	(3)
	$NO+ClO \rightarrow NO_2+Cl$	(4)
	$Cl+O_3 \rightarrow ClO+O_2$	(5)
	$ClO+O \rightarrow Cl+O_2$	(6)
b	Governing [Cl+ClO]	
	$HCl+OH \rightarrow Cl+H_2O$	(7)
	$ClONO_2+h\nu \rightarrow ClO+NO_2$	(8)
	$HOCl+h\nu \rightarrow Cl+OH$	(9)
	$ClO+NO_2+M \rightarrow ClONO_2+M$	(10)
	$ClO+HO_2 \nrightarrow HOCl+O_2$	(11)
	$Cl+CH_4 \rightarrow HCl+CH_3$	(12)
	$Cl+HO_2 \rightarrow HCl+O_2$	(13)
c	$HCl+ClONO_2 \rightarrow Cl_2+HNO_3$	(14)

has large positive activation energy[9], with the result that ψ is strongly dependent on [ClO] at low temperature, as shown in Fig. 4. [ClO] is not simply proportional to total ClX, because $ClONO_2$ formation (reaction (10)) intervenes. Throughout the stratosphere, $\chi \ll 1$, so that [ClO]~[Cl+ClO]. From a steady-state analysis of the reactions given in Table 1*b*,

$$[Cl+ClO] \sim \frac{k_7[HCl][OH]+j_8[ClONO_2]+j_9[HOCl]}{k_{10}[NO_2]+k_{11}[HO_2]+\chi(k_{12}[CH_4]+k_{13}[HO_2])} \quad (4)$$

Values of ψ, χ and [Cl+ClO] obtained from equations (2), (3) and (4) are in good accord with full one-dimensional model results for late summer in Antarctica[6]. Neglecting seasonal effects other than those resulting from temperature and from variation of $[NO+NO_2]$, it is possible to solve simultaneously for $[NO_2]$ and [ClO], and to derive L. Results are shown in Table 2 as relaxation times[8], $[O_3]/L$, for various conditions. The spring values (lines 2, 3 and 4) are highly dependent on ClX amount (compare columns *a* and *b*), the autumn values (line 1) much less so. At Argentine Islands, the sensitivity to ClX growth should resemble that seen in line 2, attributable solely to low temperature. Lines 3 and 4 show the enhanced sensitivity possible at stations within the Antarctic Circle, such as Halley Bay, arising from slow release of $[NO+NO_2]$ following the polar night. It remains to be shown how stable O_3 budgets were achieved with the relaxation times for the lower chlorine level (Table 2, *a*).

Much O_3 destruction is driven by visible light, but production requires radiation below 242 nm. On the dates shown (Table 2), destruction persists for some 11 h, while, because of the long UV paths, production is weak (except around noon) at 29 km, and is virtually absent below that altitude. Line 1 of Table 2 then demands O_3 transport in autumn from the upper to the lower stratosphere, which is consistent with inferred thermally-driven lagrangian-mean circulations[10]. A mean vertical velocity of 45 m per day is in good accord with calculations of net diabatic cooling[11] and gives a realistic total O_3 decay rate in an otherwise conventional one-dimensional model[6]. The short

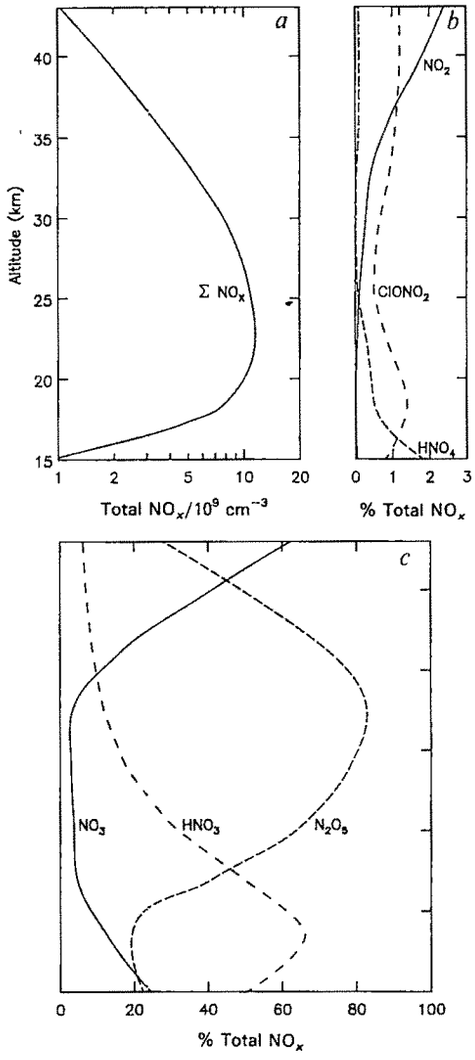


Fig. 3 NO_x during the polar night. *a*, Total NO_x cm^{-3}, from 15 to 43 km *b*, NO_2, $ClONO_2$ and HNO_4 as percentages of total NO_x. *c*, NO_3, HNO_3 and N_2O_5 as percentages of total NO_x

relaxation times in the lower stratosphere in autumn are tolerable, with adequate transport compensating for lack of O_3 production.

In early spring, on the other hand, wave activity scarcely penetrates the cold dense core of the Antarctic polar vortex and with very low temperatures the net diabatic cooling is very weak[11]. Lagrangian transport in the vortex should then be almost negligible. (The virtual exclusion of Agung dust from the vortex supports this view[5].) The final warming signals the end of this period of inactivity and is accompanied by large dynamically induced changes in O_3 distribution. However, before the warming, with low chlorine, total O_3 was in a state of near-neutral equilibrium, sustained primarily by the long relaxation times.

Table 2 Relaxation times in days, $[O_3]/L$, for maximum chlorine levels 1.5 p.p.b.v (*a*) and 2.7 p.p.b.v (*b*) (1980)

	Altitude (km)	22		25.5		29		32.5		36		39.5		43	
Date	Relative $NO+NO_2$	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
25 March	1	105	103	38	37	16	15	6.9	5.7	2.6	2.0	1.2	0.9	0.83	0.57
17 September	1	224	210	86	77	32	27	12.2	9.2	4.9	3.4	2.6	1.7	1.90	1.22
17 September	0.75	288	265	107	93	39	31	14.2	10.4	5.7	3.9	3.0	1.9	2.13	1.33
17 September	0.5	398	353	141	118	47	36	17.0	12.0	6.9	4.5	3.5	2.2	2.41	1.46

Noon values at 75.5° S, solar elevation 12.5°. Lower stratosphere at 230 K on 25 March; 190 K on 17 September. The altitudes shown apply to the summer temperature profile used in the model[6].

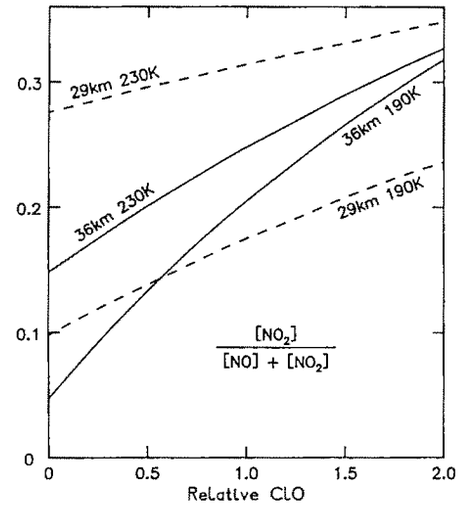


Fig. 4 $[NO_2]/[NO+NO_2]$ has the status of an efficiency factor for O_3 destruction by the NO_x cycle. In terms of the ratio ψ in the text, it is $\psi/(1+\psi)$. The figure shows how this factor varies with [ClO] at 190 K and at 230 K, at altitudes of 29 and 36 km. Values of $[O_3]$, [O], [ClO] and j_3 were taken from one-dimensional model results[6] (maximum chlorine 2.7 p.p b.v.) for noon, 25 March at 75.5° S, solar elevation 12.5°. The abscissa is [ClO] relative to the model value The rate-limiting reaction, (2) in Table 1, for the NO_x cycle has zero activation energy. Note how, nevertheless, O_3 was protected against destruction by NO_x at low temperatures in a stratosphere with small amounts of ClX, but is losing this protection as ClX grows.

With higher chlorine, relaxation times of the order seen in line 4, Table 2, entail more rapid O_3 losses. With negligible production below 29 km and only weak transport, large total O_3 perturbation is possible. The extreme effects could be highly localized, restricted to the period with diurnal photolysis between polar night and the earlier of either the onset of polar day or the final spring warming. At the pole $[NO+NO_2]$ rises continuously after the polar night, with the Sun. The final warming always begins over east Antarctica and spreads westwards across the pole. At Halley Bay the warming is typically some 14 days later than at the pole. Maximum O_3 depletion could be confined to the Atlantic half of the zone bordered roughly by latitudes 70 and 80° S.

Comparable effects should not be expected in the Northern Hemisphere, where the winter polar stratospheric vortex is less cold and less stable than its southern counterpart. The vortex is broken down, usually well before the end of the polar night, by major warmings. These are accompanied by large-scale subsidence and strong mixing, in the course of which peak O_3 values for the year are attained. Hence, sensitivity to ClX growth should be minimal if, as suggested above, this primarily results from O_3 destruction at low temperatures in regions where O_3 transport is weak.

We have shown how additional chlorine might enhance O_3 destruction in the cold spring Antarctic stratosphere. At this time of the year, the long slant paths for sunlight make reservoir species absorbing strongly only below 280 nm, such as N_2O_5, $ClONO_2$ and HO_2NO_2, relatively long-lived. The role of these reservoir species should be more readily demonstrated in Antarctica, particularly the way in which they hold the balance between the NO_x and ClO_x cycles. An intriguing feature could be the homogeneous reaction (Table 1*c*) between HCl and $ClONO_2$. If this process has a rate constant as large as 10^{-16} cm^3 s^{-1} (ref. 2) and a negligible temperature coefficient, the reaction would go almost to completion in the polar night, leaving inorganic chlorine partitioned between HCl and Cl_2, almost equally at 22 km for example. Photolysis of Cl_2 at near-visible wavelengths would provide a rapid source of [Cl + ClO] at sunrise, not treated in equation (4). The polar-night boundary is, therefore, the natural testing ground for the theory of non-linear response to chlorine[1,2]. It might be asked whether a nonlinear response is already evident (Fig. 2*a*). An intensive programme of trace-species measurements on the polar-night boundary could add greatly to our understanding of stratospheric chemistry, and thereby improve considerably the prediction of effects on the ozone layer of future halocarbon releases.

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An Ordovician ophiolite in County Tyrone, Ireland

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Ophiolites are regarded widely by geologists as remnants of old oceanic crust that has been obducted onto the continental margins during ocean or marginal basin closure and orogeny. Therefore, they provide evidence for the existence of old oceans and oceanic basins and are a key to the extension of plate tectonic modelling into pre-Mesozoic rock assemblages. In the Caledonide–Appalachian orogen, ophiolites are known although very few of these are dated well enough to provide accurate constraints on plate-tectonic kinematics. We report here the discovery of an ophiolitic sequence within the rocks of the Tyrone Igneous Complex in Northern Ireland. U/Pb dating combined with field evidence indicates that the Tyrone ophiolite was formed and then obducted onto the northern margins of the Iapetus Ocean close to 470 Myr BP (Arenig).

The Tyrone Igneous Complex (Fig. 1) occurs in an approximate along-strike continuation of the Midland Valley of Scotland; it is partly overlain by Upper Palaeozoic sediments within a shallow graben structure bounded by Dalradian rocks to the north and the Lower Palaeozoic of the Southern Uplands–Longford–Down zone to the south. The complex consists of three units (Fig. 1): a middle Ordovician (Llandeilo–Caradoc) volcanic sequence; a high metamorphic grade gneiss unit 'the central inlier'; and a unit of gabbros and dolerites previously called 'the basic plutonic complex'[1]. Opinions on the age relationships and significance of the units have varied. Originally the basic plutonic complex and volcanics were grouped together as Llandeilo–Caradoc and regarded as being altogether younger and in tectonic contact with the central gneisses[2]. Alternatively, the basic complex has been correlated with layered basic intrusions of Connemara and Aberdeenshire. This led to the view that both basic rocks and gneisses were older than and in unconformable contact beneath the Ordovician volcanic sequence[3].

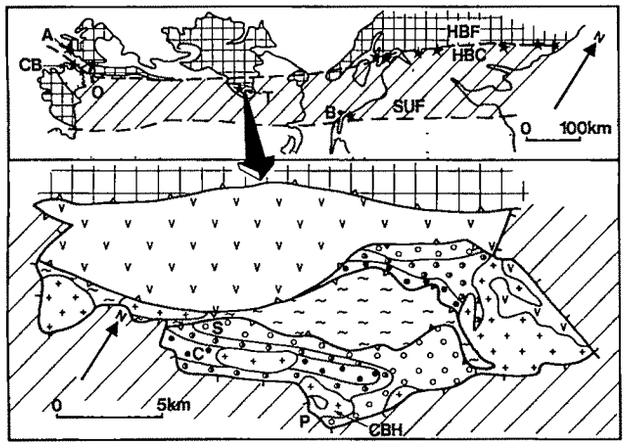


Fig. 1 *a*, Outline geological map of the Midland Valley zone in the British Isles (diagonal ruling). Southern Uplands zone (stipple). Dalradian and older (cross hatch). HBF, Highland Boundary Fault; SUF, Southern Uplands Fault; Caledonian ophiolites: T, Tyrone; B, Ballantrae; HBC, Highland Border Complex; A, Achill Island; CB, Clew Bay; O, Ox Mountains. *b*, Outline geological map of the Tyrone Igneous Complex. Dalradian (cross hatch); Upper Palaeozoic-Tertiary cover (diagonal ruling); Llandeilo-Caradoc volcanics (V); granite (+); central inlier gneisses (~); ophiolite sheeted dykes (●); ophiolite transition (◑); ophiolite gabbros (○); tectonic contacts (barbed lines); unconformites (toothed lines); C, Carrickmore; P, Pomeroy; CBH, Craigballyharky pluton; S, the Scalp.

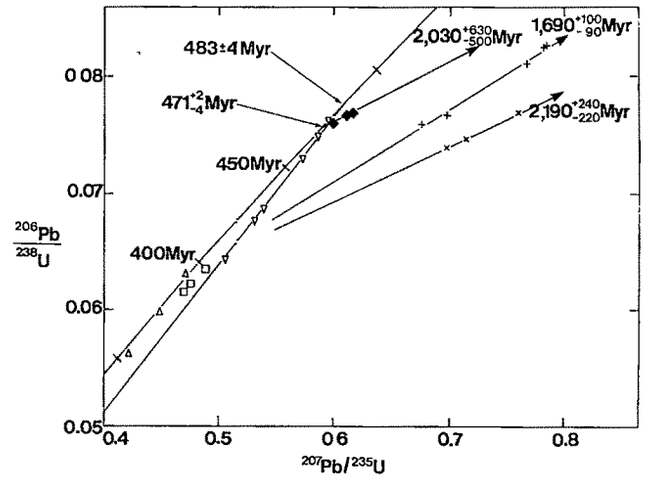


Fig. 2 U/Pb concordia diagram showing data for zircons from the Craigballyharky tonalite and, for comparison, other local rock types, namely, the Ballantrae complex[12] (the nearest other ophiolite), late Caledonian (~400 Myr BP) granites (Distinkhorn[28] and Loch Doon[10]), a greywacke from the Southern Uplands of Scotland[10] and a granulite facies xenolith from the Midland Valley of Scotland[11] ◆, Craigballyharky tonalite R.C.2005; ▽, Ballantrae trondhjemite R.C.1502; □, Loch Doon granite R.C.1812; △, Distinkhorn granite R.C.965; +, S. Uplands greywacke R.C.1813; ×, Granulite facies metasedimentary xenolith, Partan Craig R C 1861.

Reconnaisance remapping and reassessment of the 'basic plutonic complex' (D.H.W.H.) indicates that this is part of an ophiolite 35 × 10 km in area. The lowermost exposed part of this is essentially gabbroic with variably developed cumulate layering[4] as well as leucogabbros and isotropic and pegmatoid gabbros. Overlying this and previously unrecognized as such is a sheeted dyke complex. This is most clearly seen in Carrickmore Quarry [609 721] (Irish National Grid), which exposes 100% dolerite made up of parallel dykes averaging 1 m in thickness. These intrude each other to produce both two-sided chills and, more commonly, the classic one-sided chilled margins typical of sheeted dyke complexes. Elsewhere in the Carrickmore area and along strike for 18 km to the north-east and in another slice of the ophiolite to the north, this dolerite is found and is composed entirely of dykes cutting dykes. The dykes in the complex have general north-east south-west trends and are vertical-to-moderately south dipping. In the lower parts of the sheeted complex the dykes are often deformed parallel to their margins. Overlying the dolerites and so far recorded in only two localities (Carrickmore[2], and Craigballyharky [732 755]) are a few metres of spilitized basaltic pillow lavas with thin chert screens and fragments.

Separating the sheeted dyke complex from the gabbros is a complicated transition zone. This contains early sheeted-dyke dolerites, often strongly deformed, which are cut and intruded by isotropic and pegmatitic gabbros as irregular bodies and veins. This zone is probably the result of repeated intrusion of the gabbro magma chamber in the vicinity of the spreading centre into the overlying sheeted-dyke complex. The deformed dolerite dykes probably represent ductile extension fault development sub-parallel to the dykes, a consequence of newly formed oceanic lithosphere subsiding away from the ridge crest during cooling and crystallization[5].

Within the lower part of this zone and in the gabbro unit, at, for example, the Scalp[4] [636 745], there are common small intrusive sheets, bodies and net vein systems of tonalite, quartz diorite and trondjemite. These have complex time relationships with the basic rocks and the deformation and are interpreted as silicic differentiates from the developing ocean crust. Apart from older records of small serpentine occurrences in Tyrone and the identification in the present study of clinopyroxenite pegmatites (thought to be typical of the gabbro-peridotite contact in ophiolites[6]), there are no ultrabasic rocks present. This is probably the result of thrusting, because all the external contacts of the ophiolite are tectonic.

A feature of the entire basic complex that previous models could not easily explain is the widespread development of moderate-to-high-grade metamorphism. The gabbros and dolerites are irregularly uralitized to epidote amphibolite metamorphic assemblages without, in general, any accompanying deformation. This is typical of the metamorphism of many ophiolites and is probably the result of metasomatic metamorphism by circulating hot brines in the oceanic environment[7].

The sequence described above, which occurs in two tectonic slices separated by the central inlier, is clearly part of an ophiolite[8]. The bottom of the ophiolite has been removed

Table 1 U/Pb isotope data for zircons from Craigballyharky pluton, Tyrone

Size fraction (μm)*	Pb (p.p.m)	U (p.p.m.)	$^{206}Pb/^{204}Pb$	Atom. % ^{206}Pb	^{207}Pb	^{208}Pb	Atomic ratios $^{207}Pb/^{206}Pb$	$^{207}Pb/^{235}U$	$^{206}Pb/^{238}U$	Apparent age (Myr BP) $^{207}Pb/^{206}Pb$	$^{207}Pb/^{235}U$	$^{206}Pb/^{238}U$
−85	12.4	138	2,429	74.38	4.30	21.42	0.057939	0.61533	0.077020	528	487	478
−85+70	14 7	151	2,403	68.25	3 94	27.81	0.057699	0.61236	0.076967	518	485	478
−53	14.9	161	2,260	70.77	4.03	25.20	0.056945	0.59819	0.076183	489	476	474

* All size fractions analysed were non-magnetic at a side inclination of 1°. Uncertainty in $^{207}Pb/^{235}U$ and $^{206}Pb/^{238}U$ estimated at 0.3% and 0.2% (1σ) respectively. Regression of data using the method of York[27] yields upper intercept of $2{,}030^{+630}_{-500}$-Myr BP and lower intercept of 471^{+2}_{-4}-Myr BP (SUMS = 0 52) All isotope measurements were made using a VG Micromass MM308 mass spectrometer.

tectonically and much of the uppermost (basalt-sediment) part has been removed by tectonism or erosion or both. The Tyrone ophiolite is not equivalent to the Connemara and Aberdeenshire layered basic complexes by virtue of its structure and its non-intrusive nature.

The Tyrone ophiolite is cut by a number of small granitic plutons, many of which are biotite and hornblende granites and granodiorites and may be Devonian in age[2]. The Craigballyharky pluton however, which occurs near the village of Pomeroy [732 755], is a tonalite quartz-diorite granodiorite body that intrudes the ophiolite. The marginal quartz diorite of the Craigballyharky intrusion has a hybrid igneous character that has been interpreted as the result of interaction and mixing between the ophiolitic basic country rocks and the intruding central tonalite magma[9]. To achieve such mixing, the basic rocks themselves must have been at quite a high temperaure. As there is no evidence in the area for a separate post-obduction pre-granite heating event of the ophiolite, this high temperature must have been a relic of the ophiolite ocean floor metamorphism. Thus the age of emplacement of the tonalite must approximate the age of the ophiolite itself or be slightly later than it.

In Table 1 we report U/Pb isotope data for three zircon size fractions from a sample of the main tonalite unit at Craigballyharky. Techniques are described elsewhere[10] and are summarized in the table footnote. The results are moderately discordant and when plotted on a conventional concordia diagram (Fig. 2) define a discordia line with a lower intercept of 471^{+2}_{-4} Myr BP and an upper intercept of $2{,}030^{+630}_{-500}$ Myr BP (2σ).

We interpret the lower intercept as the age of intrusion of the Craigballyharky tonalite. This, from the foregoing arguments, places close constraints on the age of the ophiolite itself, which was formed at or just before 471^{+2}_{-4} Myr BP (Arenig) The ophiolite is therefore older and quite distinct from the Llandeilo-Caradoc (~455 Myr BP) volcanic sequence with which it has been correlated in the past.

The upper intercept indicates that the source of the magma and/or the material assimilated by the magma contained zircons of early Proteozoic age. This result is very similar to that obtained for zircons in Ordovician greywackes from the Southern Uplands of Scotland[10] and also from a metasedimentary granulite-facies xenolith from a vent in the Midland Valley of Scotland[11]. The U concentrations of the Craigballyharky zircons (Table 1) are among the lowest recorded from rocks of the British Isles and are very similar to those of zircons from the Ballantrae trondjemite[12] and to zircons from, again, the Midland Valley granulite-facies xenolith[11]. Such low concentrations are expected from granitic regimes that have been melted from basic oceanic crust or from granulite facies continental crust. A plausible explanation to these data, therefore, is that the tonalite represents a mixture of both a mafic component from a relatively young ocean crust and a felsic component from old (Proterozoic) continental crust. We suggest that this crust was part of the northern continental margin to the Iapetus Ocean at this time. Thus, the tonalite rose through the margin and cut the ophiolite very soon after it had been obducted. Hence ophiolite formation and obduction occurred close together in time at 471^{+2}_{-4} Myr BP (Arenig).

The age of the Tyrone ophiolite compares well with the age of the Bay of Islands complex (475–515 Myr BP)[13,14], Ballantrae (~480 Myr BP)[12,15], Shetland (465–479 Myr BP)[16] and Thetford in Quebec (~490 Myr BP)[17]. The Tyrone data provides an important link between the Newfoundland and Scottish ophiolites and confirms that, during the late Tremadoc-Arenig, ophiolite formation and obduction was probably widespread along the Caledonide-Appalachian system. In the British Isles it is perhaps significant that the Caledonian ophiolites north of the Iapetus suture all lie in the Midland Valley of Scotland and its extension into Ireland. Thus, in Scotland the ophiolitic fragments of early Arenig age or older that occur in the Highland Border complex[18] may be comparable with at least part of the Ballantrae ophiolitic sequence seen further to the south[12,15] (Fig. 1). In Ireland, Tyrone, the largest of these ophiolites, has obvious similarities in both age and setting to Ballantrae. Further west in Ireland the undated Clew Bay ophiolitic melange[19] and the Achill Island serpentinitic melange[20] are disrupted Caledonian ophiolites that may be compared with the Highland Border rocks. Also, nearby in the Ox Mountains, small scraps of Caledonian serpentinite are recorded[21]. One view of these ophiolite occurrences in the Midland Valley is that a closed ocean or oceanic basin is concealed at the site of the Highland Boundary or Southern Uplands fault[19,22]. An alternative view, presented here, is that the Midland Valley basement is part of the Proterozoic North American continental margin to Iapetus, onto which ophiolites were obducted from the south during the Ordovician The Highland Boundary and Southern Upland fault have been identified recently[23,24] as long lived Palaeozoic transcurrent faults. We suggest that the Midland Valley became detached from the North American continent along these faults by sinistral movements, probably culminating in the early Devonian[25,26], which displaced this allochthonous terrane northeastwards into its present position.

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Seismic sequences in Greece interpreted in terms of barrier model

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Between 1978 and 1981, five shallow main shocks occurred in Greece and the surrounding region. Here we consider the space–time distribution of the foreshocks and the aftershocks of these earthquakes by interpreting the migration characteristics of the epicentres in terms of the barrier model[1]. This model suggests that segmented ruptures can occur in a seismic fault but that the rupture front may be stopped by a barrier, or may propagate over the whole fault plane leaving the barrier unbroken. These unbroken barriers result in aftershocks due to the concentration of stress there, and cause migration of major events along a plate boundary. This migration may be an important factor for the prediction of earthquakes.

According to the barrier model, segmented ruptures could occur in a seismic fault, that is, slip could occur in cracks during the fault rupture process, while the region between cracks remains unbroken after the rupture. A rupture front may be

stopped by a barrier, but elastic waves generated by the slip can break the fault plane ahead of the barrier. Then, the rupture can propagate over the whole fault plane to leave the barriers unbroken The residual stress over the fault plane immediately following the rupture is not uniform. Consequently, the stress will concentrate at the unbroken barriers[2] and cause aftershocks. Barriers also cause migration of major events along a plate boundary, such as the westward migration of great earthquakes[3] on the Anatolian fault which took place between 1939 and 1944.

During 1978–81, five shallow main shocks with surface wave magnitudes ≥ 6.5 occurred in Greece and the surrounding area. Table 1 lists the focal coordinates (φ, λ, h), the surface wave magnitude (M_s), number of observations (N), mean error of the origin time (r.m.s.), and mean error in the location (ERH, km) for the main shocks.

To determine accurate locations of the epicentre and the focal depth, we used the HYPO 71 computer program[8], adopting a two-layer crustal model above a half space[9] with the following thicknesses (d_i) of the layers, and velocities (V_i); $d_1 = 19.0$ km, $V_1 = 6.0$ km s^{-1}, $d_2 = 12.0$ km, $V_2 = 6.6$ km s^{-1}. The P-wave velocity in the half space has been taken as 7.9 km s^{-1}. The accuracy in the determination of the focal parameters has been improved by using time delay corrections[10] on the arrival times at the seismological stations in Greece and neighbouring countries. The average ERH for all the shocks of the five seismic sequences is satisfactory for our purpose, being 2.0 ± 0.9 km.

Figure 1 is a map of Greece and of the surrounding area showing the strikes of the faults producing the five seismic sequences The main source parameters of these earthquakes are known[11]. To investigate the space-time distribution of the epicentres of the five seismic sequences, the position of the epicentre projection on the strike direction of the fault plane, which is the largest dimension of the aftershock area, is plotted against time for each sequence. The distances from the epicentre, x, are always measured from a point, A, which is located at one of the two ends of the fault's strike, because the epicentre migration takes place parallel to this direction.

Figure 2 shows the space-time diagrams of the five seismic sequences. In three of these, the main shock was preceded by smaller shocks. In the Thessaloniki 1978 seismic sequence (Fig. 2*a*), these shocks migrated towards the main shock epicentre, that is, from east to west, while in Montenegro 1979 and Magnesia 1980 seismic sequences (Fig. 2*b*, *c*) these shocks are very closely related, in space and time, with the main shock. This clearly shows that these earthquakes are real foreshocks of the three main shocks.

In all five cases, after the main shock, the aftershock activity expanded from the focus of the main shock to one (Fig. 2*a*, *c*, *d*) or both ends (Fig. 2*b*, *e*) of the fault, reaching these ends after 2 h, 24 min, 7 h, 15 min and 30 min respectively

Figure 2 shows that parts of the faults do not have aftershocks. For example the central parts of the faults which produced the Monte-Negro 1979 (Fig. 2*b*), the Magnesia 1980 (Fig. 2*c*), the Alkyonides Gulf 1981 (Fig. 2*d*) and the Northern Agean 1981 (Fig. 2*e*) seismic sequences. These are the parts of the faults which presumably slipped smoothly during the main shocks. The dashed circles in Fig. 2*d* denote shocks related to an antithetic fault parallel to the main fault.

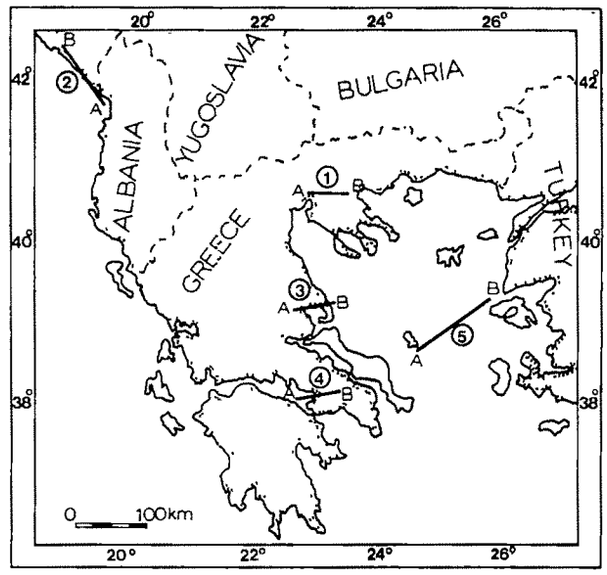


Fig. 1 Index map indicating the strikes, AB, of the main faults of the five seismic sequences Migration of these shocks in each sequence was investigated along these strikes Positions 1–5 correspond to *a*–*e* of Fig. 2

The aseismic slip induced high tectonic stress at one or both ends of the fault where the clustering of aftershocks is observed The clustering probably indicates the sites of the barriers where high stress was induced following the main shocks.

After a time interval, which varies from sequence to sequence, the stress reached the barrier's ultimate strength, breaking the strongest barrier and producing the largest aftershock in each seismic sequence.

Three out of five main shocks with $M_s \geq 6.5$ were preceded by recorded foreshocks. These foreshocks started 5 to 42 days before the main shocks and were either very close to the epicentres of the main shocks or their epicentres migrated to the epicentres of the main shocks. Detailed studies of foreshocks could, therefore, contribute to the prediction of the main shocks.

In three of the seismic sequences, the focus of the main shock was located in the middle section of the fault; in the other two cases, it was located near one end of the fault. Thus, the focus of the main shock can be anywhere in the fault.

In all five cases, the aftershock activity expanded to one or to both ends of the fault after the main shock and reached these ends within one day. In all five cases, the largest aftershocks occurred near the one end of the fault within a time period which varied from sequence to sequence between 15 min and 38 days after the main shock. This observation shows that a continuous monitoring of the aftershock activity can lead to the prediction of the epicentre of the largest aftershocks which sometimes produce damage comparable with that of the main shock.

The barrier model gives a general explanation of the occurrence of the shocks of the seismic sequences, but detailed knowl-

Table 1 Data for the main shocks of the five sequences

No	Date	Origin time	Location φ (°N)	Location λ (°E)	h (km)	M_s	N	r.m.s (s)	ERH (km)	Ref.
1	20 June 1978	20.03.23	40.73	23.25	8	6 5	385	—	—	4
2	15 April 1979	06:19:41	41.93	18.99	1	7.1	33	0 46	1.4	5
3	9 July 1980	02:11:57	39.28	23.11	9	6.5	17	0.50	2.4	6
4	24 February 1981	20:53.37	38.16	22.88	10	6 7	36	0.39	1 5	7
5	19 December 1981	14:10.52	39.08	25 24	3	7 3	36	0.43	1.3	*

* Our determination.

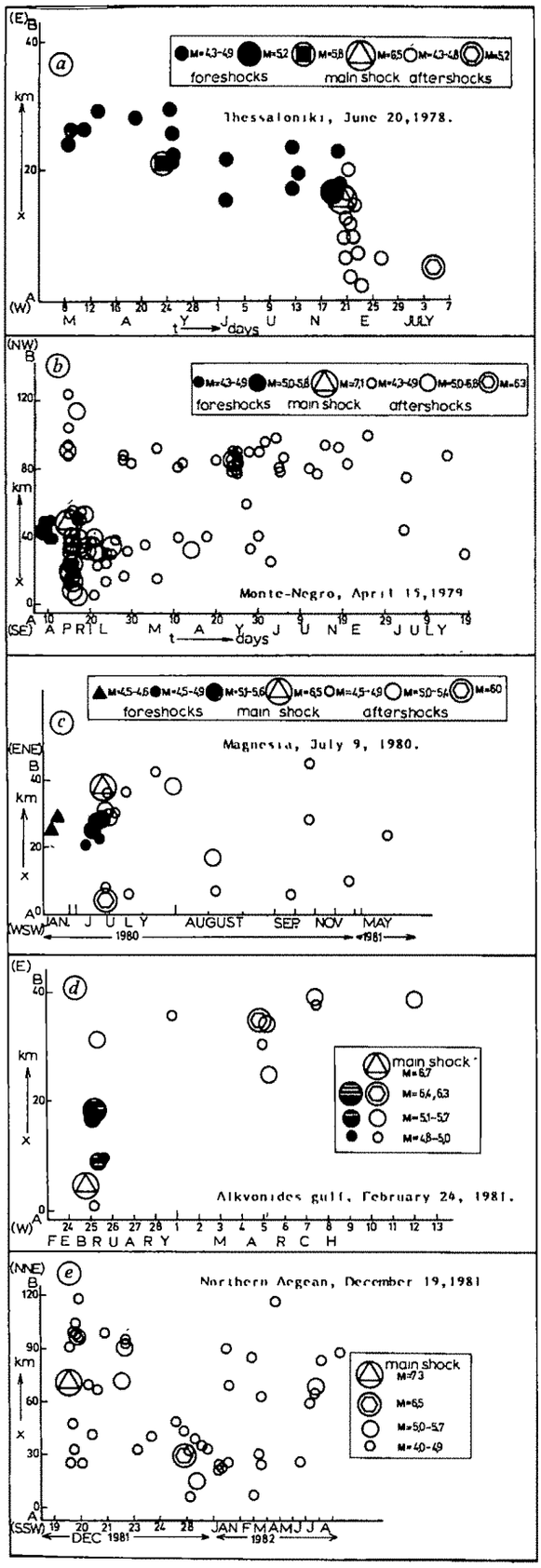


Fig. 2 Space-time distribution of the five seismic sequences. *a*, Thessaloniki, 1978; *b*, Montenegro, 1979; *c*, Magnesia (central Greece), 1980; *d*, Alkyonides Gulf, 1981; *e*, northern Aegean, 1981

edge of the space-time distribution of shocks would improve this model.

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The origin of authigenic ankerite from the Ninian Field, UK North Sea

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The carbonate minerals ankerite and ferroan dolomite are widely reported in studies of concretions[1,2] and of sandstone hydrocarbon reservoirs[3–8]. These minerals are often thought to have formed relatively late in the sandstones' diagenetic history and in the Gulf Coast their occurrence has been linked to clay mineral diagenesis in the surrounding shales[9]. I describe here authigenic ankerite from the Ninian Field[10]. Ankerite formation was probably synchronous with hydrocarbon emplacement and is related to clay mineral diagenesis in the hydrocarbon source rocks of the Viking Graben. It occurs throughout the reservoir sandstones but is particularly abundant below the oil–water contact. This distribution reflects hydrocarbon emplacement arresting diagenesis at progressively deeper levels of the reservoir. The resulting preferential cementation may adversely affect transmissibility between the aquifer and the oil column and consequently could reduce hydrocarbon recovery.

Two forms of authigenic ankerite are observed in cores from Wells 3/3-1, -2 and -3 from the Ninian Field in the northern North Sea. The reservoir comprises Middle Jurassic Brent Group sandstones[10] and is characterized by a complex diagenetic mineralogy including clay minerals, quartz and feldspar overgrowths, siderite, pyrite, calcite and barytes[11]. The two forms of ankerite occur irrespective of depositional facies and enclose and replace the earlier formed authigenic minerals. The first form is concretionary and occurs in finer-grained sediments. It comprises large poikilotopic crystals several millimetres in length which totally fill pore space and constitute 23–50% of the bulk rock volume. The second form is discrete pore-filling rhombs, 20–40 μm in length which form up to 1% of the bulk rock volume above the oil–water contact, but up to 5% below.

Carbon and oxygen stable isotope ratios of ankerite samples are compiled in Table 1. Carbon isotope ratios can provide information on the origin of the bicarbonate in carbonate minerals[1]. Carbonates precipitating from seawater have a $\delta^{13}C$ of 0‰ PDB. Also, carbonates incorporating bicarbonate from specific bacterial degradation reactions have specific $\delta^{13}C$ compositions of −20 to −25 or +15‰ (ref. 1) or intermediate values if bicarbonates are liberated by more than one mechanism. The carbon isotope compositions of these ankerite samples ($\delta^{13}C$ −7.1 to −13.6) are not indicative of a specific source of bicarbonate. These values could result from precipitation in pore waters supplied by two sources of bicarbonate. Alternatively, this composition could reflect the dissolution, equilibration and reprecipitation, of *in situ* carbonate cements of one

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isotopic composition with CO_2 produced during abiotic degradation of organic matter or hydrocarbon maturation.

Oxygen isotope ratios can, in certain circumstances, provide information on the temperature of mineral precipitation. During isotopic equilibrium precipitation, the $\delta^{18}O$ composition of a carbonate is related to both the temperature of precipitation and the composition of the fluid from which it precipitated. Assuming the pore waters to have been Jurassic seawater ($\delta^{18}O = 1.2‰$)[12] the temperature at which ankerite precipitated is estimated to be ~115 °C. Because the isotopic composition of the pore waters from which ankerite precipitates is unknown this temperature is unlikely to be accurate. However, the isotopic composition is consistent with precipitation from isotopically light, and/or hot pore waters[1,3,6]. Interpretation of the isotopic data confirms petrographical observations. Ankerite authigenesis was late in the diagenetic sequence and occurred at a 'temperature' approaching the present reservoir temperature of 100 °C (ref. 10). Precipitation probably involved CO_2 generated in a hotter, more deeply buried hydrocarbon-source rock in the Viking Graben and was related to Tertiary hydrocarbon migration into the reservoir[10,13-15].

Electron microprobe analysis reveals various chemical compositional trends within individual crystals. These trends reflect local fluctuating pore-water compositions during precipitation. However, the data as a whole exhibit a gradual decrease in $CaCO_3$ content with increasing depth (Fig. 1). It is suggested that this gross compositional change reflects evolving pore-water composition. When ankerite precipitation began throughout the reservoir pore waters were more calcium-rich. As hydrocarbons filled the reservoir, the precipitation of ankerite continued but pore-water composition changed progressively. The last ankerite precipitation occurred below the present oil–water contact, in relatively calcium-depleted pore waters.

Dissolution of, and equilibration with, earlier formed calcite or siderite respectively could have supplied the Ca^{2+} or Fe^{2+} required for ankerite precipitation. The replacement of calcite certainly may be implied from the high-minus cement porosities of the concretionary ankerites. However, ankerite, siderite and calcite coexist in the water. If dissolution and equilibration did take place, calcite dissolution would have become unfavourable during later precipitation when equilibrium was reached. This would explain the chemical compositional trends seen in the ankerite. Mg^{2+}, however, must have been introduced from outside the system, there being no precursor Mg^{2+}-bearing minerals present. In the Gulf Coast[3,9], ankerite formation in sandstones is related to mudrock diagenesis in the surrounding shales. The replacement of smectite by illite releases Mg^{2+} and Fe^{2+} into solution[9], at temperatures which mark the onset of hydrocarbon migration[9,13,14]. In both the hydrocarbon source rocks of the Viking Graben, and elsewhere in the North Sea, the replacement of smectite by illite has occurred and cation release is likely to have overlapped hydrocarbon generation[13,14].

It is postulated that ankerite began to form in the Ninian Field during hydrocarbon emplacement, as the invading pore waters reached equilibrium with the formation. In view of the sealed nature of the reservoir during hydrocarbon accumulation, fluid flow must have been convective[16]. Poikilotopic ankerite began to form by nucleating on and replacing preexisting authigenic calcite. Ubiquitous pre-filling rhombs also formed at this time. Ankerite precipitation continued below the oil–water contact as hydrocarbons arrived but was arrested as the oil column grew progressively while the reservoir filled. As Fe^{2+} and Mg^{2+} continued to arrive in the hydrocarbon-bearing fluids, pore-water compositions changed, leading to formation of progressively Ca^{2+} poorer ankerite with increasing depth (Fig. 1). The earlier formed cements now occur in the oil-bearing sandstones and the discrete pore-filling ankerite rhombs both in the oil and below the oil–water contact in the aquifer, having formed as saturated pore waters were displaced downwards by the accumulating hydrocarbons. Late stage ferroan carbonates and ankerites are reported from reservoirs elsewhere[3-6]. In view of the widespread association of altering smectites and maturing source rocks[9,13], many may have a similar origin, although this has not been widely recognized[17].

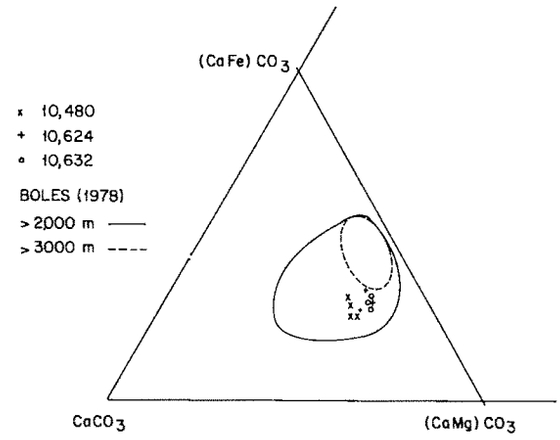


Fig. 1 Chemical compositional trends of ankerite from the Ninian Field. Data points are labelled in core or drillers depths. The oil–water contact in 3/3-3 occurs at 10,503 feet drillers depth (3201.3 m). Electron microprobe analyses were performed with a Links Systems Model 290 energy dispersive analyser fitted to a Cambridge Instruments Geoscan. The operating conditions were beam voltage 15 V, specimen current 2.5 nA and beam width 1 μm. The raw data were recalculated with a Links Systems ZAF 4 software package. Depths of the three cores in metres are 3,194.3 m, 3,238.2 m and 3,240.6 m, respectively.

In the Gulf Coast, ankerite authigenesis is reported to be succeeded by the replacement of kaolinite by chlorite[9]. This reaction is also related to the release of Fe^{2+} and Mg^{2+} during smectite's transformation to illite[9]. In the Ninian Field and in Brent Group sandstones elsewhere, chlorite is only reported to occur in the Broom Formation[11] and it is not interpreted as replacing kaolinite[11]. This could be due to the sediments not having reached the appropriate pore-water conditions. Alternatively, the Gulf Coast model may not be universally applicable as shown by the doubt concerning the original concept of the reaction's structural mechanism[18].

In the Ninian Field, porosities at the oil–water contact are not reduced significantly by ankerite precipitation[10]. Indeed, ankerite is disseminated throughout the reservoir sequence. Authigenesis here has been promoted by metal cations and bicarbonate released from hydrocarbon-source rocks, and progressively arrested by the arrival of hydrocarbons. By contrast, with rapid hydrocarbon accumulation and a plentiful supply of bicarbonate and metal cations, ankerite-forming pore waters could be displaced into the aquifer thus concentrating its formation at the oil–water contact. Also, light hydrocarbon generation and emplacement before clay mineral rection releasing cations

Table 1 Stable isotope ratios for authigenic ankerite

Well	Core depth (feet)	$\delta^{13}C$	$\delta^{18}O$	Precipitation temperature (°C)
3/3-1	9,916	−9.7	−15.2	144
3/3-3	10,330	−7.1	−10.3	96
3/3-3	10,456	−13.6	−12.9	120
3/3-3	10,502	−9.4	−10.5	98

The samples were cleaned with 1% sodium hypochlorite[19] to remove hydrocarbons and organic matter and reacted with phosphoric acid at 25 °C for 2 h. The CO_2 evolved was analysed with a Micromass 903 mass spectrometer and the raw data corrected using the normal methods[20,21]. Stable isotope ratios are reported as δ‰ variations from PDB standard, corrected with a fractionation factor of 1.01109 (ref. 22). Hypothetical precipitation temperatures for ankerite were determined assuming ankerite–water fractionation to be similar to dolomite–water[23]. Depth given in feet; equivalents in metres are 3,022.4 m, 3,148.6 m, 3,187.0 m and 3,201.0 m, respectively.

would restrict ankerite anthigenesis to below the oil–water contact. Thus, whilst high porosity and effective permeability might exist within both the oil column and the aquifer, connection between the two could become impaired. This possibility should be evaluated during reservoir development planning because any such transmissibility barrier could restrict the flow into the reservoir of either a natural aquifer or injection water. Both the loss of pressure support and the disruption of an injection pattern could adversely affect hydrocarbon recovery.

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Anomalous ^{13}C enrichment in modern marine organic carbon

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Marine organic carbon is heavier isotopically (^{13}C enriched) than most land-plant or terrestrial organic C[1]. Accordingly, $\delta^{13}C$ values of organic C in modern marine sediments are routinely interpreted in terms of the relative proportions of marine and terrestrial sources of the preserved organic matter[2,3]. When independent geochemical techniques are used to evaluate the source of organic matter in Cretaceous or older rocks, those rocks containing mostly marine organic C are found typically to have lighter (more-negative) $\delta^{13}C$ values than rocks containing mostly terrestrial organic C. Here we conclude that marine photosynthesis in mid-Cretaceous and earlier oceans generally resulted in a greater fractionation of C isotopes and produced organic C having lighter $\delta^{13}C$ values. Modern marine photosynthesis may be occurring under unusual geological conditions (higher oceanic primary production rates, lower P_{CO_2}) that limit dissolved CO_2 availability and minimize carbon isotope fractionation[4].

In a recent compilation of geochemical analyses of samples of middle Cretaceous organic C-rich strata from numerous Deep Sea Drilling Project (DSDP) sites and several land sections[5], we failed to observe a consistent relationship between organic carbon $\delta^{13}C$ and independent geochemical indicators of marine

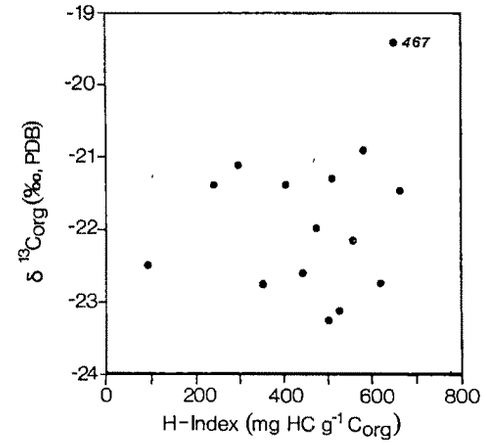


Fig. 1 Plot of $\delta^{13}C$ of organic C against Rock-Eval pyrolysis hydrogen index for organic C-rich sediments of Pleistocene–Miocene age from offshore California, DSDP Leg 63, Site 467. Hydrogen index is pyrolytic hydrocarbon yield per unit organic carbon in mg per g.

and terrestrial organic matter for samples older than Miocene. In fact, the Cretaceous sequences examined in the greatest detail usually showed the reverse of the expected relationship; that is, samples with the lightest (or apparently terrestrial) $\delta^{13}C$ values had chemical compositions indicating the greatest contributions of marine organic matter.

This apparently anomalous ^{13}C depletion in organic C of ancient sediments has been noted by others[6], and is particularly troublesome in rocks older than Silurian[7], or before the development of land plants. We conclude that organic C derived from marine plants in rocks that are Cretaceous or older typically have $\delta^{13}C$ values that are ~5–7‰ more negative than marine organic carbon in Holocene sediments.

Other techniques used to evaluate marine against terrestrial organic matter include atomic H/C ratios[8], microscopic examination of isolated organic-matter particles[9], abundance of phenolic lignin degradation products[3], organic $^{15}N/^{14}N$ ratios[10] and ^{13}C-nuclear magnetic resonance spectroscopy[11]. In general, the relative proportions of marine and terrestrial organic matter indicated by these techniques are consistent with the proportions inferred from bulk organic C $\delta^{13}C$ values in modern marine sediments.

For the data sets that we have compiled[5], the most common measure of H content is the H index from Rock-Eval pyrolysis, which has been shown to correlate with atomic H/C ratios[12]. We plot organic C $\delta^{13}C$ against H index or atomic H/C of organic matter and observe either a positive correlation or no correlation for organic C rich sequences of Holocene–Miocene age. Figure 1 shows a typical lack of correlation for Holocene–Miocene sediments from offshore southern California[5]. Here, essentially all of the organic matter is from marine productivity induced by upwelling. This marine organic matter has $\delta^{13}C$ of ~−23 to −21‰, regardless of H content.

For Cretaceous and older organic C-rich sequences, the data commonly show a negative correlation between H index and $\delta^{13}C$ of organic matter. Figure 2 illustrates data from four different middle Cretaceous organic C-rich limestone sequences. These data sets were selected to minimize possible effects of differences in preparation procedures and lithology. In general, middle Cretaceous organic C-rich strata in the North American Basin of the western North Atlantic Ocean contain predominantly terrestrial organic matter, the abundance of which is greatest at those sites closest to the North American continental margin[9,13]. In contrast, middle Cretaceous strata at DSDP sites adjacent to the African continental margin contain predominantly marine organic matter[9,13]. Figure 2*a* shows $\delta^{13}C$ and H index data for interbedded Neocomian white limestones and black marlstones of the Blake-Bahama Formation[14] cored at DSDP Site 603, one of the most terrestrially influenced

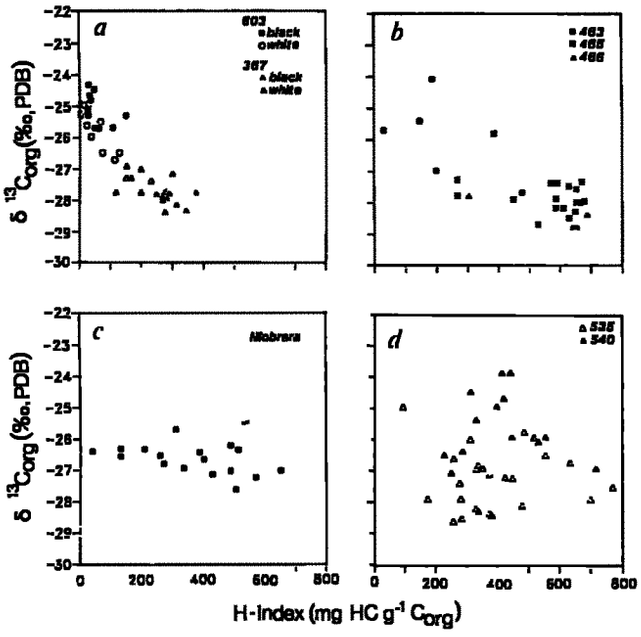


Fig. 2 Plots of $\delta^{13}C$ of organic C against Rock-Eval pyrolysis hydrogen index for *a*, samples of Neocomian black marlstone and white limestone from DSDP Sites 603, North American Basin, western North Atlantic and 367, Cape Verde Basin, southeastern North Atlantic[5], *b*, samples of middle Cretaceous organic C-rich limestone from DSDP Sites 463, Mid-Pacific Mountains, and 465 and 466, southern Hess Rise, western North Pacific[19], *c*, Upper Cretaceous Niobrara Limestone, Colorado[5], and *d*, samples of middle Cretaceous limestone from DSDP Sites 535 and 540, eastern Gulf of Mexico[21]

localities in the North American Basin. Also shown on Fig 2*a* are data on coeval strata from DSDP Site 367 in the Cape Verde Basin off north-west Africa, which contains the most abundant marine organic matter of any DSDP Site in the North Atlantic[9,13]. At both sites, much of the organic matter was redeposited by turbidity currents from continental slope settings[15,16]. The $\delta^{13}C$ and H index data show distinctly different trends for these two sites (Fig 2*a*) At Site 367, the organic matter in the black marlstone is H-rich and isotopically light ($\delta^{13}C$ of −28‰) and, at Site 603, the organic matter in the black marlstones is H-deficient and isotopically heavier ($\delta^{13}C$ of −25‰). The white limestones at both sites probably contain an oxidized residual mixture of marine and terrestrial organic matter, with possibly more marine influence at Site 367

Figure 2*b* shows the relationship between $\delta^{13}C$ and H index for organic C-rich limestones of early Aptian to late Albian age from the Mid-Pacific Mountains and southern Hess Rise These limestones were deposited on the flanks of seamounts and volcanic plateaus that were formerly under shallower water and under the equatorial high productivity zone during the middle Cretaceous[17]. The organic matter from these sites, especially from southern Hess Rise (Sites 465 and 466), is predominantly H-rich marine organic matter[18]. The relationship between isotope composition and H content of the organic matter is the same as that shown in Fig. 2*a*, that is, the most H-rich, most marine organic matter is isotopically the lightest. Figure 2*c* and *d* are data sets of samples from mid-Cretaceous limestones that apparently contain mostly marine organic matter. Figure 2*c* shows results of analyses of 17 samples from a quarry of the Coniacian-Santonian Niobrara Limestone deposited in the western interior seaway of the United States. The organic matter in these samples is mainly marine and the variation in H index is probably attributable to differing degrees of oxidation[19]. The tendency for the more H-rich organic matter to be isotopically lighter in the Niobrara may be a result of diagenesis rather than source of the organic matter. Samples of the early Cenomanian to late Albian limestone from DSDP Site 540 in the Gulf of Mexico (Fig. 2*d*) have $\delta^{13}C$ values that range between ~−27 to −24‰ with a H index range of 220–700. Other organic geochemical analyses[20] suggest that the organic matter is marine in origin, with lower H contents attributable to deposition under more oxidizing conditions. Samples of Cenomanian–Berriasian limestone from the nearby Site 535 also contain predominantly marine organic matter[21] and have organic C $\delta^{13}C$ values that range from −28 7 to −25‰. The differences between the $\delta^{13}C$ values from Sites 540 and 535 may be attributable to age differences and secular variations in $\delta^{13}C$ of the ocean–atmosphere C reservoirs, because the more-negative organic C $\delta^{13}C$ values at Site 535 are matched by correspondingly more-negative carbonate-C $\delta^{13}C$ values[20].

We conclude from numerous data sets, such as those illustrated in Fig 2, that Cretaceous organic matter, interpreted to be largely marine by H richness or by other geochemical or optical indicators, has $\delta^{13}C$ values of −29 to −27‰, and organic matter, interpreted to be largely terrestrial, has $\delta^{13}C$ values of −25 to −24‰ Deposits of mixtures of terrestrial and marine organic matter, therefore, show a negative correlation between organic C $\delta^{13}C$ and H content (H index or atomic H/C). From this we conclude that Cretaceous marine organic matter was, on average, 5–7‰ lighter than Holocene marine organic matter.

There are several potential factors that could cause shifts in the $\delta^{13}C$ of marine organic matter, such as· (1) early to late diagenetic effects, (2) sea-surface temperature changes and their possible influence on CO_2 solubility and C isotope fractionation during photosynthesis; (3) changes in dominance of marine phytoplankton species and related isotope fractionation factors, (4) global or local changes in the $\delta^{13}C$ of oceanic dissolved CO_2; and (5) changes in general levels of productivity and the influence of CO_2 availability on isotope fractionation during planktonic photosynthesis Thus, either the primary isotope composition of Cretaceous organic matter was the same or similar to that of the Neogene through Holocene and was modified during accumulation and diagenesis, or the isotope composition was different (~5–7‰ more negative) for some primary reason

Factors (1)–(4) above probably can account for only minor changes of 1 or 2‰ in the $\delta^{13}C$ of marine organic carbon[5] Various diagenetic processes can alter the $\delta^{13}C$ of organic C by removing or adding ^{13}C-enriched or depleted fractions of the organic matter, but consistent unambiguous diagenetic trends in modern and Neogene sediments are difficult to document[1,22] Hatcher *et al.*[11] showed a 4‰ decrease in $\delta^{13}C$ of organic C of algal sapropelic sediment in a saline lake in Bermuda, but a diagenetic shift of this magnitude has not been observed in normal marine sediments Cretaceous palaeotemperatures at low latitudes were as warm or warmer than those of today[23,24] If temperature change was the primary reason for the different $\delta^{13}C$ of organic carbon in the Cretaceous, then according to previous hypotheses[25,26] we would expect marine organic C to have been isotopically heavier, not lighter as observed. In addition, evidence now suggests that there is not a direct simple relationship between surface water temperature and $\delta^{13}C$ of marine organic matter[27]. Changes in C isotope fractionation as a result of plankton evolution or changes in the dominance of a particular phytoplankton group cannot be eliminated completely, but such changes are not supported by the Cenozoic palaeontological record and are considered unlikely to be the cause of the shift in the $\delta^{13}C$ of marine sedimentary organic matter Secular variations in the $\delta^{13}C$ of marine carbonates[28] indicate that the $\delta^{13}C$ of total dissolved CO_2 in surface ocean water changed by 2–3‰ during the Cretaceous. However, the average Cretaceous carbonate $\delta^{13}C$ is ~1.5‰ heavier than modern pelagic carbonates, so that the effect of secular $\delta^{13}C$ variations would be to make Cretaceous marine organic matter heavier rather than lighter as observed Kuspert[29] observed 4‰ lighter $\delta^{13}C$ in both organic and carbonate C of organic matter-rich beds in a Jurassic shale sequence, which he attributed to local contributions of oxidized organic C to the water column during episodes of anoxic stagnation. The main effect of local

or global $\delta^{13}C$ variations in dissolved CO_2 probably has been to induce variation of as much as 2-3‰ in the record of the isotope composition of marine organic matter through time.

The most probable cause of significantly lighter $\delta^{13}C$ of marine organic C in Cretaceous, compared with modern, sediments is the different set of conditions under which marine photosynthesis operated. Namely, the higher atmospheric CO_2 concentrations[30,31] and the overall lower levels of oceanic productivity[32] that prevailed during the Cretaceous. Photosynthetic C isotope fractionation is maximized under conditions that promote high CO_2 availability. In modern oceans, Antarctic diatoms with an abundant supply of CO_2 provided by upwelling of cold CO_2-rich bottom waters in the Antarctic Divergence have more negative $\delta^{13}C$ than the plankton in low-latitude warm water with less available CO_2 (ref. 27). During much of the Cretaceous, the combination of low surface productivity and higher atmospheric CO_2 should have resulted in an ocean that had more available CO_2 and an increased fractionation of carbon isotopes in marine plankton.

Our interpretation of generally increased marine photosynthetic carbon isotope fractionation during Cretaceous (and earlier) time has several implications. The utility of $\delta^{13}C$ values alone for organic matter provenance studies should be considered carefully. The technique works reasonably well for Neogene-Quaternary sequences, but certainly not in the same way for Cretaceous sequences and probably not for much of the Phanerozoic. Variable marine photosynthetic fractionation of C isotopes in the past affects quantitative models of the C cycle. Most models assume a constant fractionation between CO_2 and organic matter and interpret $\delta^{13}C$ variations with time in terms of variations in the proportions of reduced and oxidized carbon reservoirs. Variable isotope fractionation during marine photosynthesis adds a significant complication to these models.

More work is necessary to document the preliminary conclusions that we offer here, both for the Cretaceous and for other times in the Phanerozoic. We emphasize that such geochemical results must be carefully collected and understood in the context of their facies and palaeogeographic settings.

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Seasonal variations in natural abundance of ^{15}N in particles sinking to the deep Sargasso Sea

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Temporal variations in ^{15}N natural abundance have been used to study both nitrogen cycling and particle dynamics in the ocean[1]. Changes in the $\delta^{15}N$ $(1{,}000 \times [(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{atmospheric\ N_2} - 1])$ of particulate matter are related, in part, to variations in the input of nitrogen to the euphotic zone that have been observed to regulate the downward transport of particles[2]. Here we report the first long-term data set for seasonal variations in the $\delta^{15}N$ of sinking particulate matter sampled using a sediment trap. These variations co-vary with the magnitude of the particle flux. We propose that the $\delta^{15}N$ of sinking particles is a monitor for NO_3^- flux into the euphotic zone during the formation of these particles on a timescale directly related to the magnitude of the particle flux.

Deuser and Ross[3] have reported seasonal variations in the flux of particulate matter into the deep ocean at a site in the Sargasso Sea. The fluxes of both biogenic and abiogenic components have been correlated with the total mass flux[4-6]. It was suggested that this seasonal variation in particle flux occurred in response to a seasonal cycle in primary production, as has been observed by Menzel and Ryther[7]. The main forcing of the cycle in primary production was the convective mixing in winter of the upper water column and subsequent injection of nutrients into the euphotic zone. However, an internal record in the sediment trap samples of the processes directly related to primary production has not been found.

Considering the role of nitrogen in determining the magnitude of primary production, it seems that variations in the natural abundance of ^{15}N may be related to variations in particle flux. Seasonal changes in $\delta^{15}N$ values for suspended particulate organic nitrogen (PON) in a Gulf Stream warm-core ring have been reported[1]. Low values for the $\delta^{15}N$ of euphotic-zone PON occurred when NO_3^- had been mixed into the euphotic zone. A similar observation was made by Wada and Hattori in the North Pacific Ocean[9].

Measurements of ^{15}N natural abundance were made on particulate matter collected in a sediment trap (described previously[6]) moored at a depth of 3,200 m, ≈45 km south-east of Bermuda in the Sargasso Sea over a 6-yr period. The sediment trap was retrieved and re-deployed approximately once every two months so that the integrated flux over this period was sampled. No preservative was used, however, temperature at this depth is approximately 2.6° C. $\delta^{15}N$ measurements were made only on the <37-μm size fraction, which contains >90% of the organic matter captured. Analyses for $\delta^{15}N$ were made using a Vg Micromass 602E dual-inlet double-collector mass spectrometer. Details of the sample preparation will be reported

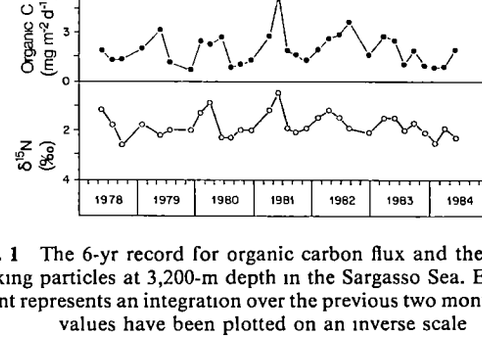


Fig. 1 The 6-yr record for organic carbon flux and the $\delta^{15}N$ of sinking particles at 3,200-m depth in the Sargasso Sea. Each time point represents an integration over the previous two months. $\delta^{15}N$ values have been plotted on an inverse scale

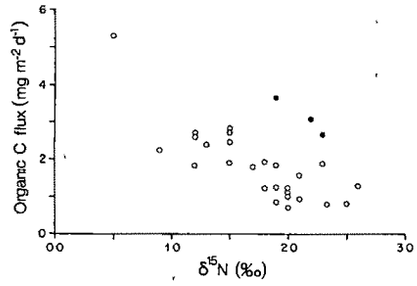


Fig. 2 Organic carbon flux versus $\delta^{15}N$. A linear regression of all points is statistically significant ($r = -0.62$ $n = 31$). Filled circles represent samples collected when the minimum in $\delta^{15}N$ preceded the maximum in organic carbon flux (see text) Excluding these points, $r = -0.80$ ($n = 28$).

elsewhere[10]. Values for $\delta^{15}N$ are relative to the ^{15}N content of atmospheric N_2, with a reproducibility of ±0.2‰.

The time series data for both $\delta^{15}N$ and organic carbon flux are presented in Fig. 1. Note that $\delta^{15}N$ values are plotted on an inverse scale. Every year a maximum in organic carbon flux occurs in late spring to early summer, associated with a minimum in $\delta^{15}N$. In the autumn of 1983, there seems to be a small secondary maximum in organic carbon flux, corresponding to a minimum in $\delta^{15}N$. In 1981 and 1983, the minima in $\delta^{15}N$ co-occur exactly with the maxima in organic carbon flux. In 1979, 1980 and 1982, the minima in $\delta^{15}N$ precede the maxima in organic carbon flux by ~2 months. There is a significant correlation between organic carbon flux and $\delta^{15}N$ ($r = -0.62$, $n = 31$, Fig. 2). Part of the scatter can be attributed to the phase lag in 3 out of 5 years for which there is a complete record.

The annual cycle in particle flux may be considered to have two phases: a high-flux phase during which destratification has mixed nutrients into the euphotic zone and a low-flux phase characterized by undetectable nutrient concentrations in the euphotic zone. During the low-flux phase there is a small turbulent flux of nutrients across the seasonal thermocline, immediately used by phytoplankton. Because almost all the NO_3^- entering the euphotic zone is transformed into particulate nitrogen by this means, the potential for isotope fractionation is small. Assuming that the material collected in the sediment trap has undergone little degradation, the $\delta^{15}N$ of particulate nitrogen sinking during the low-flux phase should be similar to the $\delta^{15}N$ of NO_3^- below the euphotic zone. We do not yet have data for the $\delta^{15}N$ of NO_3^- in this region; nevertheless, the almost-constant $\delta^{15}N$ values for September–December of each year (except 1978) support the above hypothesis.

During the high-flux period, NO_3^-, which has been transported into the euphotic zone, is used by phytoplankton over several months, permitting isotope fractionation between NO_3^- and the particulate matter produced. As NO_3^- is depleted, the $\delta^{15}N$ of NO_3^- in the euphotic zone is expected to increase, possibly reaching high values before depletion occurs. The $\delta^{15}N$ of sinking particulate nitrogen is expected to be low in the first half of the high-flux period and, subsequently, to increase as NO_3^- is depleted from the euphotic zone. Integrated sampling over two months may not have provided sufficient resolution for the observation of this pattern.

Other factors will modify the predicted variation in time of the $\delta^{15}N$ of sinking PON. Wind events occurring throughout the high-flux period pump NO_3^-, relatively low in ^{15}N, into the euphotic zone, thereby mitigating the effect of NO_3^- uptake by phytoplankton. Consequently, the $\delta^{15}N$ of sinking PON tends to remain low during the high-flux period, as was observed for 1981. Variations in the sequence of wind events may be reflected in the time variation of the $\delta^{15}N$ of PON during the high-flux period.

These results have implications for the study of marine geochemical cycles. The seasonal decrease in $\delta^{15}N$ associated with an increase in particle flux represents in many respects a 'natural tracer experiment'. Studying seasonal variations in the $\delta^{15}N$ of other reservoirs of particulate matter (living and detrital) should yield valuable information on the exchanges and transformations that take place among them. Of particular interest is the interaction between the small suspended particles at depth (>150 m) and the downward flux of large particles[8]. Similarly, the mechanisms that package suspended matter in the euphotic zone into larger particles can be studied by examining covariations in $\delta^{15}N$ between these compartments.

If the sediments preserve a $\delta^{15}N$ record, these results suggest a means by which primary production in the ocean of the past can be studied. Such work would have ramifications for present studies of the relationships between world climate and global biological and chemical cycles. Further work in other regions is required to determine the generality of the correlation between particle flux and ^{15}N natural abundance.

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Photoadaptation of high Arctic ice algae

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In aquatic systems, the layer that is suitable for positive net photosynthesis (the euphotic zone) is usually considered to extend from the surface down to the depth of penetration of 1% of the surface irradiance, which corresponds to ~15 μE m^{-2} s^{-1} at solar noon during late summer in open waters in the high Arctic[1]. In polar regions, vernal blooms of epontic algae (unicellular algae associated with the lower interface of sea ice whose photosynthetic characteristics are not well known[2–4]) develop under conditions where they are only rarely or briefly exposed to light levels exceeding 1–2% of that incident at the surface. I report here that epontic algae from the Canadian Arctic show unusually high photosynthetic efficiencies normalized to pigment content, which increase with a decrease in the light levels at which the populations are growing. Photosynthesis was measurable at light intensities well below ambient (0.01% of surface irradiance). In the most shade-adapted populations[5] under deeper snow cover, photosynthesis was optimal at light intensities close to the maximum ambient level, and inhibited at higher intensities. Furthermore, even after almost 2 months of exposure to elevated light levels (≈ 3–4% of surface irradiance), biomass and productivity normalized to biomass were still lowest in algae from an area almost free of snow cover. Taken together, these results indicate that epontic algae from the high Arctic can be considered as an obligate shade flora genetically constrained to very low photon fluxes.

The main study site was ~1 km offshore from Sight Point on the southwestern tip of Resolute Bay, Northwest Territories, Canada (74°40′N, 94°54′W). At this site, annual sea ice was

Table 1 Photosynthetic parameters for natural assemblages of ice algae from four light regimes defined by snow cover

Snow depth (cm)	Maximum ambient light ($\mu E\ m^{-2}\ s^{-1}$)	P_s^B	α	β ($\times 10^{-3}$)	P_m^B	I_m*	I_k*	I_b*	I_c*
20	≈5	0 047 (±0.003)	0.094 (0.018)	0.44 (0.17)	0.046	2 7	0.5	107	NM
11	≈11	0.090 (±0 003)	0 046 (0.003)	0.61 (0 12)	0 084	8.5	1.8	148	NM
3	≈23	0 045 (±0.003)	0 010 (0.001)	0 04 (0.09)	0 044	25 0	4 3	1,125	0.18
0	≈50	0 025 (±0.001)	0.007 (0.001)	0.01 (0.03)	0.025	23.0	3.3	2,500	NM

The initial slope is α; P_s^B is the maximal photosynthetic rate without photoinhibition, β describes the slope at high intensities, P_m^B is the assimilation value; I_m, I_k, I_b and I_c are irradiances (see text). Standard deviations are given in parentheses for three primary (fitted) parameters. Units for P_s^B and P_m^B=mg C (mg chlorophyll a)$^{-1}$ h^{-1}, units for α and β=mg C (mg chlorophyll a)$^{-1}$ h^{-1}($\mu E\ m^{-2}\ s^{-1}$)$^{-1}$. NM, not measurable.
* Units=$\mu E\ m^{-2}\ s^{-1}$ (E=Einstein).

~1.8 m thick and natural snow cover ranged from ~2 cm to 30 cm. Simple manipulations of the surface snow cover allowed considerable control over the light conditions of the epontic algae. Algal material was collected from the bottom few centimetres of ice with a SIPRE corer or an ice chisel. Algal assemblages with different light histories were taken from areas at discrete depths in the snow within a snow drift stabilized by a snow fence or from a 'cleared' patch of ice which was kept relatively free of snow with a snow blower. (The snow fence anchors the snow drift and prevents drift migration (much like sand dunes) during storms of erosion and/or deposition.) A transverse section of the two-sided drift is triangular, with the central apex at 30 cm height sloping down over 5–7 m to a height of 2–4 cm. Such a low-profile snow fence allows for an unusually high degree of control over the light environment within the stabilized snow field. Ambient light levels for the ice algal populations were inversely proportional to the depth of the overlying snow cover (Table 1). Algal samples were collected from 27 to 31 May 1984 after at least 6–7 weeks of growth in their respective light environments.

In all cases the algal assemblages were dominated by pennate diatoms, with *Nitzschia*, *Navicula*, *Amphipora* and *Pleurosigma* the most abundant genera. Although the communities were similar in species composition at each snow depth, standing stocks were markedly different. The species composition and levels of biomass were similar to those found in previous studies of high Arctic sea-ice communities[6–9]

Dense algal suspensions from the lower 2–5 cm of ice were diluted with two parts of surface sea water (water/ice = 2:1), and determinations of photosynthesis/irradiance relationships were done in a 'Photosynthetron'[10] with ≈1.4 μCi $NaH^{14}CO_3$ ml^{-1} (1-h incubations at −1 °C). Irradiance measurements were made with a Licor 2π sensor before each experiment. Fluorimetric estimates[11] of chlorophyll a were used to normalize the rates of photosynthesis. Details of curve-fitting, parameter estimation and statistical considerations of photosynthesis/irradiance relationships have been described previously[1,12,13].

Photosynthesis/irradiance curves (Fig. 1a–d) show that very low ambient light levels are sufficient to saturate carbon fixation in the ice algae studied. The fitted and derived parameters for the curves and environmental data describing the four cases are summarized in Table 1. The populations from different snow depths (representing different light levels) display increasing degrees of photoadaptation that are consistent with their previous light environments. The initial slope, α, (a measure of photosynthetic efficiency) and the so-called adaptation parameter[14], $I_k (\equiv P_m^B/\alpha$, where B is chlorophyll biomass and m denotes maximal photosynthetic rate), both decrease by almost an order of magnitude over the natural range of ambient light levels and snow cover (Table 1). The ice algae from areas with deep snow cover (> 10 cm) exhibited exceptionally high photosynthetic efficiencies (α) at extremely low levels of irradiance (≈0.1–1.0 $\mu E\ m^{-2}\ s^{-1}$ of photosynthetically active radiation, PAR).

Photoinhibition was pronounced only in the assemblages from regions of moderate and heavy snow cover, as shown by their relatively low values of I_b ($\equiv P_s^B/\beta$, where s denotes the light-saturated photosynthetic rate), an index of photoinhibition[12] (Table 1) Values of I_b for Arctic phytoplankton range from ~900 $\mu E\ m^{-2}\ s^{-1}$ for assemblages from depths receiving 1% of the surface irradiance to >5,000 $\mu E\ m^{-2}\ s^{-1}$ for populations from 30–50% light levels[15]. Except for the assemblage from the 'cleared' region, values of optimal irradiance, I_m, for the algal assemblages were very similar to the actual maximum light levels

Table 2 Parameters for photosynthesis–irradiance curves of various types of polar microalgae

Organism	P_s^B	α	β ($\times 10^{-3}$)	P_m^B	I_m	I_k	I_c	Source
Arctic ice algae	0 052 (±0 027)	0 039 (0.041)	0 27 (0.30)	0 050 (0.025)	15 (11)	2.5 (1 7)	≤0 2	Present study (all light levels)
Antarctic ice algae	—	0.023 (0.009)	0.20 (0.11)	0 103 (0.048)	24 (8)	4.8 (1.7)	—	Ref. 19 (two light levels)
Antarctic ice algae	—	—	—	—	≈20	≈10	≈1 0	Ref. 16
Arctic phytoplankton	1.32 (±0.19)	0.011 (0 002)	0 17 (0 21)	1.22 (0.18)	587 (117)	117 (15)	6.1	Ref 1 (50% light level)
Arctic phytoplankton	1.29 (±0.51)	0.008 (0.002)	1.18 (0.79)	0.79 (0.21)	359 (89)	112 (34)	1 4	Ref 1 (1% light level)
Antarctic phytoplankton	—	0.009 (0 007)	—	1.93 (1 35)	117 (33)	31 (9)	—	Ref 24
Antarctic benthic diatoms	—	0.022 (0.005)	Negligible	0 21 (0 03)	—	11 (1)	—	Ref. 23 (one light level)

Mean values (±s d.) are shown. Units have been standardized with the following conversion factors to correspond to those given in Table 1 1 W m^{-2}=4 66 $\mu E\ m^{-2}\ s^{-1}$; 1 klux=17.5 $\mu E\ m^{-2}\ s^{-1}$, 1 ft candle=0 19 $\mu E\ m^{-2}\ s^{-1}$. I_m is optimal irradiance; I_k, the 'light-adaptation' index; I_c, the compensation intensity in units of $\mu E\ m^{-2}\ s^{-1}$ (see Table 1 and text)

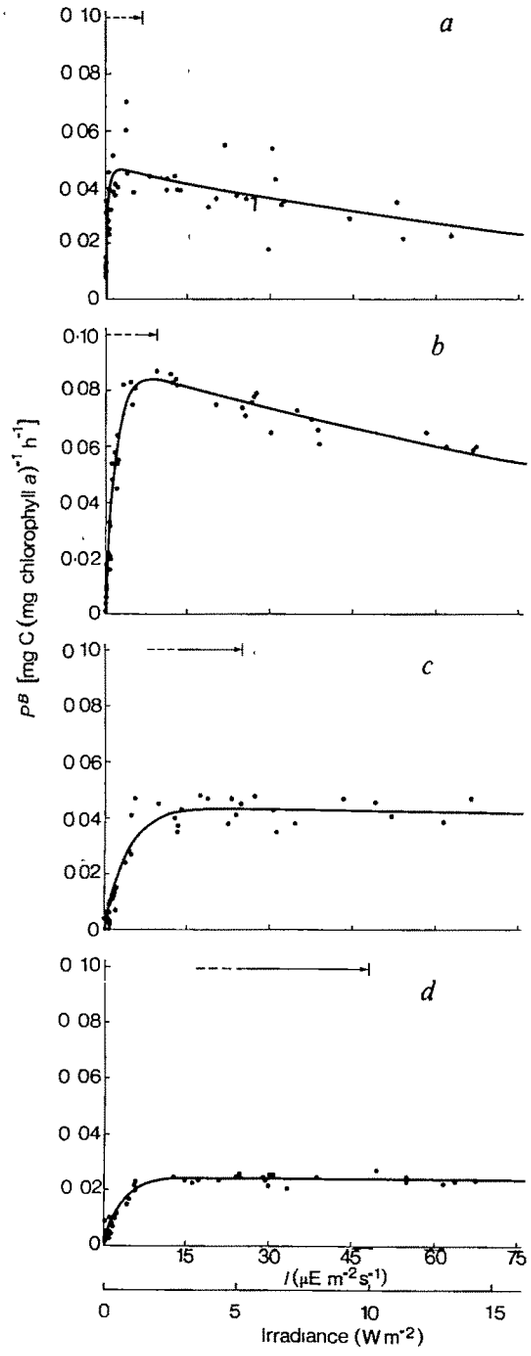


Fig. 1 Photosynthesis/irradiance curves for four populations of ice algae from different snow depths (or light levels). *a–d*, 20, 11, 3 and 0 cm, respectively. Maximum *in situ* light levels measured at solar noon for each nominal snow depth are indicated by the arrows in the upper portion of each panel.

recorded *in situ* at those snow depths (Table 1). Close correspondence of the derived irradiance parameters, I_m and I_k, to their respective light environments indicates advanced states of photoadaptation in these populations.

Maximum photosynthetic rates, P^B_m, measured at all snow depths were low, but they are representative of the lower end of the range of values reported by others studying ice algae[4,6,7,16–19]. Previous work[20] and limited evidence from the present study suggests that assimilation values may vary directly with nutrient status. The values of P^B_m for the algal populations also parallel the 1984 distributional pattern of biomass (chlorophyll) with snow cover, when the lowest levels were found under little or no snow. Maximum apparent growth rates calculated from increases in chlorophyll concentration over a few days and assuming no losses, were ~0.22 and 0.14 doubling per day for the combined data from moderate to heavy snow cover and little or no snow, respectively. That the algae with little or no snow cover were unable to achieve relatively more rapid growth and/or production rates suggests that they represent an obligate shade flora, genotypically adapted to very low photon flux densities[21].

These data indicate extreme degrees of photoadaptation and, in fact, the derived irradiance parameters (I_m, I_k and I_c) describing photosynthetic performance at low light levels are the lowest values of this sort reported for marine microalgae to date [4,21,22]. (The term adaptation is used here 'in the physiological sense, that is, as a physiological adjustment to the surrounding conditions'[5].) Table 2 shows some of the most relevant information on the photosynthetic responses of other natural assemblages of polar marine microalgae[1,16,19,23,24]. Phytoplankton from both poles apparently are adapted to light levels that barely overlap with the maximal levels of irradiance in epontic systems: for example, even the relatively low I_k for Antarctic phytoplankton[24] is slightly above the highest values of ambient light that we measured under slight (2–4 cm) snow cover (Fig. 1*c*). With respect to photosynthetic efficiency of light utilization, the epontic algae under heavy snow cover (Table 1) yield values of α that are 4–10 times higher than those of polar phytoplankton (Table 2). In addition, the compensation intensity, $I_c(\equiv|R^B/\alpha|$, where R^B is the ordinate intercept), for the ice algae was considerably lower than values for arctic phytoplankton (Table 2).

Previous work on epontic algae has emphasized the importance of snow cover in regulating the light environment[2–4,6,7,25]. Removal of snow cover over established populations of ice algae may result in photoinhibition, pigment degradation or habitat destruction (that is, melting of the soft interfacial ice) from excessive absorptive heating[2–4,16,25]. The present experimental results emphasize the importance of photoinhibition, and preliminary calculations on the magnitude of absorptive heating by this intense chlorophyll maximum lend support to the idea of habitat alteration.

The ice algae studied here have adapted to a point where they can flourish in a marginal environment at near freezing temperature with very low levels of light. In terms of overall efficiency of light conversion, their optimal irradiances are well within the dynamic range proposed by Radmer and Kok[26] for the most energetically efficient photosynthesis. Moreover, high Arctic ice algae are apparently not able to function effectively in light conditions exceeding ambient levels, yet in the Antarctic similar ice algae are able to achieve very high levels of biomass approaching theoretical limits (that is, maximum chlorophyll per unit area) in some cases[18].

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Early hominid from Baringo, Kenya

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Palaeontological evidence and comparative molecular studies of modern hominoids suggest that major evolutionary changes occurred in the African Hominoidea between 14 Myr and 4 Myr[1–3]. Unfortunately, very few relevant fossils are known from that time interval[4–13]. Rocks exposed in the fault scarps of the Tugen Hills in the north Kenya Rift Valley west of Lake Baringo are important as the major known outcrops of fossiliferous sediments spanning this otherwise poorly known time period[13,14]. As reported here, a new fossil hominid from Tabarin, within this sequence, establishes the presence of the family in Africa as much as 5 Myr ago. Before this find the oldest well-documented occurrence of hominids was the *Australopithecus afarensis* material from Laetoli, Tanzania[15]. The Tabarin specimen is up to one million years nearer the divergence point between hominids and the African apes. In several features it resembles *A. afarensis* rather than other hominoids.

Tabarin (Baringo Palaeontological Research Project site. BPRP K077; UTM 36N ZR 186844) is a newly discovered fossil locality about 2 km north of Rondonin, north-west of Lake Baringo (Fig. 1). It is an outcrop of the Chemeron Formation[16] *sensu* Martyn (refs 17, 18; see also refs 13, 19). At the base of the local section is a flow of the Kaparaina basalt which separates the sediments from those of the Lukeino Formation beneath. The basalt is overlain by some 20 m of fine- to medium-grained sediments, followed by a tuffaceous unit ~10 m thick. Much of this is ignimbritic and bakes the underlying sediments, but the upper part is a coarse pumice tuff, and pumiceous patches also occur at the base. After successive units comprising 20 m of mainly yellow and orange silts and sands, there is a dark brown coarsely bedded ferruginous conglomerate containing poorly sorted igneous clasts; this is fossiliferous, and on the basis of location and matrix it seems that much of the surface fossil material also derives from this unit. The succession of overlying sediments, some also containing fossils, continues towards the Saimo Fault of the main Tugen Hills range.

Isotopic determinations (K/Ar) on the tuff unit underlying the main fossiliferous horizon give dates of 4.96 ± 0.03 Myr (on anorthoclase) and 5.25 ± 0.04 Myr (on sanidine) (G. Curtis and R. Drake, personal communication). Because the fossiliferous sediments are apparently conformable on the surface of the dated tuff, it is reasonable to suppose that the hominid is not appreciably younger than these isotopic dates. The faunal evidence is entirely compatible with this date and independently places an upper limit on the age of 4.15 Myr.

The fossil fauna from Tabarin is abundant and varied (Table 1), including lacustrine animals and those that would be found along a lake margin and in its catchment area. Particularly crucial for dating purposes is the suid *Nyanzachoerus jaegeri*, and the proboscideans, notably *Anancus kenyensis*. The most

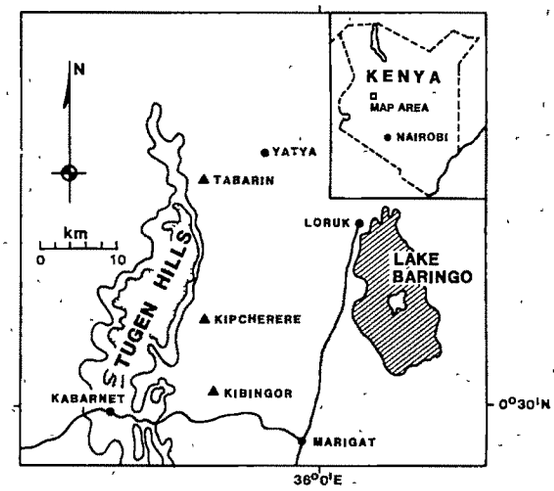


Fig. 1 Map of the area to the west of Lake Baringo, showing the Tugen Hills and the location of Tabarin

specific fossil is *N. jaegeri*, which is found in sediments older than 4.15 Myr (corrected for new constants) in the Mursi Formation at the Omo[20,21], and at Aterir which is dated at about the same age[14,20]. It is not found in the Hadar Formation[20,22]. It is, however, known from the Aramis Member and the Beeraryada beds that underlie the Kalaloo beds of the Sagantole Formation in the Middle Awash Complex[23–25]. These units lie beneath the Cindery Tuff dated at 3.8–4.0 Myr[11,26]. It does not occur in the Laetoli fauna at 3.7 Myr[15,20]. *Anancus kenyensis* is not known later than 4 Myr (despite ref. 27) and is not part of the Laetoli fauna at 3.7 Myr, although it is found in the older Lower Laetoli Beds (J. Harris, personal communication). In Ethiopia it is not found in the Hadar Formation[22]. It is known in the Omo sequence only in the Mursi Formation, dated at older than 4.15 Myr[21,28]. Kalb *et al.* report a single uncollected specimen from the Kalaloo beds of the Sagantole Formation, Middle Awash Complex[25], which is stratigraphically immediately above the Cindery Tuff horizon. The rest of the Tabarin fauna is compatible with this interpretation (see Table 1).

The hominid specimen (KNM-TH 13150; Fig. 2) was found by Kiptalam Cheboi. It was a surface find but its situation and matrix unequivocally link it to the conglomeratic unit and the rest of the fauna.

From other sites in Africa, several of them in the Tugen Hills succession, are a very few other late Miocene and Pliocene hominoid specimens, which cannot reliably be allocated to taxa[4–13]. Most definite Pliocene hominid material comes from Laetoli in Tanzania dated at 3.7 Myr[15], and from the Afar region of Ethiopia ranging in age from ~3.3 Myr to 2.8 Myr[29,30]. The material shows a considerable variation in size and morphology, and has been described as a single species, *Australopithecus afarensis*, with this variation being explained as sexual dimorphism[31]. In addition, the species is postulated to be the ancestral form from which all later hominids were derived[32,33]. Despite, or perhaps because of, its relative abundance, the material has been subject to various other interpretations: for example, some acknowledge that it represents the common ancestor of later hominids yet regard it as nothing more than a subspecies of *Australopithecus africanus*[34]. Others differ primarily in believing that the initial hominid radiation had already taken place. Of these, some imply that the *A. afarensis* material represents only one species but suggest that it was directly ancestral to, or shared a common ancestor with, the robust australopithecines[35]; others are convinced that the variation in the sample indicates that there are two or more sympatric species, the ancestors of both robust australopithecines and later *Homo*[36–39].

The Tabarin specimen closely resembles relevant specimens from the smaller end of the size range of *A. afarensis*, for example

Table 1 Fossil fauna from Tabarin and Sagatia

	Tabarin	Sagatia
Mollusca		
Bivalvia	×	
Pisces		
Siluriformes		
cf. *Clarias*	×	×
Reptilia		
Chelonia		
Testudinidae		×
Trionychidae	×	×
Pelomedusidae	×	×
Crocodilia	×	×
Mammalia		
Primates		
Cercopithecidae		
Papionini		×
Hominidae		
cf. *Australopithecus afarensis*	×	
Carnivora		
Hyaenidae		×
Felidae		
cf. *Panthera*		×
Small species		×
Mustelidae		
Enhydriodon cf. *campani*		×
Another species; family indeterminate		×
Proboscidea		
Gomphotheriidae		
Anancus kenyensis	×	×
Elephantidae		
cf. *Primelephas gomphotheroides*	×	×
Deinotheriidae		
Deinotherium bozasi	×	×
Perissodactyla		
Equidae		
Hipparion	×	×
Rhinocerotidae	×	×
Artiodactyla		
Suidae		
Nyanzachoerus jaegeri	×	×
Hippopotamidae	×	×
Bovidae		
Several species	×	×
Rodentia		
Hystricidae		
Hystrix	×	

Fossil fauna from Tabarin and additional species found at Sagatia, a site 1.5 km to the south at the same stratigraphical level as Tabarin. There are also well-preserved leaf impressions and coprolites. Tabarin has an archaic elephant, probably the same species as that found at other Chemeron Formation sites, but which is not known from Hadar or Laetoli, and which Maglio[44] considers to be typical of ~5 Myr. The papionine is consistent with an early Pliocene age (M. G. Leakey, personal communication). The *Enhydriodon* resembles a form found at Kanapoi. The hyaenid resembles one from Kanapoi which also occurs at Kaiso (J. Barry, personal communication).

A.L. 288-1i and A.L. 128. It is smaller than the type specimen (LH-4) and the larger material from Hadar; it is also smaller than the Lothagam mandible (KNM LT-329). In detail it has several characters which typify *A. afarensis* rather than other hominoids: on the mandibular corpus these include a large buccal eminence and hollowing of the lateral contour. The teeth have a strong buccal flare. The differential proportions of mesial and buccal roots produce a serrate pattern, and there is a bifid mesial root apex on M_3 (refs 33, 40–43).

However, many of the parts diagnostic of *A. afarensis* are not available on this specimen, and while time and geography should not be used taxonomically, it is true that increasing distance in time or space increases the probability that a specimen, particularly one preserving a small number of morphological traits, represents something significantly different from other known forms. As the Tabarin specimen is up to a million years older than the oldest specimens attributed to *A. afarensis*, increasing the record of the Hominidae as a whole back in time by 25%,

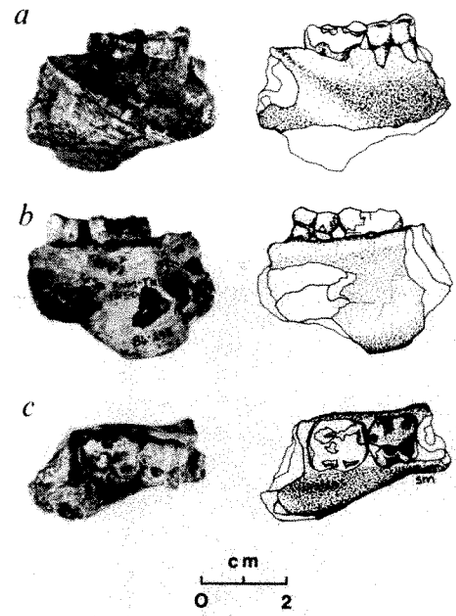


Fig. 2 The hominid specimen from Tabarin (KNM-TH 13150): buccal (*a*), lingual (*b*) and occlusal (*c*) views. The specimen consists of a fragment of right mandibular corpus with M_1 and M_2, and some of the alveolus and a root fragment of P_4. The mesial break extends from the P_4 alveolus diagonally and distally to below M_2. Part of the buccal surface of the corpus is lost ventrally. This damaged area links the mesial to the distal fracture, which extends distal to M_2. Weathering has removed a uniform and extremely thin layer of surface bone from much of the lingual surface, although the contours are preserved. The whole of the cortex has been lost over a small area of the lingual surface extending from the mesial fracture to below mid-M_2. The fractured margins, including the broken edge of the P_4 root, are worn. There is no obvious postmortem distortion. The basal surface of the corpus is present distal to M_2, where the corpus is 26.9 mm in height perpendicular to the alveolar margin. The root of the ramus is present, with the oblique line extending at ~45° to the alveolar margin into a flared buccal prominence. The prominence and extra-molar sulcus lose distinction below M_1, where the buccal face becomes slightly concave mesially. From the alveolus P_4 can be seen to have a bifurcated root, the mesial being more buccally placed than the distal. M_1 is intact but for the loss of the mesial margin of the protoconid, and measures 11.1 mm mesiodistally and 10.4 mm buccolingually. Wear has exposed small dentine islands on metaconid and hypoconulid. Larger exposures on protoconid and hypoconid are almost united. The lingual border is steep, with a shallow lingual groove. There is a buccal groove on the more inclined and flared buccal surface. Most of the occlusal enamel of M_2 has been lost through postmortem damage, as has that on the buccal surface and on the lingual face of the entoconid. A small section of the contact facet for M_3 remains distally. The tooth measures 13.1 mm mesiodistally, and 11.4 mm buccolingually. The mesiodistal dimension is slightly reduced by interproximal wear with M_1, where the contact facet measures 4.5 mm buccolingually. As in M_1, the lingual face is steep with a lingual groove; the buccal face slopes and has a buccal groove. Mesial roots on both M_1 and M_2 are broader than distal roots. Although weathering has removed the surface of the M_3 alveolus, the form of the remaining bone suggests that the mesial root was directed somewhat posteriorly and had a bifid apex.

its attribution may change when further material is available. More detailed discussions of the relationship of the Tabarin hominid to other late Miocene and Pliocene hominoids will appear elsewhere.

The specimen is important in that it resembles later known hominids from a time estimated by comparative molecular studies to be very close to the divergence point with African apes[1–3]. Additional material from Tabarin and other new sites in the area will be critical to understanding the details of the early phase of hominid evolution and discriminating between

the various hypotheses that have arisen around specimens described as *A. afarensis*. Further material may show the same large range of variation as these later specimens or it may provide more explicit evidence of an early radiation in the Hominidae between 5 Myr and 3.7 Myr.

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Chromosome Y-specific DNA in related human XX males

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Human 'XX males' are sterile males whose chromosomes seem to be those of a normal female. About 1 in 20,000 males has a 46, XX karyotype, and most cases are sporadic, that is, they are without familial clustering[1]. It has long been argued that maleness in XX males may result from the undetected presence of a small, testis-determining fragment of the Y chromosome[2], and there is strong evidence for this in sporadically occurring XX males[3–5]. Indeed, the genomes of three of four sporadic XX males tested were found to contain certain Y-specific DNA sequences[6]. A pedigree in which three XX males occur has been interpreted as being consistent with autosomal recessive inheritance of maleness[7,8], and it has been argued that the basis of XX maleness in this family is fundamentally different from that in the sporadic cases[9]. However, we report here that these related XX males, like the sporadic cases, contain portions of the Y chromosome. The portion of the Y chromosome present in one of the three XX males differs from that present in the other two.

In one of the few families reported to contain two or more XX males, the pattern of inheritance suggests a dominant male-determining locus segregating according to an autosomal mode[10]. Affected siblings[11] and affected identical twins[12] have also been described. The family reported here has been described previously[7,8]. Briefly, two XX males are second cousins, and they are distantly related to a third XX male via a common male ancestor (Fig. 1*a*). The mothers of two of these XX males are related in several ways. It has been argued that an autosomal[8] or 'pseudoautosomal'[9] recessive male-determining gene is segregating in this family.

In the present study, DNAs from three related XX males and their relatives were tested for the presence of certain Y-specific sequences using probes derived from the human Y chromosome. Some of these Y-specific DNA sequences were previously found to be present in, and others absent from, the genomes of sporadic XX males[6]. We have reported previously the absence in these three related XX males of the Y-specific 15-kilobase (kb) *Taq*I fragment at the X-Y homologous locus *DXYS1* (ref. 13). DNAs prepared from cultured fibroblasts or from peripheral blood leukocytes were digested with the restriction endonuclease *Taq*I and gel transfers[14] of the resulting fragments were hybridized with radiolabelled DNA probes detecting Y-specific *Taq*I fragments. Table 1 summarizes the results of these tests and Fig. 1*b*, *c* shows autoradiograms of some of the original data. All three XX males carry certain Y-specific sequences. The two XX males who are second cousins, X-1 and X-2, seem to carry similar if not identical portions of the Y chromosome; that is, their genomes contain the two Y-specific *Taq*I fragments detected by probe 47c, but they are negative for all other Y-specific sequences tested. (In this respect, they resemble the sporadic XX male '3' previously reported by Guellaen *et al.*[6].) X-3, the distantly related XX male, is positive not only for the two Y-specific *Taq*I fragments detected by 47c, but also for some of the Y-specific *Taq*I fragments detected by probes 50f2, 52d and 118. (The patterns observed were identical with those seen in sporadic XX male '4' reported by Guellaen *et al.*[6].) None of the three XX males is positive for all of the Y-specific sequences. We conclude that each carries only a part of the Y chromosome.

By this test, there is no evidence of abnormality at the DNA sequence level in any of the relatives. The fathers of the XX males and a brother, X-4, are positive for all of the Y-specific sequences tested, while the mothers and a sister, X-5, are negative for all of the Y-specific sequences. Moreover, the intensities

of the Y-specific bands (relative to autosomal or X-specific bands) in the fathers are similar to those seen in normal unrelated males (data not shown). Thus, as the fathers all have a normal male karyotype, it is likely that the Y-specific DNA sequences they display are present in normal copy number and only on the Y chromosome. As in *Sxr* mice[15,16], 'sex reversal' in these humans may be the result of an X; Y translocation. However, unlike *Sxr*, duplication of the testis-determining portion of the Y chromosome in carrier males is apparently not involved.

We conclude that these three related XX males are males because, like the three sporadic XX males reported by Guellaen *et al.*[6], they carry a testis-determining fragment of the Y chromosome. Note that the patterns of Y-specific fragments shown by these related XX males are among the patterns shown by sporadic XX males. In the case of each of the three XX males, a new mutation must have occurred in the father, during or before the meiotic divisions. That mutation apparently involved breaking the Y chromosome and perhaps translocating one of the resulting pieces to the X chromosome. We detected no Y-specific sequences in any of the three mothers. (Guellaen *et al.*[6] previously reported the same finding in the mother of one sporadic XX male.) These results seem incompatible with a maternal contribution to XX maleness in this family; such a contribution had been suggested by weak positivity for the H-Y antigen in the mothers[8]. If there is an inherited defect predisposing to XX maleness in this family, it is operating in the fathers, in whom Y chromosomes are broken.

The two closely related XX males (X-1 and X-2) carry a similar if not identical portion of the Y chromosome, and they are related through males only. Thus, it is possible that the Y chromosome common to their fathers is susceptible to the sort of gross mutation described above, perhaps at a particular site on the Y chromosome. Indeed, a Y-linked mutation predisposes to reciprocal X; Y translocation in the mouse[17]. If a Y-linked mutation is operating in the fathers of the two closely related XX males, the occurrence of XX maleness in a distant relative (X-3), related through a lineage that includes females as well as males, may be merely coincidental. This is consistent with the finding that X-3 carries a different, albeit overlapping, fragment of the Y chromosome.

Using X-linked restriction fragment length polymorphisms, we have shown previously that most, if not all, XX males, including the three studied here, inherit one X chromosome from their father and one from their mother[13]. However, like many XX males, X-3 does not express his father's allele for the dominant, X-linked marker *Xg*. (*Xg* segregation is uninformative in the other two XX males.) As reported above, X-3 has inherited part of his father's Y chromosome. This is reminiscent

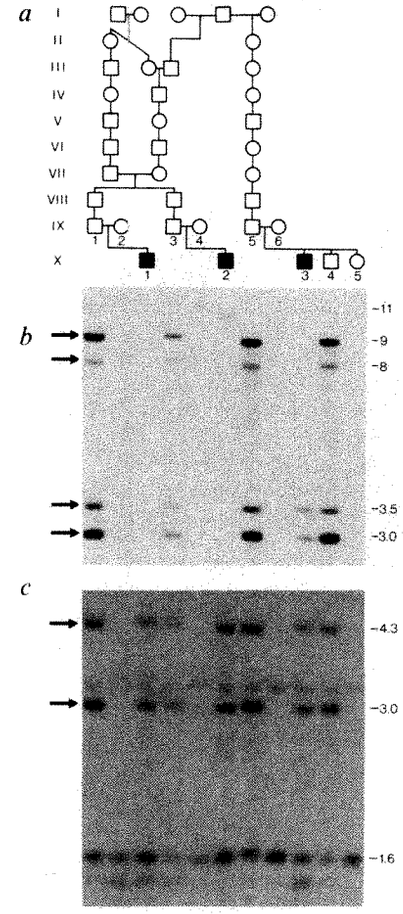


Fig. 1 *a*, Simplified paternal pedigree of XX males X-1, X-2 and X-3 (black squares) (from ref. 8). *b*, *c*, Hybridization of *Taq*I-digested DNAs from the XX males and their relatives with 50f2 (*b*) and 47c (*c*). Each lane corresponds to the individual immediately above that lane in the pedigree shown in *a*. DNA samples were digested with *Taq*I, electrophoresed on agarose gels, transferred[14] to Pall Biodyne (1.2-μm pores) nylon paper and hybridized with radiolabelled probes largely as described previously[13]. However, following transfer, the air-dried papers (DNA-coated side up) were exposed to germicidal (short-wave) ultraviolet light at an intensity of 400 μW cm^{-2} for 1 min; they were not baked. The arrows on the left indicate the Y-specific *Taq*I fragments detected. The sizes of restriction fragments (in kilobase pairs) are shown on the right.

Table 1 Y-specific DNA sequences in XX males and their relatives

	Probes							
Individual	47c	50f2	52d	118	27	49f	12f3	pDP34*
Normal male	2	4	3	8	1	3	1	1
Normal female	0	0	0	0	0	0	0	0
IX-1 (GM2672) father	2	4	3	ND	ND	ND	ND	1
IX-2 (GM2671) mother	0	0	0	ND	ND	ND	ND	0
X-1 (GM2626) XX male	2	0	0	0	0	0	0	0
IX-3 (GM2624) father	2	4	3	ND	ND	ND	ND	1
IX-4 (GM2625) mother	0	0	0	ND	ND	ND	ND	0
X-2 (GM2670) XX male	2	0	0	0	0	0	0	0
IX-5 (LGL118) father	2	4	3	ND	ND	ND	ND	1
IX-6 (LGL117) mother	0	0	0	ND	ND	ND	ND	0
X-3 (LGL115) XX male	2	2	2	4	0	0	0	0
X-4 (LGL116) brother	2	4	3	ND	ND	ND	ND	1
X-5 (LGL119) sister	0	0	0	ND	ND	ND	ND	0

Genomic DNAs from normal unrelated males and females and from the indicated members of the pedigree in Fig. 1*a* were digested with *Taq*I, blotted and hybridized with probes detecting Y-specific sequences. Methods were as described for Fig. 1*b*, *c*. The number of male-specific *Taq*I fragments detected by each probe are shown. ND, not done.

* The data for probe pDP34 (*DXYS1*) are from ref. 13.

of our finding of a sporadic XX male who expresses his father's allele for 12E7, a Y-linked marker, but fails to express his father's *Xg* allele[5]. Both cases can be explained by an interchange of a portion of the Y chromosome with a portion of the paternal X chromosome[2]. However, such an interchange cannot easily account for XX males X-3's apparent failure to express his father's allele for the dominant, X-linked marker *Xm* (ref. 1), which maps to the long arm of the X chromosome[18]; *Xg* maps to the short arm. It is possible that the Xm typing of this family was in error. Unfortunately, as Xm antiserum is no longer available, the typing cannot be repeated.

Taken together, our findings and those of Guellaen *et al.*[6] suggest that, though variable in size, the segment of the Y chromosome present is similar in at least some familial and sporadic XX males. It is likely that that segment of the Y chromosome carries the genetic information which is male-determining.

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X-linkage of steroid sulphatase in the mouse is evidence for a functional Y-linked allele

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In the human there is an X-linked gene affecting steroid sulphatase (STS) activity which, when deficient, is associated with X-linked congenital ichthyosis[1,2]. The gene (*STS*) is located on the distal tip of the short arm[3–6] and is only partially inactivated when it is on the inactive X-chromosome[7–10]. In the mouse, the genetics of *STS* are not clear; the results of one study using XX:X0 oocyte comparisons indicated X-linkage[11], but three other studies using STS variants have produced segregation data compatible with autosomal linkage of murine *STS*[12–14]. Here we present the results of STS assays of crosses of deficient C3H/An male mice[12] to normal X0 animals which demonstrate X-linkage of *STS* in the mouse and indirectly indicate the existence of a functional *STS* allele on the Y-chromosome which undergoes obligatory recombination during meiosis with the X-linked allele.

Table 1 Results of crosses of STS-deficient C3H/An with normal mice

Cross		No. of offspring			
		Females		Males	
Female	Male	STS$^+$	STS$^-$	STS$^+$	STS$^-$
*C3H/An (STS$^-$)	BALB/c (STS$^+$)	13	0	17	0
*BALB/c (STS$^+$)	C3H/An (STS$^-$)	6	0	8	0
*C3H/An (STS$^-$)	Feral mouse (STS$^+$)	3	0	5	0
*C3H/An (STS$^-$)	C57B (STS$^+$)	Not tested		7	0
*(C3H/An × C57B) F_1 (STS$^+$)	C3H/An (STS$^-$)	10	13	13	15
*(C3H/An × C57B) F_1 (STS$^+$)	C57B (STS$^+$)	16	0	17	0
C3H/An (STS$^-$)	(C3H/An × BALB/c) F_1 (STS$^+$)	2	6	2	4
C3H/An (STS$^-$)	(BALB/c × C3H/An) F_1 (STS$^+$)	1	6	2	1

STS assays were carried out on sonicated spleen extracts using ^{3}H-dehydroepiandrosterone sulphate as substrate, according to Shapiro *et al.*[32]. Pure-breeding STS$^+$ mice have STS activity of >250 pmol per h per mg protein and pure-breeding STS$^-$ mice (C3H/An) have <7 pmol per h per mg protein. The STS activity of STS$^+$ hybrid mice varied from 70 to 160 pmol per h per mg protein.
* Unpublished data from Balazs *et al.*[12].

When C3H/An STS-deficient mice are crossed with STS normal animals in various combinations, the results seem compatible with simple autosomal inheritance of STS deficiency (Table 1). However, one litter from a cross of an STS-deficient female with a supposed pure-breeding STS normal male yielded one STS-deficient female. If the exceptional female was X0, it would suggest that STS deficiency follows an unusual form of sex-linked inheritance. Unfortunately, materials were not available for cytological analysis of the exceptional animal nor could the male parent be tested for possible heterozygosity.

We therefore instituted crosses of C3H/An STS-deficient males with STS normal X0 mice marked with the tabby (*Ta*) gene. The X-linked tabby marker permits separation of mice carrying *Ta*, in either the hemizygous or homozygous condition, from heterozygotes and wild-type mice by its characteristic coat markings. From these crosses, three classes of offspring are expected with regard to tabby and sex: *Ta*/+♀♀, +/0♀♀ and *Ta*/Y♂♂. Table 2 gives the results of these crosses with respect to tabby, sex and STS level. If the STS variant we are studying is autosomal, there should be no differences in STS levels between the three classes of offspring. On the other hand, if the STS variant lies on the X-chromosome, then the X0 progeny should be STS-deficient, in contrast to the normal or intermediate values for the *Ta*/+ and *Ta*/Y animals. In fact, the X0 animals show STS values not significantly different from their deficient male parent. The absence of STS-deficient animals among 15 *Ta*/Y and *Ta*/+ siblings argues against the possibility that the X0 parents are heterozygous for STS deficiency at an autosomal locus. These results demonstrate X-linkage of the C3H/An STS deficiency.

The studies reported here (Table 1) and elsewhere[12] using the C3H/An STS-deficient mouse strain in various crosses yield results compatible with simple autosomal inheritance of the deficient *STS* gene in the C3H/An strain. However, when deficient C3H/An males are crossed to STS normal X0 mice, the segregation pattern observed in the offspring demonstrates X-linkage of the *STS* deficient gene (Table 2). These apparently contradictory results are best explained by postulating a functional Y-linked *STS* gene that undergoes obligatory recombination with its X-linked allele. As both the C3H/An and BALB/c Y chromosomes exhibit recombination with the X-linked *STS*

Table 2 Levels of STS activity resulting from crosses of STS^+ X0 to STS^- XY mice

	STS activity (pmol per h per mg protein)				
	Parents		Offspring		
	STS^+	STS^{-*}			
Sex chromosomes:	X0	XY	X0†	XX	XY
Tabby genotype:	*Ta*/0	+/Y	+/0	*Ta*/+	*Ta*/Y
	198	25	27	310	189
	119	42	23	260	188
	217	52	36	162	236
	281		62	274	176
	198		16	114	234
	162		10	244	140
	134				131
					313
					258
Mean STS value	187	40	29	227	207

Each STS value represents an individual mouse. Tabby X0 mice (F_1 mice from C57BL/6J×CBA/Ca crosses) were obtained from Jackson Laboratories (Bar Harbor, Maine). The STS assay is a modification of the technique of McNaught and France[33]. Ear punches of 2 mm diameter were placed in 100 μl of buffer (0.1 M Tris-HCl *p*H 7.2) and frozen on dry ice. The samples were then thawed and sonicated on ice for 5 s at 250 W and placed on ice. This cycle was repeated four more times. 'Cold' oestrone sulphate (4 ng) and ^{3}H-oestrone sulphate (2 μCi, specific activity 52.5 Ci mmol^{-1}; NEN) were added to 50 μl of the extract on ice. A zero-time sample of 26 μl of the assay mixture was added to 25 μl of 0.1 M NaOH. The rest of the reaction mixture was incubated at 37 °C for 40 min, then the reaction was stopped by adding 25 μl of 0.1 M NaOH. Petroleum ether (350 μl) was added to each sample and the mixtures vortexed for 30 s. The samples were placed on dry ice and an aliquot (100 μl) of the organic phase was removed and added directly to 5 ml of toluene/Omnifluor for radioisotope counting. Protein assays were done according to the Lowry method[34].

* The values reported here are for the three STS^- males used in the above crosses. All STS^- mice (C3H/An) have low STS levels which do not overlap with those of either the pure-breeding STS^+ strain or the F_1 animals. Duplicate assays varied by <10%.

† One X0 offspring was examined cytologically and the modal chromosome count was found to be 39.

Y-linked *STS* gene. However, another closely linked gene, gene (Table 1, crosses shown in lines 7 and 8) the recombinational property postulated here is not a unique characteristic of the C3H/An strain.

Ultrastructural studies of crossing-over in the XY pair of various mammals, including the mouse[15], suggest that there is partial synapsis of the X and Y, followed by chiasma formation. A model including a single obligatory crossover between the X and Y[16–18] would predict that genes distal to the crossover will be transmitted to both male and female offspring and appear to be autosomally inherited. The *Sxr* ('sex-reversed') mouse[19] is a precedent for this possibility since the *Sxr* rearrangement is passed equally to male and female offspring[20,21]. *Sxr* has been mapped to the distal tip of the X and Y coincident with the proposed X-Y pairing region of the mouse. Accordingly, the *STS* locus should map to the same region. It should be pointed out that our data on the *STS* locus in the mouse directly contradict the theory of Ashley[22], who suggests that synapsis between the X and Y chromosomes is non-homologous and that regular crossing-over does not occur between the X and Y chromosomes.

In man, the X-linked *STS* locus maps to the distal tip of the short arm of the X, which is the presumed pairing region of the human sex chromosomes[3–6]. The X-linked recessive pattern of inheritance of human STS deficiency precludes a functional *MIC2*, which codes for the red blood cell antigen 12E7, does have an expressible allele on the Y chromosome in human[23]. Obligatory recombination proximal to this locus appears to be precluded by results from studies of a polymorphism of 12E7 which shows a complex sex-limited expression of variation in 12E7 levels[24]. Therefore, if there is an obligatory crossover between the human X and Y, it would have to be distal to 12E7 and *STS*. These observations do not exclude the possibility of occasional exchange between the X and Y in human, which has been shown to occur in the origin of some XX males[25–30].

With functional alleles on the X and the Y chromosomes in the mouse, dosage compensation is not required to effect equality of expression of X-linked alleles in females and males[31]. In fact, the similarity of STS values in X0 STS^+ parents and XX STS^+ daughters seems to exclude the *STS* locus from X-inactivation. We know from coat colour markings that the tabby gene in the XX daughters is being randomly inactivated; if the *STS* locus followed the same pattern, then the STS values of the XX daughters would be approximately half those of the X0 mothers (Table 2). Definitive evidence for or against X-inactivation of the *STS* locus will require some form of cloning study in heterozygotes.

Thus, we have shown by crossing STS normal X0 mice with C3H/An STS-deficient males, that the STS deficiency of the C3H/An mice is controlled by an X-linked gene. This result, in conjunction with previous data suggesting autosomal inheritance of the C3H deficiency, implies the existence of an *STS* allele on the Y chromosome that undergoes obligatory recombination.

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Does interocular transfer occur in visual navigation by ants?

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If an animal that learns to respond to a visual stimulus presented to only one eye can continue to respond accurately when the stimulus is presented to the other eye, it is said to exhibit interocular transfer (IOT). IOT has been studied extensively in many groups of vertebrates[1–11], but there is only one conclusive report of IOT in an invertebrate (*Octopus*)[12]. Here we demonstrate that an insect, the desert ant *Cataglyphis*, exhibits IOT when it navigates by the pattern of polarized light in the sky, but shows no IOT when it uses landmarks for orientation. This marked difference in behavioural performance may help to elucidate the neural strategies adopted by insects when using celestial (polarization) and terrestrial cues for navigation.

Insects possess multi-faceted compound eyes which endow them with full panoramic vision[13,14]. The present experiments used the long-legged Saharan desert ant, *Cataglyphis fortis*, in which the visual fields of the two eyes cover 93% of the unit sphere[15]. As the remaining 7% of 'dead space' is confined completely to parts of the unit sphere that lie below the horizon, desert ants can view simultaneously all parts of the celestial hemisphere as well as the entire skyline extending above the horizon. Ants use visual skymarks such as the Sun and the extensive pattern of polarization as directional cues[16]. A desert ant can easily be trained to find an artificial food source established, say, 20 m to the north of its underground colony[17]. Having arrived there, the ant will grasp the piece of bait (say, a small cube of cheese) with its mandibles and run home along a straight path, selecting the proper home direction (in this case south) even if the insect is displaced to unknown territory and prevented from viewing either landmarks or the Sun; the celestial pattern of polarization suffices as a compass cue[17].

To test IOT, we trained ants after covering one of the compound eyes (and its three ocelli) with a light-tight cap. Thus, these ants had to learn their home direction using only one eye (the 'trained eye'). After training was completed (Fig. 1, test A), we removed the cap from the 'naive eye' and occluded the other (trained) eye. The eye caps could be removed carefully using fine forceps, without damaging the corneal surfaces of the eyes. In scanning micrographs, eyes from which caps had been removed could not be distinguished from untreated eyes. Ants with the eye caps exchanged oriented in the homeward direction just as the controls did using their trained eyes (Fig. 1, test B1).

In these tests, the ants used both eyes to navigate until we started to train them to an artificial food source; only then was one eye occluded. In the following experiment, one eye was covered before the ant could acquire any visual information. This was done by rearing the ants in a laboratory colony and providing them with eye caps shortly after they had hatched from their pupal cases. The monocular ants were then transferred to a natural colony in the desert, where they remained underground until they began their foraging lives about 28 days later[18]. These animals were still able to perform IOT when the trained eye was covered and the naive eye opened for the first time in the ant's life, using only the pattern of polarized light in the sky (Fig. 1, test B2).

In contrast, if the ants used landmarks to pinpoint the position of their nest, IOT did not occur. This was demonstrated by marking the position of the nest by a set of artificial landmarks, consisting of three black cylinders (11° high and 6° wide), which were positioned at the corners of an equilateral triangle centred on the nest entrance (Fig. 2*a*). Ants were tested which had already returned from the food source to the nest; such ants have reset their path integration (celestial navigation) system to zero and rely exclusively on landmarks for finally locating the

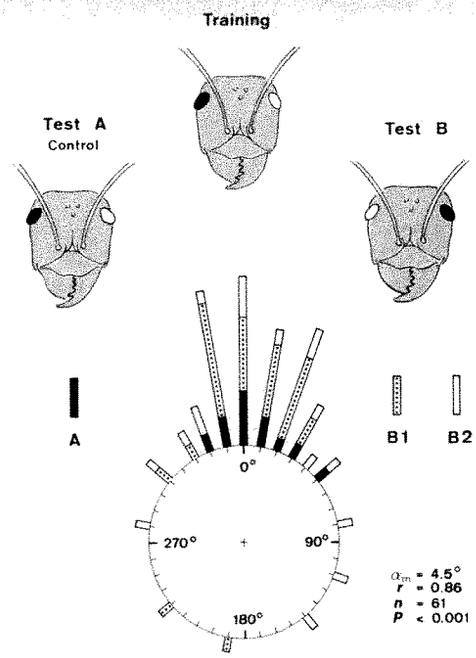


Fig. 1 Ants exhibit interocular transfer of visual information deduced from the pattern of polarized light in the sky. The bars indicate walking directions of monocular ants which can see only the pattern of polarized light in the sky. Landmarks and the Sun were occluded[17]. One eye was covered with a thin coat of fast-drying paint (Testors Matte Enamel 1149 Black) applied while the ant was immobilized, its head viewed through a dissecting microscope. 0°, Homeward direction. A, Control experiment: trained eye open; $n = 11$. B, Tests for interocular transfer: naive eye open. The naive eye was occluded while training the ant to the 0° compass course (B1; $n = 32$) or during the entire lifetime of the ant before the experiment (B2; $n = 18$). α_m and r are the direction and length of mean orientation vector, respectively[25]. If one eye is occluded, ants, as well as other arthropods, deviate by an error angle towards the seeing side. In *C. fortis*, this error angle, ε, was determined before the experiment ($\varepsilon = 37.2 \pm 4.3°$ (±s.e.), $n = 50$) and then used to correct the ants' compass bearings. In all tests, homeward orientation was highly significant ($P < 0.001$; Rayleigh test[25]).

position of their nest[16]. The distance between the nest entrance and each cylinder was 2 m.

Monocular ants learned the configuration of landmarks while performing four to six orientation runs which led them to a distance of up to 3 m from the nest; only then did they begin foraging up to distances of more than 50 m. In the critical test, ants and landmarks were transferred to a nearby plain which had been provided with a grid of white lines to facilitate the recording of the ant's walking trajectories[17]. When the ants captured directly at the nest entrance were released anywhere near the triangle of cylinders, they immediately headed for the centre of the triangle and consistently searched there by walking in narrow loops around the point where they expected the nest to be (Fig. 2*b*, *c*). In contrast, when the trained eye was occluded and the naive eye opened, the ant was unable to locate the centre of the triangle to which it had been trained, but searched around the point where it was released (Fig. 2*d*). However, if the eye cap was immediately exchanged, leaving the trained eye open and the naive eye occluded, the ant again oriented perfectly by searching the centre of the triangle (Fig. 2*b*, *c*). Even when eye caps were changed from eye to eye several times, the same result was always obtained; the ant oriented perfectly when using the trained eye, but was disoriented completely when using the naive eye.

It may generally be assumed that IOT for some function occurs when the locus of that function is downstream from the place where information from the two eyes is combined. Lack of IOT would indicate some contribution from monocular mechanisms peripheral to binocular convergence. The landmark experiments indicate that the way in which the image of the

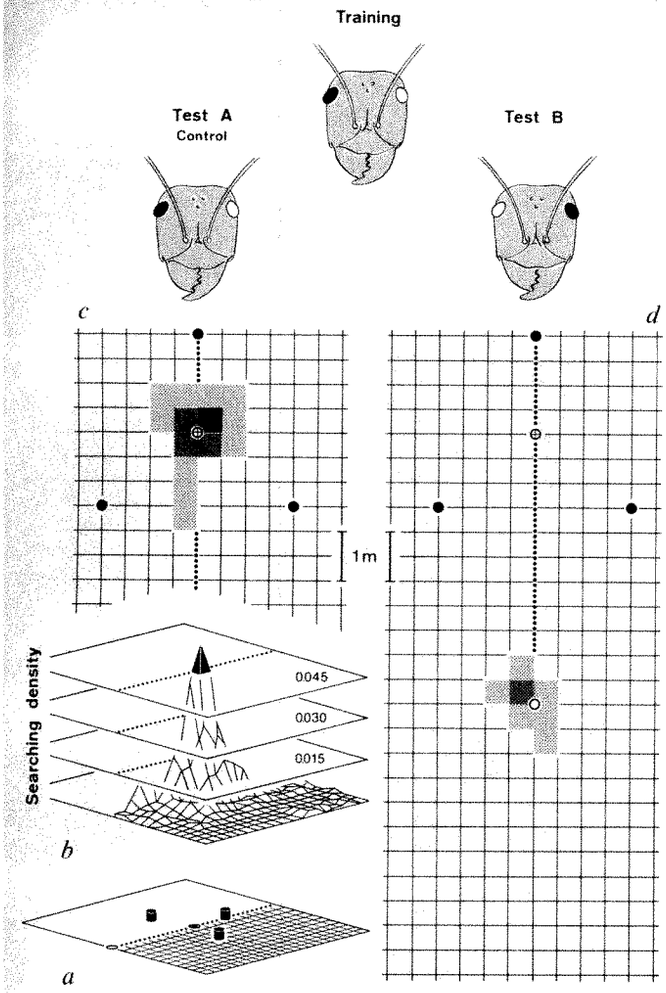


Fig. 2 Ants do not exhibit interocular transfer of visual information deduced from landmarks. *a*, Landmark configuration. Monocular ants were trained to the centre ⊕ of the triangle marked by three black cylinders (11° high, 6° wide as seen from the centre of the triangle). In the tests following the training procedure, the ants were released outside the triangle (at ○). *b*, *c*, Test A, control experiment; trained eye open. Searching densities (*SD*) are shown in three-dimensional (*b*) and two-dimensional (*c*) plots. They were determined as follows. In the field, the ants' paths were recorded at reduced scale by using a grid of white lines painted on the hard desert ground. Later, for each of the sampling squares, the total pathlengths of all ants tested were determined by using an electronic digitizer, and the relative pathlengths per sampling square (searching densities) were computed. In *c*, *d*, the shaded areas are those that were searched by the ants most extensively: $SD > 0.045$, $0.045 > SD > 0.03$, $0.03 > SD > 0.015$. *d*, Test B, test for interocular transfer; naive eye open. The ants no longer recognized the configuration of landmarks. They searched about the point of release, but not in the centre of the triangle. In total, 30 ants were tested for 5 min each.

skyline is stored within the ant's visual system depends crucially on the information gained by each eye. A monocular ant, while turning around and scanning the entire skyline with one of its eyes, does not use the landmark information it obtains to form a central 'binocular' image that could be read and used by the other eye as well. This is consistent with the recent view that insects use internally stored images of landmarks to guide them home[19,20]. In this context, it is interesting that birds which exhibit IOT when discriminating between shapes and colours[7-9] also completely lack IOT when visual spatial memories are involved in the task to be solved, for example, when food sources must be located on the basis of landmark information[21].

On the other hand, a monocular ant can read a learned compass direction from the pattern of polarized light in the sky irrespective of whether it uses the trained eye or the naive eye. In the latter case, this might be explained very simply. In bees, and probably also in ants, the ability to use polarized sky patterns as a compass is restricted to a small specialized part of the retina located at the uppermost dorsal margin of the insect's eye[22]. According to our current understanding of how this specialized area is used as a celestial compass[23,24], the compass information read from the sky by one eye is identical to that read by the other eye. It seems that one eye can substitute for the other when the areas of the two eyes involved convey identical messages to the nervous system. In these experiments, IOT has been inferred from unilateral stimulation by light[26], but as the contralateral eye was not occluded, one cannot dismiss the possibility that the response was due to stimulation of the rather large binocular field of view or to stray light artefacts.

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Voltage-dependent potassium currents in cultured astrocytes

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Astrocytes are a major cell type in the mammalian central nervous system (CNS), yet their functions remain uncertain. There are two principal classes of these glial cells—protoplasmic astrocytes, found mainly in grey matter, and fibrous astrocytes, which occur mainly in white matter[1]. Recently, these two types of astrocytes have been distinguished in cultures of developing CNS and have been shown to be biochemically distinct[2]. Because both types contain large numbers of glial filaments *in vitro* and hence appear 'fibrous', we will refer to them as type 1 (protoplasmic) and type 2 (fibrous) astrocytes[2]. Most type 2 astrocytes in culture share several properties with neurones[2]; for example, they have a process-bearing morphology and bind tetanus toxin and the monoclonal antibody A2B5 (ref. 3), both of which recognize specific gangliosides[3,4] and were initially considered to be neurone-specific markers in the CNS[3,5,6]. We have therefore investigated whether type 2 astrocytes also share electrophysiological properties with neurones. Using intracellular microelectrode and 'whole-cell' (patch-clamp) recording techniques, we have now found that both type 1 and type 2 astrocytes in culture have time- and voltage-dependent potassium ion conductances which, until recently, were considered to be confined largely to electrically excitable cells.

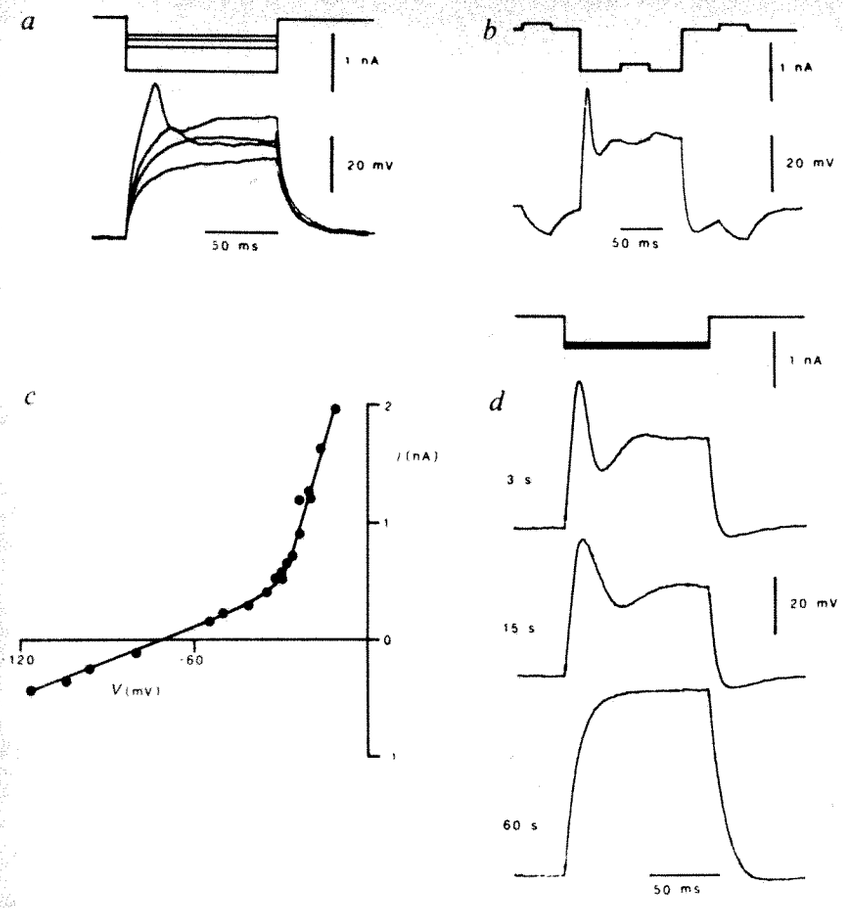


Fig. 1 *a*, Voltage response (lower trace) of a type 2 astrocyte to depolarizing current pulses of varying amplitude (upper trace). Resting potential, −78 mV. *b*, Hyperpolarizing current pulses delivered before, during and after a depolarizing pulse (upper trace) show that the membrane conductance is increased during the plateau phase of the voltage response (lower trace). Resting potential, −75 mV. *c*, Current-voltage (*I*-*V*) plot for depolarizing and hyperpolarizing current pulses shows an increase in membrane conductance for depolarizations to >−40 mV. The membrane potential was measured at the end of the current pulse, on the plateau phase of the voltage response. *d*, Membrane responses to depolarizing current pulses injected from a 2 M CsCl-filled intracellular microelectrode at the specified times after electrode impalement. Note the initial complex nature of the depolarizing response and its disappearance with time. The current pulse for the middle voltage trace is slightly smaller than for the other two traces. Resting potential, −80 mV. Except where noted, intracellular microelectrode studies were made on cells bathed in a recording solution of (mM): 145 NaCl, 5 KCl, 10 $CaCl_2$, 5 HEPES-NaOH *p*H 7.4. The use of a high calcium concentration improved the quality of electrode impalement and gave more negative and more stable membrane potentials. Microelectrodes of resistance 30–45 MΩ were filled with 3 M KCl except in *d*. Current pulses were delivered through the recording electrode which was connected to a bridge circuit. Temperature, 19–21 °C.

Cells from 7-day-old Sprague-Dawley rat optic nerves were dissociated and cultured on poly-L-lysine-coated plastic culture dishes in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS) as previously described[2,7]. We have previously shown that there are no neurofilament-containing neurones in such cultures[2] and that >90% of the process-bearing cells are type 2 astrocytes, as determined by labelling with A2B5 antibody and antibodies against glial fibrillary acidic protein (GFAP)[8]. The remaining process-bearing cells are oligodendrocytes, as determined by their labelling with antibody against galactocerebroside (GC)[8,9].

After 2–5 days in culture, cells with a typical type 2 astrocyte morphology[2] were examined electrophysiologically. Resting membrane potentials, measured with an intracellular microelectrode, were in the range −65 to −85 mV (mean ± s.d. = −79.6 ± 5.9 mV; $n = 29$). When the cells were polarized by injecting current pulses, a 'passive' membrane response was noted for small depolarizing or hyperpolarizing responses; however, with larger depolarizing pulses the response was more complex and a delayed reduction in the size of the depolarization was observed (Fig. 1*a*). This decline in amplitude to a plateau level was associated with an increase in membrane conductance, which was detected in two ways. First, when small hyperpolarizing current pulses were delivered before, during and after the depolarization (Fig. 1*b*), the evoked hyperpolarization was reduced during the plateau phase of the response. Second, when the relationship between the amplitudes of the injected current and the membrane potential near the end of the pulse was examined, a clear increase in membrane conductance was noted with depolarizations beyond −40 mV (Fig. 1*c*).

This voltage-dependent response was not affected by removal of external Na^+ (by replacement with *N*-methyl-D-glucamine), by addition of tetrodotoxin (TTX, 10 nM), by reducing the external Ca^{2+} level to 10^{-8} M with added EGTA or by addition of the Ca^{2+}-channel blocking agents La^{3+} (1 mM), Cd^{2+} (10 mM) and nifedipine (1 μM) to a nominally 'calcium-free' solution. In contrast, the response was attenuated by addition of the K^+-channel blocking drugs, tetraethylammonium (TEA, 10 mM) and 4-aminopyridine (4-AP, 5 mM). Furthermore, the voltage-activated increase in membrane conductance was greatly reduced when cells were impaled with microelectrodes containing Cs^+, which blocks K^+ channels in many cell types[10,11]. Figure 1*d* shows the response of a type 2 astrocyte to similar current pulses delivered 3, 15 and 60 s after penetration with a CsCl-filled microelectrode; a reduced voltage- and time-dependent response occurred as Cs diffused into the cell from the pipette. These findings suggest that the channels mediating the response to depolarization are similar to voltage-gated K^+ channels found in neurones and muscle cells.

To investigate this voltage-dependent response further, type 2 astrocytes were studied by the patch-clamp method with 'whole-cell' voltage-clamp recordings[12]. The measured resting potential, with 145 mM KCl in the patch pipette ($[K^+]_o = 5$ mM), was −80.1 ± 3.5 mV (mean ± s.d., $n = 21$), which is close to the value calculated from the Nernst equation for an exclusively K^+-permeable membrane. Figure 2*a* shows a family of voltage-clamp currents evoked by depolarizing voltage steps from a holding potential of −80 mV. After the initial capacity current, time- and voltage-dependent outward currents occurred with depolarizations to >−40 mV. This is shown graphically in Fig. 2*b*, where the current amplitude at the end of the depolarizing pulse is plotted against membrane potential. The voltage-dependent response was altered when Cs^+ (10 mM), TEA (10 mM) or 4-AP (2 mM) was included in the internal solution of the patch pipette; depolarizing steps now no longer evoked a persistent outward current. However, with Cs^+ present, an outward current was observed which peaked within a few milliseconds and then rapidly became inactivated, declining exponentially to a steady level with a time constant of ~30 ms (Fig. 2*c*). Unlike the persistent outward current, this transient current was abolished if the holding potential was set to levels >−40 mV. Because, in our experimental conditions, this current is outward at negative membrane potentials, it also must be carried largely by K^+ ions.

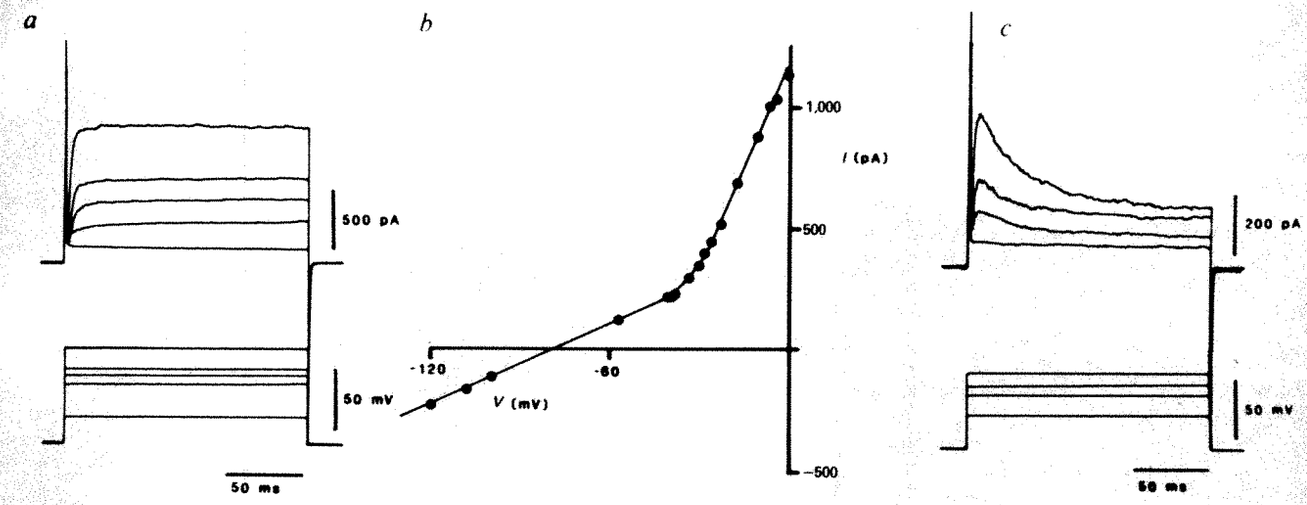


Fig. 2 Voltage clamp studies on type 2 astrocytes made with the whole cell (patch clamp) recording method. *a*, *c*, Outward currents (upper traces) evoked by depolarizing voltage command steps (lower traces) from a holding potential of −80 mV; time-dependent currents are evoked by depolarizations to > −40 mV. Cell resting potentials: *a*, −80 mV; *b*, −83 mV. In *c* the solution in the patch pipette was supplemented with 10 mM CsCl. *b*, *I*-*V* plot of 'steady-state' current for the cell shown in *a*. For these studies, cells were bathed in a recording solution containing 2 mM $CaCl_2$. Patch pipettes (3–8 MΩ resistance) were filled with (mM): 145 KCl, 5 NaCl, 1 EGTA, 5 HEPES-KOH *p*H 7.4. TEA (10 mM), 4-AP (2 mM) or CsCl (10 mM) was added to this solution as required. Gigaohm seals (>25 GΩ) were formed between the patch pipette and the membrane surface by gentle suction; increased suction then ruptured the underlying membrane to give whole-cell recordings. No series resistance or capacity compensations were made for the illustrated records. Temperature, 20–22 °C. The complex geometry of the cells means that although voltage control of the soma is good, the long processes will be poorly clamped. However, this does not affect the basic reported features of the evoked currents, as the kinetics and reversal potentials of the whole-cell currents and of the macroscopic currents in outside-out membrane patches are indistinguishable.

To determine whether type 1 astrocytes in culture have similar voltage-and time-dependent K^+ conductances, we examined cultures of type 1 astrocytes prepared from neonatal rat cortex by a modification of the McCarthy and de Vellis procedures[13] described elsewhere[2]. More than 95% of the cells in such cultures were $A2B5^-$, $GFAP^+$ type 1 astrocytes. Only cells that were not in contact with other cells were examined electrophysiologically, to avoid any problems introduced by electrical coupling between contiguous cells[14]. Type 1 astrocytes had similar resting membrane potentials to type 2 astrocytes and also showed a voltage- and time-dependent outward current on depolarization (Fig. 3*b*). Examination of the 'tail' currents seen immediately after stepping back from a depolarized level to various holding potentials (−60 to −100 mV) indicated a reversal potential of ~−85 mV, which is consistent with these voltage-activated channels being primarily K^+-permeable.

To determine whether cultured oligodendrocytes have similar K^+ conductances, we examined oligodendrocytes in optic nerve cultures prepared from 7-day-old rats; the cells were grown in a modified Bottenstein and Sato medium containing 0.5% or no FCS for 2–5 days as described previously[8]. In these cultures, more than 95% of process-bearing cells are GC^+, $GFAP^-$ oligodendrocytes[8]. Although such cells had resting membrane potentials similar to those of type 1 and type 2 astrocytes, no voltage-dependent conductances were activated when the cells were depolarized to levels up to +20 mV. Figure 3*a* shows the membrane response to depolarizing pulses and the current-voltage relationship for a typical oligodendrocyte. Similar results with cultured oligodendrocytes have been reported by Kettenmann *et al.*[15]. The electrophysiological differences between type 2 astrocytes and oligodendrocytes must appear rapidly during development, because it has been shown that both of these cells develop within 2 days *in vitro*[7] from a common progenitor cell present in 7-day-old optic nerve[8]: in the presence of 10% FCS, the bipotential progenitor cell differentiates into a type 2 astrocyte, while in serum-free medium it differentiates into an oligodendrocyte[8]. It is not clear whether the progenitor cell itself has the voltage-dependent conductances seen in type 2 astrocytes

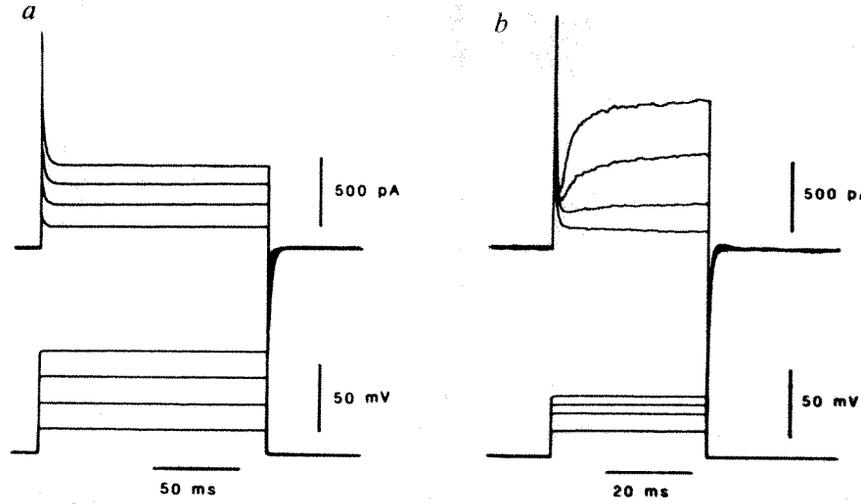


Fig. 3 Voltage clamp studies on an oligodendrocyte (*a*) and a type 1 astrocyte (*b*). Before electrophysiological examination, the flat type 1 astrocytes were usually rounded-up by a brief (~10 min) preincubation in a low-calcium-containing solution; this procedure eliminated most of the cell processes and thus improved the spatial control of membrane voltage. Whole-cell recordings show a voltage- and time-dependent K^+ current for type 1 astrocytes but not for oligodendrocytes. Holding potentials, −80 mV.

and loses them when it differentiates to become an oligodendrocyte, or whether the progenitor cell acquires these conductances on becoming a type 2 astrocyte. In either case, the changes in membrane conductance properties must occur within 2 days.

Thus, we have demonstrated voltage-activated K^+ conductances in type 1 and type 2 astrocytes in culture; these were not found in oligodendrocytes. Such conductances are activated when the cell is depolarized beyond −40 mV and seem to result from the opening of K^+ channels. The outward current normally observed in type 2 astrocytes shows little or no inactivation with time (over several seconds) and is blocked by TEA, 4-AP and Cs^+; this conductance resembles the delayed rectifier found in nerve and muscle cells. In the presence of internal Cs^+, a smaller, rapidly inactivating outward current is seen. The kinetics of this current may reflect a voltage-dependent block of the delayed rectifier-type channels by Cs^+ (see ref. 16) or the presence of a second type of K^+-permeable channel in the membrane. Our findings suggest that at least some of the neural tumour cell lines reported to contain voltage-dependent K^+ channels as well as glial cell antigens[17] arose from astrocytes or their precursor cells.

We have shown that astrocytes in culture display voltage-activated K^+ channels. This finding complements the recent reports of veratridine- and voltage-activated Na^+ channels[18,19] and voltage-dependent Ca^{2+} channels[20] in type 1 astrocytes. The biological significance of these conductances in astrocytes will remain uncertain until the functions of these cells are known.

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Expression of T-cell antigen receptor genes during fetal development in the thymus

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The T-cell antigen receptor is a heterodimeric molecule composed of α- and β-subunits of relative molecular mass 40,000–50,000 (refs 1–6). Recently, the genes encoding both the β-[7–9] and α-[10–12] chains have been cloned. By comparing amino-acid and nucleic-acid sequences, it is clear that these genes encode the α- and β-proteins of the T-cell receptor[13,14]. In addition, a third receptor-like gene, the γ-chain gene, has been identified[15], which has many structural and sequence characteristics in common with the α- and β-chain genes. The role of the γ-chain gene in T-cell development is unknown. We have reported recently that the β-chain genes are transcriptionally turned on in the thymus at about day 17 of fetal development[16]. Here we report that the α-chain also is transcriptionally activated during this time, but that the γ-chain gene is active in the thymus at day 14, reaches a peak steady-state level at day 15 and rapidly declines thereafter. If the γ-chain gene has a functional role, it would seem to be involved very early in T-cell development, before the mature T-cell receptor is expressed.

The mouse thymus is initially colonized by blood-borne precursors during days 11 and 12 of gestation[17]. Cell-surface molecules with the characteristics of the T-cell antigen receptor do not appear until day 17 (ref. 16) and functional cells do not appear in appreciable numbers until day 19 (refs 18, 19). Consistent with this, the α and β loci are transcriptionally active around day 17 of gestation (Fig. 1); this is evident not only in the absolute levels of messenger RNA, but also in the amounts of the larger transcripts (1.6, 1.3 and 1.5 kilobases (kb) for α, β and γ, respectively) relative to those of the smaller transcripts (1.3, 1.0 and 1.2 kb for α, β and γ, respectively). This is important because in the case of the β-chain gene, the larger transcript represents the full-length functional mRNA, whereas the smaller mRNA is an aberrant transcript representing only rearrangements of the diversity-joining-constant gene regions[20,21]. As reported previously[16,22], the levels of β-chain mRNA reach a peak in the adult thymus and drop substantially in peripheral T cells, in contrast to steady-state levels of α-chain mRNA, which reach a maximum by day 19 and remain high in peripheral T cells.

From the data presented in Fig. 1, it is impossible to compare the relative amounts of α, β and γ mRNA because of the different sizes of probes used and the different exposure times. A more quantitative estimate of the relative levels of expression is obtained from the number of clones of each of these genes present in a complementary DNA library. A library of 5×10^4 independent cDNA clones was prepared from day 17 fetal thymocytes, containing 50 (0.1%) β-chain clones and 5 (0.01%) clones each of the α- and γ-chains. (In a similar experiment, Chien *et al.*[10] found frequencies of 0.01% (α), 0.2% (β) and 0.002% (γ) in the adult thymus and 0.03% (α), 0.1% (β) and 0.002% (γ) in peripheral T cells.) These differences in expression between day 17, adult thymus and peripheral T cells are reflected in the hybridization intensities shown in Fig. 1. The inverse relationship in the expression of the α- and γ-chain genes is striking. The γ-chain gene is transcribed at high levels early in differentiation and then transcription declines rapidly at the time when the α-chain gene is becoming activated.

These data further support our previous conclusions[16] that the mature receptor seen on peripheral T cells is first expressed in the thymus at ~day 17 of gestation. The role of the γ-chain gene is unknown. Our data are consistent with a change in receptor repertoire during ontogeny, as suggested in some models[23,24]. One speculation, consistent with the expression of the α-, β- and γ-chain genes during ontogeny, is that the β- and γ-proteins form receptors that have specificity for major histocompatibility complex (MHC) antigens either alone or in combination with a ubiquitous cell-surface antigen; in other words, perhaps the β/γ combination is a MHC-restricted receptor specific for a nonpolymorphic cell-surface determinant. Thymocytes with the β/γ combination specific for self-MHC antigens are induced to differentiate. One of the differentiation steps would include the turning-off of the γ-chain gene and turning-on of the α-chain gene. The new α/β receptor would now (or perhaps after another round of selection) have specificity for a wide range of non-MHC antigens and for self-MHC. The binding site for self-MHC would be contributed by the β-chain, whereas the binding site for non-MHC would be formed by both the α- and β-chains. Although this, in effect, suggests the

presence of two binding sites, the overall functional specificity would depend on the specific α/β combination. Such a model is compatible with what is known about the structure of the β-chain variable region[25], as well as the extent of variability of the other two genes[10,11,15]. This would explain the lack of observed mixing of specificities in somatic cell hybrids (ref. 26 and W. Haas, personal communication). There is no direct evidence either for or against such a model. More information is needed concerning the expression of the γ-chain on the cell surface and the repertoire of the three genes before and after thymic selection.

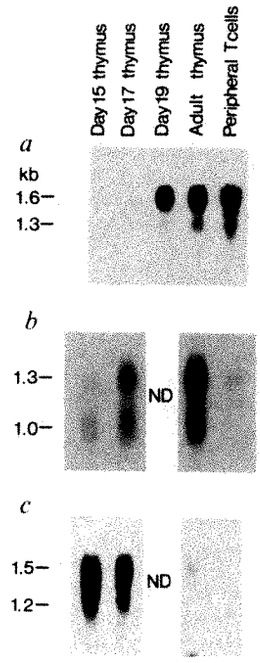


Fig. 1 Northern blot analysis of T cells at various stages of development. Total RNA (20 μg) was separated on 1.2% glyoxal gels[27], transferred to Gene Screen (NEN) and hybridized with an α-chain probe (*a*), β-chain probe (*b*), or γ-chain probe (*c*). *b*, From ref. 16; ND, not done.

Methods. B10.BR fetal thymocytes were obtained from timed matings where the day of vaginal plug detection was designated as day 0. RNA was isolated by the guanidinium isothiocyanate and CsCl gradient centrifugation procedures[28,29]. RNA blots were hybridized in 1 M NaCl, 50 mM Tris *p*H 7.5, 1% SDS, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll, 100 μg ml^{-1} denatured salmon sperm DNA, 10% dextran sulphate and 1.5×10^6 c.p.m. ml^{-1} oligo-labelled[30] probe (10^9 c.p.m. μg^{-1}) at 68 °C overnight. Filters were washed in 0.3 M NaCl, 60 mM Tris *p*H 8, 2 mM EDTA twice at 25 °C for 10 min and twice at 68 °C for 30 min, and twice in 10 mM NaCl, 6 mM Tris *p*H 8, 2 mM EDTA at 25 °C. Exposure was overnight with intensifying screens (Ilford Fast Tungstate). Sizes of RNA molecules were estimated from denatured phage DNA restriction fragments of known lengths run in parallel on the same gel. All probes are gel-purified inserts. The α-chain probe is the 300-base pair (bp) *Nco*I/*Ava*II fragment from the constant portion of the α-chain cDNA clone T1.2 (ref. 12). The β-probe is the 300-bp insert of the 4.4-kb cDNA clone from the constant portion of the β-chain gene starting ~50 bp downstream of the joining (*J*) gene segment (M. Kiefer and M.S., unpublished results). This clone was isolated together with the 4.1-kb β-chain cDNA clone, as described previously[16]. The γ-chain probe represents the 1.3-kb insert of the Tγ5 cDNA clone containing an almost full-length γ-chain cDNA as determined by comparison of this clone with published[15] γ-chain cDNA clones. The Tγ5 cDNA clone was isolated from an EL-4 cDNA library with a 33-residue oligonucleotide specific for the constant (C_γ) gene segment (M.S., W. Bannwarth and M. Kiefer, unpublished results). This cDNA clone has a 105-bp deletion starting at amino acid −4 and extending to amino acid 32 (numbered according to ref. 15; A. Traunecker, personal communication), presumably a result of aberrant splicing.

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Note added in proof: Since the submission of this manuscript, Raulet and co-workers[31] have reported results consistent with the data presented here.

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Interleukin-2 stimulates association of protein kinase C with plasma membrane

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Interleukin-2 (IL-2) is a regulatory peptide important for the growth and differentiation of antigen-specific T lymphocytes and large granular lymphocytes[1,2]. Interaction of IL-2 with its specific receptor results in the promotion of S-phase progression as well as, in certain circumstances, the production and release of γ-interferon (IFN-γ)[3–5]. Although the binding of IL-2 with high-affinity specific receptors has been well characterized[6,7], the intracellular mechanisms by which this ligand–receptor interaction promotes growth and differentiation are unknown. Here, we present evidence that IL-2/receptor interaction produces a rapid and transient redistribution of protein kinase C (PK-C) from the cytosol to the plasma membrane. Phorbol myristate acetate (PMA) also induces PK-C transposition in an analogous manner, except that PMA-induced PK-C transposition to the plasma membrane is apparently protracted. As phorbol esters have been shown to mimic IL-2 in the regulation of cellular proliferation as well as IFN-γ production[8–10], the activation of PK-C by either phorbol esters or IL-2/receptor interaction seems to have a crucial role in signal transduction elicited by these extracellular messengers.

Figure 1 shows the time course of IL-2-induced protein kinase C activity. IL-2-dependent murine CT6 cells grown in 5 units of IL-2 per 10^5 cells were washed in serum-free medium three times, 24 h before experimental stimulation. The cells were

washed twice more, resuspended to 1×10^7 cells ml^{-1}, and incubated at 37 °C for 2 h. Approximately 500 units of recombinant IL-2 were added per 10^7 cells and the PK-C-specific activity in the cytosol and plasma membrane were compared at the indicated time intervals. Within 3 min of IL-2 stimulation, an increase in PK-C-specific activity of over fourfold was observed in the plasma membranes of CT6 cells compared with unstimulated controls. Increases in membrane-associated PK-C activity of over 10-fold were observed by 10 min after IL-2 stimulation, followed by a rapid decline of plasma membrane PK-C activity at 20 min and a return to initial PK-C membrane-specific activity by 60 min. Concomitant with the rapid increases of PK-C membrane-associated activity, there was a precipitous decline in PK-C activity within the corresponding cytosol preparations obtained at the same time intervals. Although a decline in cytosolic PK-C activity was seen as early as 1 min after IL-2 stimulation, the lowest PK-C cytosolic activity was observed at 10 min, corresponding to peak plasma membrane-associated activity. Cytosolic PK-C activity progressively increased to basal levels by 60 min in the continuous presence of IL-2. In the same experiment, we compared the effects of PMA with those of IL-2. As reported previously[11], PMA (10 ng per 10^6 cells) stimulated a rapid (1 min) association of PK-C with particulate membranes. The PMA-stimulated membrane association of PK-C was approximately twofold greater than that stimulated by IL-2 and exhibited a more protracted dissociation from CT6 plasma membranes. CT6 cytosolic PK-C activity rapidly declined within 1 min of phorbol ester stimulation and was depressed to 50–115 pmol throughout the experiment. Similar data were obtained in a previous study in which murine thymoma EL4 cells were stimulated with phorbol esters[11]. The present results indicate that the decrease in cytosolic PK-C activity and consequent membrane association is not limited to phorbol ester-treated EL4 cells or parietal yolk sac (PYS-2) cells[11,12]. Indeed, IL-2, a growth-stimulating peptide, induced a substantial decrease in cytosolic PK-C activity with a concomitant increase in the plasma membrane-associated activity (Fig. 1). Attempts to dissociate PK-C from the membrane fraction of PMA- or IL-2-treated CT6 cells with high-salt (0.3 M NaCl)-Tris buffer or with buffer containing metal chelators (1 mM EDTA and EGTA) have been unsuccessful (data not shown), suggesting that PK-C has become a tightly associated integral membrane component.

To further investigate whether there is a direct correlation between IL-2-induced PK-C membrane association and IL-2-induced stimulation of proliferation of CT6 cells, we compared the dose-response curve for PK-C membrane association with that for IL-2-induced proliferation. Figure 2 shows the results of a typical experiment comparing the effects of recombinant IL-2 on PK-C plasma membrane association and CT6 proliferation; the titration curves have similar slopes. Concomitant with the increased membrane association of PK-C, there was a decrease in cytosol-specific activity, with half-maximal translocation of kinase activity in both membrane and cytosol occurring within an equivalent range of IL-2 activity (65–85 units). These dose-response kinetics further reinforce the notion that cytosolic PK-C became associated with the plasma membrane. In a related study, crude homogenates were preapared from control, IL-2- or PMA-stimulated CT6 cells and the total homogenate was extracted (cytosol plus membrane) with Nonidet P-40 (NP40). The resulting detergent-solubilized, DEAE-purified total PK-C activity from either control, IL-2- or PMA-stimulated CT6 cells agreed within ±15%, indicating that neither IL-2 nor PMA significantly altered total enzyme activity (data not shown). Also, the 50% effective dose (ED_{50}) of IL-2 for both PK-C membrane association and IL-2 bioactivity (measured by the proliferative response of CT6 cells) titrated within the same range of IL-2 concentration (80–145 units), suggesting a close correlation between the activation of PK-C membrane association and those events involved in IL-2-induced proliferation of CT6 cells.

Phospholipid enzymatic degradation, particularly phosphatidylinositol and phosphatidylcholine turnover stimulated by a wide variety of neurotransmitters, hormones, growth factors and toxins, appears to generate transmembrane control of cellular functions[13–17]. Phosphatidylinositol (PI) turnover, specifically, has been shown to activate a novel protein kinase (PK-C) by the generation of a transient metabolite of PI degradation, diacylglycerol[18,19]. The activation of PK-C seems to play a crucial part in the signal transduction elicited by extracellular messengers[13]. Although PK-C is normally found in a functionally inactive form in the cytosol of a variety of resting cell types, phorbol esters have been shown to stimulate a rapid protracted association of PK-C with plasma membrane, whereupon the lipid-rich environment plus Ca^{2+} serve as cofactors for the activation of enzymatic kinase activity[11,12]. The process of phorbol ester activation of PK-C activity is believed to be independent of phosphatidylinositol hydrolysis and diacylglycerol formation. Phorbol esters apparently displace the physiochemical association between diacylglycerol and PK-C and, as a result, activate PK-C in the absence of phosphoinositides[20,21]. Interleukin-2, however, probably acts through a phosphatidylinositol hydrolysis-dependent process, as indicated by

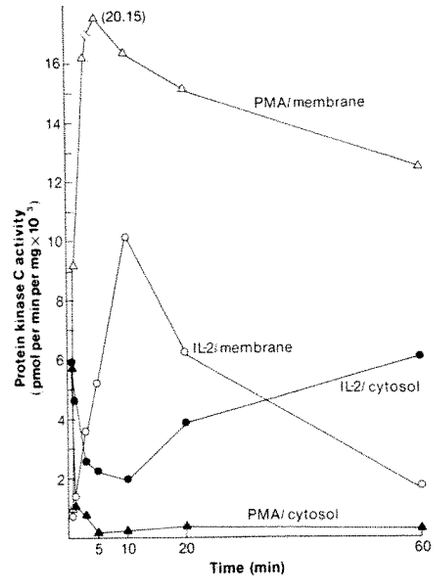


Fig. 1 Time course of association of PK-C with plasma membrane. IL-2-dependent CT6 cells were washed in serum-free RPMI-1640 medium and recultured in 1% fetal calf serum RPMI-1640 for 24 h. The cells were then washed twice and incubated at 37 °C in serum-free RPMI-1640 for 2 h before stimulation. Cells (1×10^7) were resuspended in 1 ml in Eppendorf tubes and stimulated with either 100 ng PMA or 500 U of recombinant human IL-2 (Biogen). The cells were maintained in a shaking water bath at 37 °C, removed at the indicated time intervals and washed three times in 1.0 ml serum-free RPMI-1640/1 mM sucrose. All liquids was absorbed from the cellular pellets and the cells were lysed in a 50-μl volume of distilled H_2O aspirated through a Hamilton syringe for 20 s. Immediately, the lysate was reconstituted with 1.0 ml ice-cold sample buffer (20 mM Tris *p*H 7.5, 0.33 M sucrose, 2 mM EDTA, 0.5 mM EGTA and 2 mM phenylmethylsulphonyl fluoride). Cytosol and particulate membrane fractions were prepared by centrifugation at 100,000 *g* for 60 min. Centrifuged membrane pellets were homogenized with a glass rod in 1% NP40 sample buffer, gently rotated for 30 min at 4 °C, and centrifuged at 12,000 r.p.m. for 15 min. Approximately 500 μl was passed through a 1-ml DE52 column equilibrated with sample buffer minus sucrose. Columns were washed with 15 ml sample buffer, 0.5 ml of 0.08 M NaCl, and an additional 1.5 ml of 0.08 M NaCl eluate was collected; all the PK-C activity was found in this fraction[11]. Aliquots (50 μl) were assayed for PK-C activity as described elsewhere[11,12] with 1 mM $CaCl_2$, 10 mM magnesium acetate, 100 μM [γ-^{32}P]ATP (60 c.p.m. $pmol^{-1}$), 50 μg HI histone, with or without 5 μg phosphatidylserine and 1 μg diolein. Data are expressed as PK-C-specific activity (pmol per min per mg) attributed to activity stimulated by phospholipid.

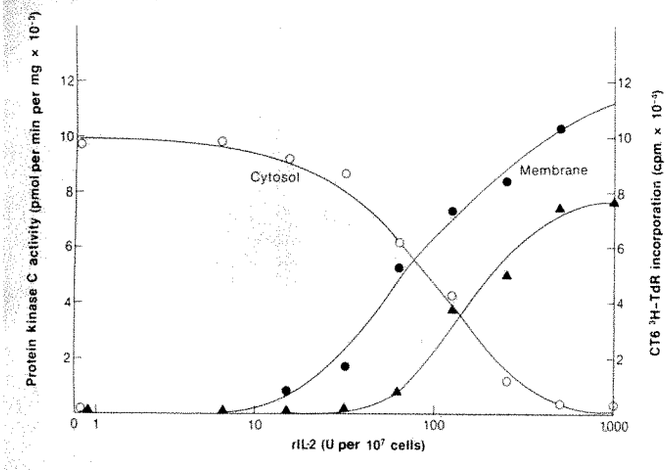


Fig. 2 Dose-response of IL-2-stimulated PK-C membrane association and cellular proliferation. CT6 cells were washed and prepared as described in Fig. 1 legend, then the indicated doses of recombinant IL-2 (rIL-2) were added and the cells were incubated for 10 min, followed by washing and lysis. Aliquots of the cell suspensions were taken and proliferation was measured in a standard IL-2 bioassay[5]. Proliferation data are expressed as the mean c.p.m. of triplicate cultures seeded with 1×10^4 cells per microculture (▲); PK-C activity in membrane (●) and cytosol (○) was determined as described in Fig. 1 legend and elsewhere[11].

the transient nature of the PK-C membrane association (Fig. 1). Preliminary experiments using isolated plasma membranes from IL-2-dependent cell lines have demonstrated that the principle metabolites formed on *in vitro* IL-2 stimulation of the isolated membranes were the PI metabolites phosphatidylinositol 4′-monophosphate and phosphatidylinositol 4′, 5′-bisphosphate (data not shown). However, diacylglycerol, a neutral lipid, could not be recolved by the single-dimension TLC analysis employed. Even though diacylglycerol is generated by PI hydrolysis, it is rapidly converted to phosphatidic acid and arachidonic acid, which may therefore explain the membrane association/dissociation kinetics observed with IL-2. Phorbol esters, on the other hand, are not readily degraded enzymatically and therefore substitute for diacylglycerol for a more protracted period[22].

Recent studies have shown that an initial event induced by growth stimulators is enhanced phosphorylation of specific membrane proteins[23,24]. The products of several different viral transforming genes have also been demonstrated to be protein kinases[25,26] and association with the plasma membrane seems to be required for *in vivo* tumour formation[27-29]. In addition, the Rous sarcoma virus transforming gene product, $pp60^{v\text{-}src}$, and the UR2 transforming protein of avian sarcoma virus have been shown to have lipid kinase activity which phosphorylates PI and diacylglycerol, suggesting a mechanism for transformation involving the initiation of PI turnover which, in turn, can activate several protein kinase activities including PK-C[30,31]. Our studies suggest that IL-2 activates a protein phosphorylation system, PK-C, which is also associated with the biological activity of tumour promoters and certain oncogenes[13-15,30,31]. The data are consistent with the notion that oncogenes may mimic or interact at different discrete events in phosphorylating systems that are normally stimulated by ligand-receptor interactions, and thereby initiate a cascading series of events associated with cellular proliferation. The finding that IL-2 initiates PK-C activation defines an event necessary for the growth of lymphoid cells, and allows the development of a model for the mechanism of action of as yet undefined oncogenic elements that may be involved in leukaemogenesis.

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Altered cytosol/membrane enzyme redistribution on interleukin-3 activation of protein kinase C

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Interleukin-3 (IL-3) is a member of a family of growth and differentiation peptides, collectively referred to as colony-stimulating factors, which regulate haematopoiesis[1-3]. IL-3 has been highly purified from medium conditioned by WEHI-3B cells, and recently the molecular cloning of complementary DNA for murine IL-3 has been reported[4,5]. IL-3 seems to stimulate a wide range of colony-forming cells derived from murine bone marrow and has consequently been studied under a variety of names, including burst-promoting activity[6], mast cell growth factor[7], P-cell stimulating factor[8] and multi-colony-stimulating factor[3,9]. Here we present evidence that IL-3-receptor interaction stimulates the rapid and transient redistribution of protein kinase C (PK-C) from cytosol to plasma membrane in FDC-P1 cells. Phorbol myristate acetate (PMA) is shown to have a similar effect in these IL-3-dependent FDC-P1 cells. Our data suggest that IL-3 and phorbol esters share a common feature of transmembrane signalling crucial for growth and differentiation.

FDC-P1 cells were established originally as a cell line dependent for its sustained growth and viability on conditioned media derived from WEHI-3 cells[10]. Ihle and colleagues established that IL-3 is the principal factor within WEHI-3 conditioned media which is responsible for the growth promotion of FDC-P1 cells[11]. In the present studies, FDC-P1 cells were washed free of support conditioned medium, rested for 2 h in serum-free media and stimulated with 100 ng PMA per 10^7 cells. At various times after PMA addition, the cells were washed, lysed and the

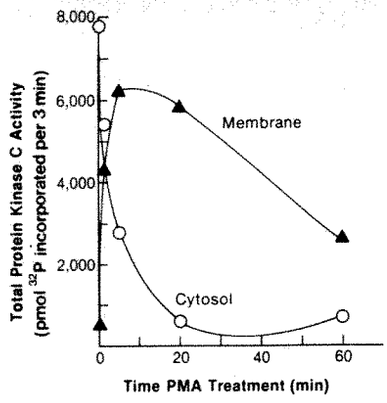


Fig. 1 Time course of the effect of PMA treatment of FDC-P1 cells on cytosolic (○) and particulate (▲) protein kinase C. FDC-P1 cells were grown in the presence of 10% FCS-RPMI 1640 and 20% WEHI-3 conditioned medium as described elsewhere[11]. The cells were washed extensively, reconstituted with serum-free RPMI-1640 at a density of 1×10^7 per experimental point, then incubated for 2 h in a 37 °C water bath in Eppendorff tubes before stimulation with 100 ng PMA. After addition of PMA, at the indicated time intervals, cells were collected and washed rapidly three times with RPMI 1640, 1 M sucrose and pelleted in a microfuge. The cellular pellet was lysed within 20 s in 50 μl of double-distilled water by passage through a narrow-gauge needle and immediately reconstituted with 1.0 ml ice-cold buffer A (20 mM Tris-HCl *p*H 7.5, 0.5 mM EGTA, 2 mM EDTA, 2 mM phenylmethylsulphonyl fluoride). Lysates were centrifuged at 100,000*g* and cytosol and membrane particulate fractions obtained. Particulate membrane fractions were solubilized with buffer A containing 1% NP40 detergent and both cytosolic and solubilized membrane fractions were fractionated by DEAE-cellulose chromatography as described in detail elsewhere[14]. Fractions eluting with 0.08 M NaCl contained all the detectable PK-C activity[14]. Aliquots (50 μl) of DEAE-purified material were assayed for cytosolic and particulate Ca^{2+}, phospholipid-dependent PK-C activity in the presence of 10 mM Mg acetate, 0.75 mM $CaCl_2$, 100 μM [γ-^{32}P]ATP (60 c.p.m. pmol^{-1}) and 25 μg of histone H1, with or without 24 μg phosphatidylserine and 1.6 μg diolein[14]. Protein kinase activity was determined by subtracting the amount of ^{32}P incorporation into histone noted in the absence of added phospholipids, from the amount of ^{32}P incorporation noted in the presence of phospholipids. Total protein kinase C activity is expressed as the pmol ^{32}P incorporated per 3 min per total volume of each cytosolic and membrane preparation.

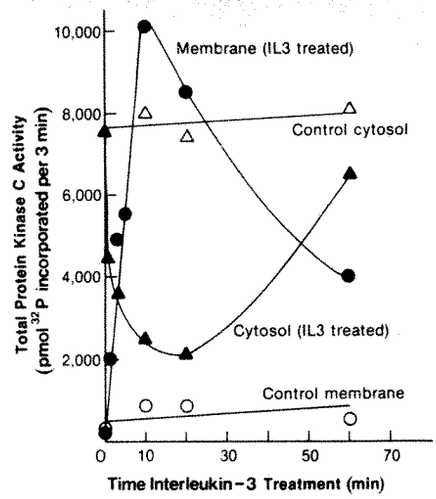


Fig. 2 Time course of the effect of IL-3 treatment of FDC-P1 cells on cytosolic and particulate protein kinase C. Washed FDC-P1 cells were prepared as for Fig. 1. Following preincubation in serum-free medium, 10^7 cells were stimulated with 100 U of homogeneous IL-3 prepared from WEHI-3 conditioned media[4]. Other cells were incubated in the absence of IL-3 to serve as the control cell population. At the indicated time intervals, the cells were washed, lysed and centrifuged at 100,000*g* to prepared cytosolic (△, ▲) and membrane (○, ●) fractions from control (closed symbols) and IL-3-treated (open symbols) cells, as described in Fig. 1 legend. Cytosol and NP40 detergent-solubilized particulate preparations were applied to and eluted from DE52-cellulose columns and protein kinase C activity was determined in the presence and absence of phospholipids as described for Fig. 1.

Ca^{2+}, phospholipid-dependent PK-C activity which was measured in the cytosol and detergent-solubilized membrane fractions was isolated.

Figure 1 shows a time-kinetic comparison of the total PK-C activity in cytosol and membrane fractions of FDC-P1 cells treated with PMA. Within 1-2 min of PMA addition, cytosolic PK-C activity had begun to fall rapidly; it had fallen 10-fold within 20 min of incubation in the presence of PMA and remained depressed for the remainder of the time course. Concomitant with the decrease in cytosolic PK-C activity, there was an equally rapid (1-2 min) increase in membrane-associated PK-C resulting in a 10-fold increase in total enzyme activity by 10 min post-treatment. Examination of the total cellular enzyme activity (membrane + cytosol) revealed no changes in total PK-C activity with PMA stimulation (data not shown). Rather, the data suggested that PMA mediates a redistribution of PK-C activity from cytosol to membrane. These findings are consistent with previous studies demonstrating similar PK-C translocations from cytosol to plasma membrane in phorbol ester-treated murine thymoma EL4 cells, interleukin-2 (IL-2)-dependent CT6 cells and parietal yolk sac cells (PYS)[12-15].

FDC-P1 cells were washed free of support WEHI-3 conditioned medium, incubated for 2 h at 37 °C in serum-free RPMI 1640, stimulated with 100 U of purified IL-3 (U = reciprocal dilution at half-maximal proliferation)[4] and the time course of cytosol- and membrane-associated PK-C activity was evaluated (Fig. 2). Unstimulated FDC-P1 cells contained ~7,500 U PK-C cytosolic activity (1 U = 1 pmol ^{32}P incorporated in 3 min). Treatment with IL-3 resulted in a precipitous decrease in cytosolic PK-C activity within 1-3 min (from 7,500 to 3,500 U), with a greater than threefold decrease within 20 min post-IL-3 treatment. This IL-3-stimulated decline of cytosolic PK-C activity was transient, however, and was followed by a return to near control levels by 60 min post-stimulation. Control cytosolic PK-C activity (no IL-3 added) did not change significantly (±15%) during FDC-P1 cell incubation, and total control membrane-associated detergent-solubilized PK-C activity was considerably lower (100-200 U) than control PK-C cytosolic activity (7,500 U).

Concomitant with the rapid loss of cytosol PK-C activity was an equally marked increase in membrane-associated activity, resulting in a >10-fold increase in membrane-associated activity within 10-20 min post-IL-3 treatment. Control (no IL-3 added) membrane PK-C activity did not change significantly during cell incubation. The return (increase) of cytosolic PK-C activity 20-60 min after IL-3 addition was associated with a corresponding decrease of membrane-associated activity. Total cellular (membrane + cytosol) PK-C activity did not change significantly during the exposure to IL-3, suggesting that the reciprocal kinetics of cytosolic and membrane-associated PK-C activity results from IL-3-mediated PK-C enzyme translocation between soluble and particulate fractions.

Figure 3 depicts the dose-response curve noted for IL-3-stimulated DNA synthesis relative to the membrane-association kinetics of PK-C activity in FDC-P1 cells. Incubation of FDC-P1 cells for 15 min with increasing concentrations of IL-3 (to 500 U) caused a progressive, IL-3 dose-dependent decrease in cytosolic PK-C activity, while particulate PK-C activity increased. The increase in detergent-solubilized PK-C activity in response to IL-3 was proportional to the dosage of IL-3 used to treat the FDC-P1 cells. The proliferative dose-response of FDC-P1 cells to IL-3 paralleled the membrane-association kinetics of PK-C,

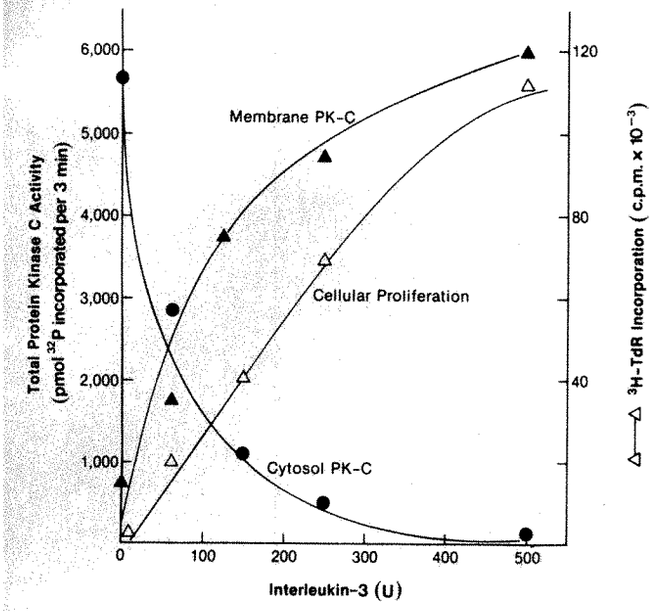


Fig. 3 Effect of treatment of FDC-P1 cells with increasing concentrations of IL-3 on the subcellular redistribution of protein kinase C activity and on DNA synthesis. To determine the dose response of IL-3 on PK-C activity, FDC-P1 cells were incubated with the indicated dosages of purified IL-3 for 15 min. The cells were washed and cytosol (●) and particulate (▲) membrane fractions prepared as for Fig. 1. Cytosolic and detergent-solubilized membrane preparations were separated by DEAE-cellulose chromatography to determine the total PK-C activity as described in Fig. 1 legend. Cellular proliferation (△) was determined by measuring the incorporation of ^{3}H-thymidine (TdR) as described elsewhere[11]. Cellular proliferation is expressed as the mean c.p.m. of triplicate cultures of 3×10^4 cells per culture.

and the IL-3 ED_{50} for both the increase in membrane-associated PK-C activity and FDC-P1 DNA synthesis was within the same dose range (200–250 U). These results suggest a quantitative relationship between PK-C membrane association and the proliferative response of FDC-P1 cells to IL-3.

The data presented here demonstrate that a haematopoietic growth factor, IL-3, stimulates a rapid and transient increase in the redistribution of PK-C activity from cytosol to the membrane (Fig. 2). IL-3 probably acts through a phosphoinositide hydrolysis-dependent mechanism involving the generation of diacylglycerol, as indicated by the transient nature of the membrane association. Phorbol esters, however, are not readily degraded enzymatically and may, therefore, become associated with the enzyme in place of the apparent physiological regulator for a protracted period, which would allow a more prolonged association of PK-C with the membrane and override other regulatory capabilities of the cell (such as the rapid metabolism of diacylglycerol) (Fig. 1)[16,17].

In related studies, IL-2, a lymphocytotropic growth factor, also stimulates transient PK-C subcellular redistribution[12]. The similarity of the IL-2-induced PK-C activation process and IL-3 suggests that growth or progression peptides share common features of transmembrane biochemical signals involved in growth promotion. Interestingly the viral transforming gene products *src* and *ros* have recently been demonstrated to possess lipid kinase activity[18,19]. This would presumably result both in the activation (and membrane association) of PK-C in response to increased diacylglycerol levels and to increased intracellular Ca^{2+} levels in response to the polyphosphoinositides[16,20]. Taken together, the data are consistent with the hypothesis that oncogenic elements mimic certain ligand–receptor interactions, thereby initiating a series of transmembrane phosphorylation events which are normally triggered by extracellular growth peptides[19–21].

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Selective inhibition of the anchorage-independent growth of *myc*-transfected fibroblasts by retinoic acid

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Fischer rat 3T3 (FR3T3) fibroblasts transfected with a cellular *myc* gene[1] can be induced to grow and form colonies in soft agar by treatment either with epidermal growth factor (EGF) alone or with the combination of platelet-derived growth factor (PDGF) and type-β transforming growth factor (TGF-β)[2]. We now show that induction of anchorage-independent growth by each of these sets of growth factors involves different cellular pathways which can be distinguished by their sensitivity to retinoic acid. Colony formation induced by the combined action of PDGF and TGF-β is 100-fold more sensitive to inhibition by retinoic acid than is colony formation induced by treatment of the *myc*-transfected cells with EGF. Moreover, retinoic acid (10^{-8} M) is inhibitory for colony growth whenever TGF-β is present, regardless of whether the effects of TGF-β are stimulatory, as occurs in the presence of PDGF, or inhibitory, as found in the presence of EGF[2].

The cellular mechanisms of induction of anchorage-independent growth by peptide growth factors such as PDGF[3–5], TGF-β[6–8] and the TGF-α family[9–11] (which includes EGF) are poorly understood. These three distinct families of growth factors[12–15] share the ability to induce phenotypic transformation of non-neoplastic fibroblasts, as measured by growth in soft agar[16–18]. The increased secretion of TGF-α[19,20], PDGF[21] and TGF-β[22] which accompanies virus transformation of cells provides further evidence for the role of these peptides in expression of the transformed phenotype. It has recently become apparent that the product of the *myc* gene, acting in the nucleus, is involved in the pathway by which a mitogenic stimulus is generated by a growth factor acting on its receptor[23–28]. For example, treatment of quiescent fibroblasts with PDGF, which induces a state competent for replication[29], results in transient expression of the *myc* gene[30]. Consistent with this observation, BALB/c 3T3 cells transfected with *myc* partially lose their requirement for PDGF[31]. Thus, whereas control cells require both PDGF and EGF for growth in monolayer culture in plasma[29], *myc*-transfected cells can grow in plasma in the presence of EGF alone[31].

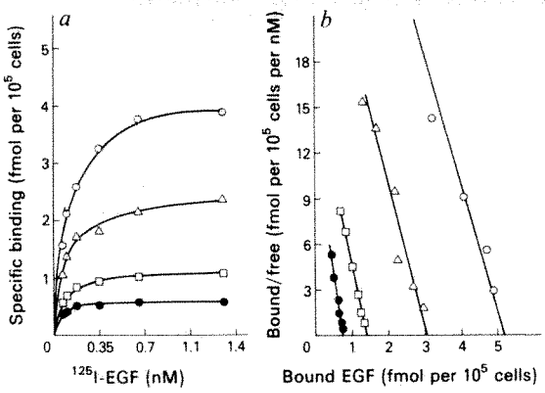


Fig. 1 Retinoic acid increases the number of cell-surface EGF receptors on Myc-1 cells. *a*, Binding of ^{125}I-labelled EGF (150 μCi μg^{-1}) to Myc-1 cells grown either in the absence of added retinoic acid (●) or in the presence of 10^{-8} M (□), 10^{-7} M (△) or 10^{-6} M (○) retinoic acid. *b*, Scatchard plot of the data in *a*.

Methods. The Myc-1 cell line was derived from FR3T3 fibroblasts by co-transfection of a neomycin-selectable marker gene and a construct in which exons 2 and 3 of a cellular *myc* gene derived from a mouse plasmacytoma were put under control of the SV40 early promoter (plasmid pSVc-myc-1)[1]. Cells were seeded in 1 ml of Dulbecco's modified Eagle's medium containing 5% calf serum at a density of 1×10^4 cells per 16-mm multiwell dish. Retinoic acid (in 20 μl of a 2% solution of dimethyl sulphoxide in medium) was added 4 h after seeding and the incubation was continued for 60 h before the binding assay[37]. Binding of ^{125}I-labelled EGF was measured at room temperature in the absence of retinoic acid. Nonspecific binding, measured in the presence of unlabelled EGF at a concentration of 1 μg ml^{-1}, was subtracted from all values. Results are the average of triplicate determinations.

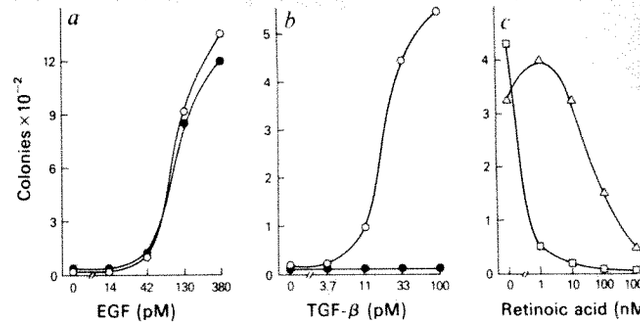


Fig. 2 Selective inhibition by retinoic acid of the anchorage-independent growth of Myc-1 fibroblasts in the presence of PDGF and TGF-β. *a*, Colony growth assayed in the presence of EGF alone. *b*, Colony growth assayed in the presence of 150 pM PDGF and the concentration of TGF-β indicated. *c*, Colony growth assayed in the presence of 42 pM EGF alone (△) or the combination of 150 pM PDGF and 100 pM TGF-β (□) and varying concentrations of retinoic acid. Myc-1 cells were assayed for the formation of colonies of >62 μm diameter after 7 days' incubation in soft agar in Dulbecco's modified Eagle's medium, 10% calf serum as described previously[37]. Purified murine EGF, human PDGF or human platelet TGF-β[7] were added at the time of the assay[2] in the presence (●) or absence (○) of 10^{-8} M retinoic acid.

Transfected NIH 3T3 or Rat-2 cells expressing an activated *myc* gene are tumorigenic and have an increased, though low (1%), cloning efficiency in soft agar compared with control cells[32]. We have shown that FR3T3 cells transfected with exons 2 and 3 of a cellular *myc* gene under control of the simian virus 40 (SV40) early promoter[1] (Myc-1 cells) do not grow in soft agar, but can be induced to form colonies by the addition of growth factors[2]; control cells transfected with the neomycin marker alone cannot form colonies. Myc-1 cells require only EGF to form colonies[2], and PDGF has little additional effect on cell growth in the presence of optimal concentrations of EGF. In contrast, PDGF is essential for anchorage-independent growth of Myc-1 cells in the presence of TGF-β[2]. This suggests that one function of PDGF may be to induce expression of other genes[33] in addition to *myc* which are required in the signalling pathways of TGF-β.

To probe for possible mechanistic differences in the action of EGF and TGF-β on anchorage-independent growth of Myc-1 cells, we investigated the effects of retinoic acid on the growth factor-dependent colony formation of the cells. Retinoic acid is known to inhibit colony growth of certain tumour cells and *in vitro* transformation of fibroblasts (reviewed in ref. 34). Previous studies suggest that retinoic acid[35-37] and TGF-β[38] may share common mechanistic elements, since they both increase the number of EGF receptors of normal rat kidney (NRK) cells. In the present study, retinoic acid treatment (60 h) of Myc-1 cells similarly resulted in a dose-dependent increase in the number of cellular EGF receptors, 10^{-8} M, 10^{-7} M and 10^{-6} M retinoic acid producing a respective two-, four- and seven-fold increase in the number of EGF receptors (Fig. 1*a*,*b*), with no apparent effect on receptor affinity. However, in contrast to the stimulatory effects of this increased number of EGF receptors on soft agar growth of NRK cells[35-37], treatment of Myc-1 cells with retinoic acid (10^{-8} M) had little effect on the dose-dependent induction of anchorage-independent growth by EGF (Fig. 2*a*). Concentrations of retinoic acid >10^{-8} M inhibited EGF-induced colony formation; 10^{-7} and 10^{-6} M retinoic acid produced 50 and 85% inhibition, respectively (Fig. 2*c*). Retinoic acid treatment of control FR3T3 cells transfected with the neomycin marker resulted in an increase in EGF binding similar to that observed in Myc-1 cells; however, the increased binding did not alter the inability of these cells to grow in soft agar either in the presence or absence of added growth factors (not shown). This suggests that these effects of retinoic acid on increasing the number of EGF receptors of transfected FR3T3 cell lines are unrelated to its effects on EGF-dependent colony growth of the cells in soft agar.

Remarkably, colony formation stimulated by treatment of Myc-1 cells with PDGF and TGF-β is ~100-fold more sensitive to inhibition by retinoic acid than is the EGF-dependent colony formation (Fig. 2*c*). Thus retinoic acid (10^{-8} M) is a potent inhibitor of colony formation stimulated by treatment of the cells with PDGF and TGF-β (Fig. 2*b*). The inhibition could not be overcome by increasing the TGF-β concentration as high as 100 pM (Fig. 2*b*), and concentrations of retinoic acid as low as 10^{-9} M still inhibited colony formation by >90% (Fig. 2*c*). Retinoic acid had little or no effect on either the number or affinity of cellular TGF-β receptors (not shown).

To study separately the effects of retinoic acid on the action of either PDGF or TGF-β on Myc-1 cells, we examined the effects of 10^{-8} M retinoic acid on colony growth in the presence of EGF with additions of either PDGF or TGF-β (Fig. 3*a*, *b*). PDGF slightly potentiated the action of suboptimal levels of EGF on colony formation (Fig. 3*a*), whereas we had shown previously that TGF-β partially inhibits the effect of EGF on growth in soft agar (ref. 2 and Fig. 3*b*). Retinoic acid had either no effect or slightly stimulated the colony growth induced by PDGF in the presence of 42 pM EGF (Fig. 3*a*). However, retinoic acid synergized with TGF-β to abolish the colony formation induced by treatment of Myc-1 cells with 420 pM EGF (Fig. 3*b*). Thus, in the absence of retinoic acid, TGF-β (10–100 pM) inhibits EGF-dependent colony formation to a maximum of 60% (ref. 2 and Fig 3*b*), but when Myc-1 cells are treated with 10^{-8} M retinoic acid, which by itself has no effect on EGF-dependent colony formation (Fig. 2*a*), concentrations of TGF-β >~10 pM result in nearly total inhibition of the colony-forming response (Fig. 3*b*).

The discriminating action of low concentrations of retinoic acid on growth factor-dependent colony formation by Myc-1 cells was observed only in conditions of anchorage-independent growth. Growth of the cells in monolayer culture was consistently inhibited by TGF-β and either unaffected or stimulated by retinoic acid (10^{-7} M), regardless of the particular combination of growth factors added (Table 1). Thus, the selective action of retinoic acid cannot be related mechanistically to the growth of cells *per se*, but rather, specifically to its effects on processes

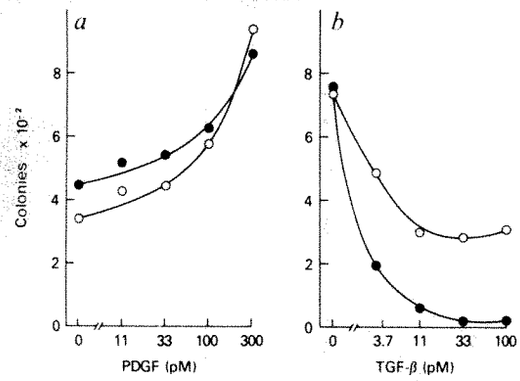


Fig. 3 Inhibition by retinoic acid is dependent on the presence of TGF-β. *a*, Myc-1 cells assayed in the presence of 42 pM EGF and increasing concentrations of PDGF. *b*, Colony growth assayed in the presence of 420 pM EGF and increasing concentrations of TGF-β. Myc-1 cells were assayed for colony formation in soft agar in the presence (●) or absence (○) of 10^{-8} M retinoic acid as described in Fig. 2 legend.

Table 1 Effects of retinoic acid on growth of Myc-1 cells in monolayer culture

Additions	No. of cells per dish ($\times 10^{-4}$) −Retinoic acid	+Retinoic acid (10^{-7} M)
None	6.6	6.5
EGF (800 pM)	10	13
PDGF (200 pM)	15	19
TGF-β (4 pM)	1.2	1.6
EGF+TGF-β	2.2	4.8
PDGF+TGF-β	7.3	13

Cells were seeded into 16-mm multiwell dishes at a density of 1×10^4 cells per well in 1 ml of Dulbecco's modified Eagle's medium containing 2% calf serum. Retinoic acid, in 20 µl 2% dimethyl sulphoxide in medium, and growth factors, in 10–20 µl 4 mM HCl, were added 16 h after seeding. Cell counts were determined in triplicate in a Coulter counter 4 days after seeding.

correlated with transformation of cells, namely anchorage independence[39].

Although PDGF, EGF and TGF-α have a consistent stimulatory effect on the growth of cells in both anchorage-dependent and independent conditions, the actions of TGF-β on cells are known to be bifunctional[2,40]. For example, we have shown here that TGF-β stimulates the anchorage-independent growth of Myc-1 cells in the presence of PDGF (Fig. 2*b*), but inhibits both the anchorage-dependent growth of the cells in the presence of PDGF or EGF (Table 1) and colony formation in the presence of EGF (Fig. 3*b*). Recently, TGF-β has been shown to be similar, or possibly identical, to a growth inhibitor isolated from African green monkey kidney cells[41]. The results presented here—that retinoic acid can selectively modulate the effects of TGF-β on *myc*-transfected cells—emphasize the distinctive mode of action of this growth factor and suggest that mitogenic effects of TGF-β on growth of cells in soft agar use different intracellular signalling pathways from those involved in transduction of mitogenic stimuli from either PDGF or EGF and their receptors. Most significantly, these results suggest that retinoic acid can be used to discriminate between different mechanisms of cellular transformation and between different pathways of growth factor action on cells.

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Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation

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Polyoma middle-T antigen is required for viral transformation of cultured cells and for tumorigenesis in animals. Like many other transforming gene products, middle-T is bound to the membrane and has an associated tyrosine kinase activity *in vitro*[1,2]. This activity seems to result from the interaction of middle-T with pp60$^{c\text{-}src}$, the cellular homologue of the transforming gene product of the Rous sarcoma virus, pp60$^{v\text{-}src}$ (refs 3–5). Both pp60$^{v\text{-}src}$ (ref. 6) and another retrovirus transforming gene product, pp68$^{v\text{-}ros}$ (ref. 7) were shown recently to have an associated phosphatidylinositol (PI) kinase activity *in vitro* and to increase PI turnover *in vivo*. These results suggest that viral transformation may be directly connected to a complex network of second messengers generated from PI turnover[8–18]. Here, we assayed for PI kinase activity in immunoprecipitates made with middle-T- or pp60$^{c\text{-}src}$-specific antisera of cells infected with polyoma virus. A PI kinase activity was detected in those immunoprecipitates which contained middle-T. Studies of mutants of middle-T defective in transformation indicate a close correlation between PI kinase activity and transformation.

Immunoprecipitates of polyoma middle-T from infected 3T3 cells catalyse phosphorylation of PI. Figure 1*a* shows the relative PI phosphorylating ability of immunoprecipitates obtained on treatment of mock-infected or polyoma-infected cells with polyoma-specific serum or preimmune serum. Immunoprecipitates made from polyoma-infected 3T3 cells with anti-polyoma serum (Fig. 1*a*, lane 4) produced 20-fold more phosphatidylinositol diphosphate (DPI) after a 20-min incubation than did immunoprecipitates made from mock-infected cells (lane 3). No increase in PI phosphorylation above background levels was seen when immunoprecipitates were made with preimmune serum (Fig. 1*a*, lanes 1, 2). Quantitation of the PI phosphorylation in 10 separate experiments gave 10–50-fold enhancement for middle-T immunoprecipitates over controls. Similar results were observed when polyoma-infected 3T6 cells, 293 cells infected with an adenovirus expressing the middle-T gene, and a polyoma middle-T transformed cell line (py14-2)[19] were compared with respective parent line or mock-infected controls (data not presented).

If the observed PI kinase activity is linked to the transformation activity of middle-T, virus mutations reducing transforming activity should reduce or eliminate the observed PI kinase activity of infected cell immunoprecipitates. Polyoma middle-T mutants defective in transformation fall into two categories: mutations that abolish transformation totally, like py1387T and NG59, and mutations that reduce but do not eliminate transforming activity, such as py1178T. py1387T contains a nonsense mutation which eliminates the last 37 amino acids at the C-terminus of middle-T, rendering it completely defective for transformation, membrane association and tyrosine kinase activity. Small-T is unaltered and large-T contains a single amino-acid substitution but is unaffected in its function[20]. Figure 1*b* shows the effect of this mutation on viral stimulation of immunoprecipitated PI kinase activity. Immunoprecipitates from cells infected with py1387T (Fig. 1*b*, lane 2) showed no increase in PI kinase activity over mock-infected control (lane *a*). Parallel infections and immunoprecipitations from ^{35}S-methionine-labelled cells indicated that py1387T-infected cells contain similar levels of large-T, middle-T and small-T to those seen in wild-type polyoma-infected cells (not shown). As judged by ^{3}H-thymidine uptake, py1387T and wild-type polyoma infection caused similar stimulation of DNA synthesis (data not shown). These results suggest that the PI kinase activity associated with polyoma immunoprecipitates may be linked to the transforming function of the virus and is not a consequence of cell stimulation by viral infection.

Experiments with the transformation-defective mutant NG59 indicate that the PI kinase activity associated with wild-type middle-T immunoprecipitates is not a consequence of membrane contaminants nonspecifically co-precipitated with membrane-bound middle-T. Like py1387T, NG59 is totally transformation defective, but unlike py1387T, the middle-T protein of NG59 associates with the membrane[21]. The mutation is a replacement of an aspartic acid residue at position 179 by isoleucine-asparagine. No PI kinase activity above background was detected using immunoprecipitates from cells infected with this virus (Table 1), even though ^{35}S-methionine labelling indicated that normal levels of mutant middle-T were present in the immunoprecipitates (not shown). Therefore, the PI kinase activity associated with middle-T is not merely a consequence of middle-T membrane association.

Unlike middle-T antigens produced by NG59 and py1387T mutants, which are totally inactive in transformation and in the standard middle-T tyrosine kinase assay *in vitro*, the middle-T product of the py1178T mutant virus (in which a Phe is substituted for Tyr 315) retains some associated tyrosine kinase activity and is partially defective in transformation[22,23]. If PI kinase activity plays an important part in transformation, then immunoprecipitates of middle-T prepared from cells infected with this virus should be less active in PI phosphorylation than wild-type immunoprecipitates; this was observed (Fig. 2*a*). Although py1178T immunoprecipitates contained some PI

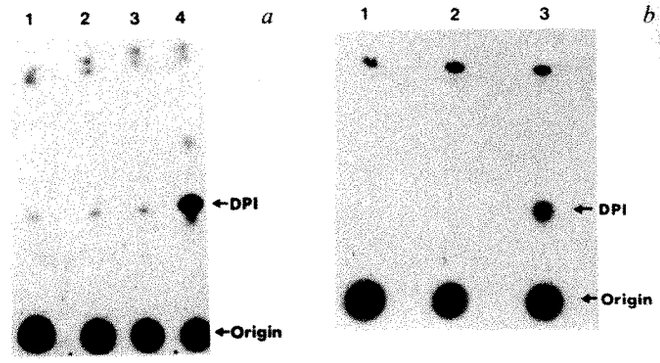


Fig. 1 Phosphorylation of phosphatidylinositol by polyoma immunoprecipitates. *a*, Autoradiograph of a TLC separation of the reaction products from PI kinase assays performed on immunoprecipitates. Lane 1, mock-infected NIH 3T3 cells precipitated with preimmune serum; lane 2, wild-type polyoma-infected cells precipitated with preimmune serum; lane 3, mock-infected cells precipitated with anti-polyoma serum; lane 4, wild-type polyoma-infected cells precipitated with anti-polyoma serum. *b*, Relative PI kinase activity of immunoprecipitates from transformation-defective versus wild-type viral infections. Immunoprecipitates using anti-polyoma serum were made and phosphorylation assays carried out as described below. Lane 1, immunoprecipitate from mock-infected cells; lane 2, immunoprecipitate made from cells infected with the transformation-defective mutant 1387; lane 3, immunoprecipitate from wild-type-infected cells. The position of migration of DPI is indicated. Spots present in all lanes near the top of the TLC are ATP contaminants which vary with the ATP shipment used, and do not depend on addition of either protein or phospholipid to the reaction mix. An additional spot appearing in *a*, lane 4, co-migrates with phosphatidic acid and seems to be due to the presence of a small amount of diacylglycerol in the added phosphatidylinositol.

Methods. NIH 3T3 cells, 24 h post-infection, were washed with cold phosphate-buffered saline, rinsed with 137 mM NaCl, 20 mM Tris *p*H 8, 1 mM $MgCl_2$, 1 mM $CaCl_2$ (buffer A), and lysed in buffer A containing 10% glycerol, 1% NP40, and 1 mM phenylmethylsulphonyl fluoride at 4 °C for 20 min. Insoluble material was removed by centrifugation at 4 °C for 10 min at 10,000*g*. The supernatants were incubated with 2 μl of rabbit anti-T serum (D. C. Pallas, M. E. Mahoney, D.R.K. and T.M.R., unpublished) or preimmune serum for 15 min at 25 °C. Sepharose Cl4B-protein A (Sigma) was added, and incubation continued for an additional 15 min. The immunoprecipitates were collected and washed once with cold phosphate-buffered saline, twice with 0.5 M LiCl, 0.1 M Tris *p*H 7.4, and finally with 10 mM Tris, 100 mM NaCl, 1 mM EDTA. Immunoprecipitates were then assayed for phosphatidylinositol phosphorylation activity: 1 mg ml^{-1} PI (Avanti) was dispersed by sonication for 15 min in 5 mM HEPES; the dispersed PI was preincubated at 4 °C with the immunoprecipitate for 20 min at a final concentration of 0.2 mg PI ml^{-1}. After preincubation, the phosphorylation reaction was started by addition of 20 μCi [γ-^{32}P]ATP and $MgCl_2$ to final concentrations of 50 μM ATP and 5 mM $MgCl_2$ in a volume of 50 μl. The reaction mixture was incubated for 20 min at 25 °C and terminated by addition of 100 μl of 1 M HCl. (Less than 10% of the ATP was consumed during the incubation as judged by chromatography on polyethyleneimine-impregnated cellulose in 2 M formate *p*H 3.5.) Phospholipids were then immediately extracted with 200 μl of $CHCl_3$/MeOH (1:1). The organic phase was washed once with 80 μl of MeOH/HCl (1:1) and spotted onto a silica gel 60 TLC plate (Merck) pretreated with 1% potassium oxalate. Phosphorylated products were separated by TLC in a $CHCl_3$/MeOH/4 M NH_4OH (9:7:2) developing solvent for 1 h and visualized by autoradiography. Unlabelled phospholipid standards were included and were visualized by exposure to iodine vapour. Identity of products was also confirmed by two-dimensional chromatography with cold standards; after chromatography in the first dimension in the system described above, plates were developed in the second dimension in $CHCl_3$/MeOH/CH_3COOH/H_2O (50:39:1:10) (not shown).

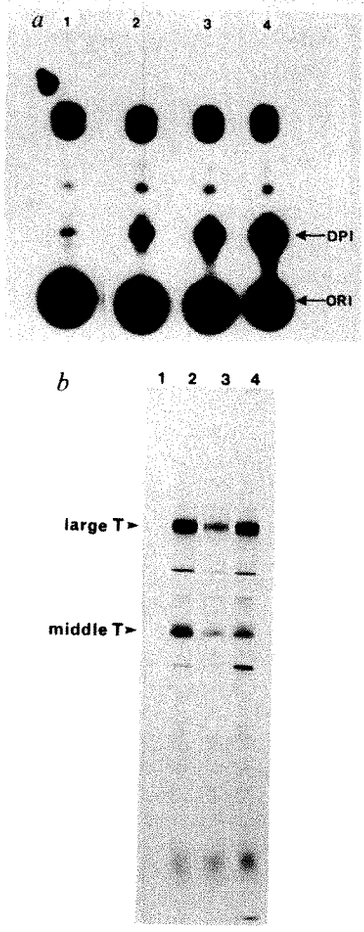


Fig. 2 Comparison of PI kinase activity and middle-T protein in immunoprecipitates from cells infected with wild-type and py1178T mutant of polyoma. NIH 3T3 cells were infected and immunoprecipitations and kinase reactions were performed as in Fig. 1 legend. The cells were labelled with 100 μCi ml^{-1} ^{35}S-methionine for 3 h before the immunoprecipitation. *a*, Autoradiogram for the PI kinase assay. Lane 1, mock-infected cells precipitated with anti-T serum; lane 2, py1178T-infected cells precipitated with anti-T serum; lane 3, cells infected with wild-type virus at 1/10th the py1178T virus multiplicity of infection; lane 4, cells infected with wild-type virus at the same multiplicity of infection as py1178T. *b*, Autoradiograph of ^{35}S-methionine-labelled proteins in the immunoprecipitates separated by SDS-polyacrylamide gel electrophoresis (10% acrylamide, procedure of Laemmli[33]). The order of lanes is the same as in *a*.

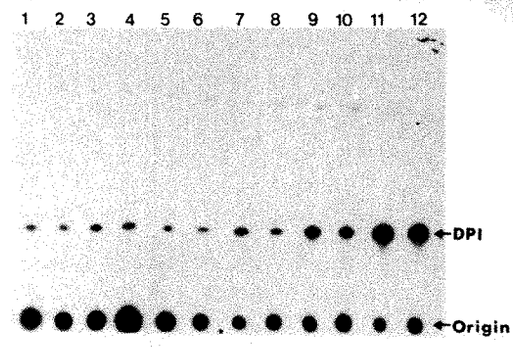


Fig. 3 PI kinase activity of immunoprecipitates made with anti-pp60$^{c\text{-}src}$ monoclonal antibody. Viral infections and immunoprecipitations were performed as described in Fig. 1 legend, except that supernatants were incubated with 3 μl of the anti-pp60$^{c\text{-}src}$ monoclonal antibody, GD11 (ref. 34), or an equal volume of rabbit preimmune serum for 30 min at 4 °C. PI kinase assays and product separations were performed as in Fig. 1. Lanes 1–6 are immunoprecipitates with control serum and lanes 7–12 are immunoprecipitates with GD11. Lanes 1, 2, 7 and 8 are from uninfected 3T3 cells; lanes 3, 4, 9 and 10 are from 3T3 cells infected with 1 plaque-forming unit per cell of wild-type polyoma virus; lanes 5, 6, 11 and 12 are from 3T3 cells infected with 10 plaque-forming units per cell of wild-type polyoma virus.

Table 1 Relative transformation abilities and PI kinase activities associated with middle-T mutants

Polyoma mutant	PI kinase activity	Transformation competence (ref.)	Association with pp60$^{c\text{-}src}$ (ref.)
RA (wild type)	100	++(29)	+(3, 4, 24)
Mock infected	7	—	—
py1387T	8	−(20)	+−/−(31)
NG59	10	−(25)	−(32)
dl45	165	++(30)	+(24, 31)
py1178T	23	+/−(22, 23)	+(31)

3T3 cells were either mock infected or infected with different polyoma mutants. Immunoprecipitations and kinase assays were carried out as described in Fig. 1 legend. For quantitation of PI kinase activity, the radioactive spot corresponding to DPI was cut out, and the silica gel was scraped into a scintillation vial and counted by liquid scintillation spectrometry. PI kinase activities are expressed as % of wild-type activity in immunoprecipitates where comparable amounts of the mutant middle-T protein to wild-type middle-T protein were present as judged by ^{35}S-methionine label. The values presented are the average of at least three separate experiments, except for d145, where the results of a single experiment are presented. References for the ability of the mutants to cause transformation and to associate with pp60$^{c\text{-}src}$ are given in parentheses.

kinase activity above background, this activity was reduced 5–10-fold relative to wild-type immunoprecipitates when infected cells were making comparable levels of middle-T, measured by incorporation of ^{35}S-methionine (Fig. 2*b*).

Cellular src (pp60$^{c\text{-}src}$) is associated with polyoma middle-T in immunoprecipitates from infected cells, suggesting that tyrosine phosphorylation of middle-T is catalysed by pp60$^{c\text{-}src}$ (ref. 3). Immunoprecipitates of pp60$^{c\text{-}src}$ were prepared from normal or polyoma-infected NIH 3T3 cells using the anti-pp60$^{c\text{-}src}$ monoclonal antibody GD11 (ref. 34). The immunoprecipitates from polyoma-infected cells (Fig. 3, lanes 11, 12) contained a PI kinase activity ~10-fold above controls (Fig. 3, lanes 5, 6). However, immunoprecipitates of pp60$^{c\text{-}src}$ from uninfected cells (Fig. 3, lanes 7, 8) or cells infected with py1387T (not shown) showed no detectable increase in PI kinase activity above controls. In five separate experiments, the pp60$^{c\text{-}src}$ immunoprecipitates from polyoma-infected cells showed a 5–20-fold stimulation in PI kinase activity compared with uninfected controls. There was no significant difference in the amounts of pp60$^{c\text{-}src}$ in immunoprecipitates from polyoma-infected cells and uninfected cells, in agreement with results of Bolen *et al.*[24]. Thus, middle-T increases the PI kinase activity of pp60$^{c\text{-}src}$ immunoprecipitates without increasing the amount of pp60$^{c\text{-}src}$.

Anti-T immunoprecipitates from cells infected with wild-type polyoma virus have at least 20-fold higher PI kinase activity than those from uninfected cells. This activity is correlated with the transforming ability of the virus. Non-transforming mutants NG59 (ref. 25) and py1387T (ref. 20) do not cause such an increase. As py1387T encodes normal large-T and small-T antigens, middle-T must be responsible for the associated PI kinase activity. py1178T has an intermediate phenotype. Its ability to transform F111 cells is severely impaired[23], but DNA containing the mutant sequence transforms Rat 1 cells normally[22]. Its PI kinase activity, like its transforming ability, is intermediate between those of wild-type and NG59 or py1387T. Like the pp60$^{v\text{-}src}$, some DPI kinase activity and diacylglycerol kinase activity is also associated with the middle-T immunoprecipitates, but these activities are at least fivefold lower than the PI kinase activity when compared at identical substrate and protein concentration (data not presented).

Parallel results were observed when the PI kinase experiments were carried out using anti-pp60$^{c\text{-}src}$ instead of anti-T serum. Background PI kinase activity made it difficult to decide whether pp60$^{c\text{-}src}$ from uninfected cells has PI kinase activity. Although the simplest model suggests that pp60$^{c\text{-}src}$ has a PI kinase activity which is activated by association with middle-T, the possibility that middle-T or an uncharacterized cellular protein associated with middle-T catalyses the PI phosphorylation cannot be excluded. Bolen *et al.*[24] have shown recently that pp60$^{c\text{-}src}$ immunoprecipitates have enhanced tyrosine kinase activity towards casein. Like pp60$^{v\text{-}src}$ (ref. 6) and pp68$^{v\text{-}ros}$ (ref. 7), the middle-T/pp60$^{c\text{-}src}$ complex may have broad specificity towards protein and lipid substrates. In our hands, the rate of phosphorylation of PI and the model substrate enolase are comparable, giving no clue to the relative importance of the two phosphorylation reactions.

The products of the phosphorylation reactions, phosphatidylinositol diphosphate and triphosphate, have themselves been implicated in the regulation of several membrane-bound enzymes, including the Ca^{2+}-ATPase[10,11] and a nuclear membrane RNA-dependent ATPase implicated in RNA transport[12,26,27]. Because polyphosphoinositides are the preferred substrates for phospholipase C[28], the increased PI kinase activity could raise cellular levels of the breakdown products diacylgycerol and inositol 1,4,5-trisphosphate; the latter compound regulates Ca^{2+} release from internal stores[8] and the former activates protein kinase C[14]. Increased levels of these potential second messengers could provide the pleiotropic response expected for transformation. Characterization of the effects of the middle-T antigen on *in vivo* PI metabolism will be required to confirm and define the physiological significance of this activity.

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Primary structure of the α-subunit of transducin and its relationship to *ras* proteins

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A group of membrane-associated guanine nucleotide binding proteins (G-proteins) are essential for transducing signals generated at cell-surface receptors into changes in cellular function and metabolism[1]. These proteins are a complex of three subunits designated α, β and γ. The α-subunit is responsible for binding guanine nucleotides and seems to be characteristic of each protein. Transducin, a member of this protein family, mediates visual transduction by coupling the signal of photolysed rhodopsin with activation of a cyclic GMP phosphodiesterase[2]. We have now cloned and sequenced the complementary DNA encoding the α-subunit of bovine retinal transducin and from this we have deduced the complete amino-acid sequence. The transducin α-subunit shares several homologous amino-acid sequences with *ras* gene products. The homologous segments correspond mostly to the regions thought to be involved in the guanine nucleotide binding and GTPase activity of *ras* proteins and to the ADP-ribosylation sites of the transducin α-subunit.

A library of cDNA clones was prepared using poly(A)$^+$ RNA from bovine retina as template and the plasmid expression vector pUC8 (refs 3, 4). Screening of the cDNA library[4] with monoclonal antibodies to the purified α-subunit of porcine retinal transducin yielded two immunologically positive clones from $\sim 8 \times 10^4$ transformants. One of these clones, pTα108, was found by nucleotide sequence analysis[5] to contain coding sequences for all six partial amino-acid sequences determined for the transducin α-subunit in the same reading frame (see below). To clone a longer cDNA sequence, we constructed another cDNA library derived from poly(A)$^+$ RNA from bovine retina, using the plasmid vector developed by Okayama and Berg[6]. Screening of this library[7] with a probe derived from the 5′-terminal region of the cDNA insert of clone pTα108 yielded 61 hybridization-positive clones from $\sim 6 \times 10^5$ transformants. From 36 randomly selected clones, we chose clone pTα13 for further study. It contained the whole cDNA sequence carried by clone pTα108 apart from the 3′-terminal 50-nucleotide sequence. The additional sequence in clone pTα108 seems to have resulted from a possible error during the construction of the cDNA expression library or DNA replication because the sequence was not present in any of the three other clones tested derived from the Okayama-Berg library.

Figure 1 shows the 2,446-nucleotide sequence (excluding the poly(dA) tract) of the cDNA encoding the α-subunit of bovine transducin, determined using clones pTα13 and pTα108. The reading frame corresponding to the six partial amino-acid sequences (amino acids 43-47, 83-96, 103-112, 139-157, 177-188 and 301-309) determined by peptide analysis (Fig. 3) was used to deduce the primary structure of the transducin α-subunit (Fig. 1). The translation initiation site was assigned to the methionine codon at nucleotides 1-3 because this is the first in-frame ATG triplet that occurs downstream of the nonsense codon TAG (nucleotide residues −81 to −79) and because the

Fig. 1 Nucleotide sequence of the cDNA for the bovine transducin α-subunit. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG triplet encoding the initiating methionine, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers; the number of the nucleotide residue at the right-hand end of each line is given. The deduced amino-acid sequence of the transducin α-subunit is shown above the nucleotide sequence, and amino-acid residues are numbered beginning with the initiating methionine. Nucleotide residue 2,309 in the cDNA insert of clone pTα13 is followed by a poly(dA) tract, which is connected with the vector DNA sequence[6,35]. The AATAAA sequence[36] is found 15 nucleotides upstream from the polyadenylation site. Nucleotides 840 (T), 1,137 (G) and 1,336 (G) have been taken from pTα13 (carrying nucleotides −137 to 2,309), whereas the corresponding residues of pTα108 (carrying nucleotides −30 to 1,453) are C, A and A, respectively; the difference in residue 840 results in no amino-acid substitution.

```
                                                                                                                5'-----ACAGCCATTGGATTCCC  -121
AGTTCTGGGGTCAGAAGGGTGTCCAGGGAGGCCAGAGGCTAGCCTGAGCCTGGCTCTGGGGAACCCCTCCATCCGGAAGAACCAACTCACGGCCCTCCTCCTTCACCTCCTGCCAGGACC    -1
  1                                  10                                  20                                  30
Met Gly Ala Gly Ala Ser Ala Glu Glu Lys His Ser Arg Glu Leu Glu Lys Lys Leu Lys Glu Asp Ala Glu Lys Asp Ala Arg Thr Val
ATG GGG GCT GGG GCC AGC GCT GAG GAG AAG CAC TCA AGG GAG CTG GAA AAG AAG CTG AAA GAA GAT GCT GAG AAA GAT GCT CGA ACC GTG    90
                                  40                                  50                                  60
Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Gln Asp Gly Tyr Ser Leu Glu
AAA CTG CTG CTT CTG GGT GCC GGT GAA TCC GGG AAG AGT ACC ATT GTC AAG CAG ATG AAG ATT ATC CAC CAG GAC GGG TAC TCA CTG GAA   180
                                  70                                  80                                  90
Glu Cys Leu Glu Phe Ile Ala Ile Ile Tyr Gly Asn Thr Leu Gln Ser Ile Leu Ala Ile Val Arg Ala Met Thr Thr Leu Asn Ile Gln
GAG TGT CTT GAG TTC ATT GCC ATC ATC TAT GGC AAC ACG CTA CAG TCC ATC CTG GCC ATT GTG CGC GCC ATG ACC ACA CTC AAC ATC CAG   270
                                 100                                 110                                 120
Tyr Gly Asp Ser Ala Arg Gln Asp Asp Ala Arg Lys Leu Met His Met Ala Asp Thr Ile Glu Glu Gly Thr Met Pro Lys Glu Met Ser
TAC GGA GAC TCT GCG CGC CAG GAC GAC GCC CGA AAG CTG ATG CAC ATG GCA GAC ACC ATC GAG GAG GGC ACG ATG CCC AAG GAG ATG TCA   360
                                 130                                 140                                 150
Asp Ile Ile Gln Arg Leu Trp Lys Asp Ser Gly Ile Gln Ala Cys Phe Asp Arg Ala Ser Glu Tyr Gln Leu Asn Asp Ser Ala Gly Tyr
GAC ATC ATC CAG CGG CTG TGG AAG GAC TCC GGT ATC CAG GCC TGT TTC GAC CGA GCC TCA GAG TAC CAG CTC AAC GAC TCT GCT GGC TAC   450
                                 160                                 170                                 180
Tyr Leu Ser Asp Leu Glu Arg Leu Val Thr Pro Gly Tyr Val Pro Thr Glu Gln Asp Val Leu Arg Ser Arg Val Lys Thr Thr Gly Ile
TAT CTC TCA GAC CTG GAG CGC CTG GTA ACC CCG GGC TAC GTG CCC ACT GAA CAG GAT GTG CTG CGC TCC CGT GTC AAG ACC ACG GGT ATC   540
                                 190                                 200                                 210
Ile Glu Thr Gln Phe Ser Phe Lys Asp Leu Asn Phe Arg Met Phe Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys
ATT GAG ACG CAG TTC TCC TTC AAG GAC CTC AAC TTT CGG ATG TTC GAT GTG GGC GGG CAG CGC TCA GAG CGC AAG AAG TGG ATC CAC TGC   630
                                 220                                 230                                 240
Phe Glu Gly Val Thr Cys Ile Ile Phe Ile Ala Ala Leu Ser Ala Tyr Asp Met Val Leu Val Glu Asp Asp Glu Val Asn Arg Met His
TTC GAG GGG GTG ACC TGC ATC ATC TTC ATC GCG GCG CTG AGC GCC TAC GAC ATG GTG CTG GTG GAA GAC GAC GAA GTG AAC CGC ATG CAC   720
                                 250                                 260                                 270
Glu Ser Leu His Leu Phe Asn Ser Ile Cys Asn His Arg Tyr Phe Ala Thr Thr Ser Ile Val Leu Phe Leu Asn Lys Lys Asp Val Phe
GAG AGC CTG CAC CTG TTC AAC AGT ATC TGC AAC CAC CGC TAC TTC GCC ACC ACG TCC ATC GTG CTC TTT CTC AAC AAG AAG GAC GTC TTC   810
                                 280                                 290                                 300
Ser Glu Lys Ile Lys Lys Ala His Leu Ser Ile Cys Phe Pro Asp Tyr Asn Gly Pro Asn Thr Tyr Glu Asp Ala Gly Asn Tyr Ile Lys
TCG GAG AAG ATC AAA AAG GCG CAC CTT AGT ATC TGC TTT CCG GAC TAC AAC GGG CCC AAC ACG TAT GAG GAC GCC GGC AAT TAC ATC AAG   900
                                 310                                 320                                 330
Val Gln Phe Leu Glu Leu Asn Met Arg Arg Asp Val Lys Glu Ile Tyr Ser His Met Thr Cys Ala Thr Asp Thr Gln Asn Val Lys Phe
GTG CAA TTC CTT GAG CTC AAC ATG CGA CGC GAC GTG AAG GAG ATC TAT TCC CAC ATG ACA TGC GCC ACC GAC ACG CAG AAC GTC AAG TTT   990
                                 340                                 350
Val Phe Asp Ala Val Thr Asp Ile Ile Ile Lys Glu Asn Leu Lys Asp Cys Gly Leu Phe
GTC TTC GAC GCT GTC ACC GAC ATC ATC ATC AAG GAG AAC CTC AAA GAC TGC GGG CTC TTC TGA GGTGCCTGAGCTCATGCGTCCCTGAGACCCTGCAGC  1089
CCTTGACACCTTGTAGCCCCAATGTGCATGACCCTATCAGTCCCCCAGGACTCCTGGGCTCCAGCCTGTGGCCCCACCAGCCACACACTCAAAGCCCTAAACCCTGCTAGCCTTGAGGCC  1209
CTATAACCCTCCCTCGTGGCTCCCAGGATTCCCAGGTCTCCGAGAGTCCAATGCCCCCAGCCACCCCAGCACTGCCCTTCACGGCCTGGCTGCACTGGCCTCCCAGACCTACCACAGCCA  1329
GCCCCAGCTAGAAGCAAGCAGGACTTGGGGCAGCTACAGCTCACGGTGACTCAGCAATACCTCTACTGACCAATCTCCTGCCTCTCTTCTGCCCCAGGAGGCCCATTACTCACCTGGTGA  1449
GTCTGGGTTCAGCAAACAGCCCTCCCTCCCAGAGCTTTATGAAGGGCAGGGGGCAGGCATGGAGCCCTTCTCTGAGCAAGCCTTCTGCTTACCATCAGCCCATTCTAGCCTTACCACCTC  1569
CATGTGACAGGGTCTGATGCCCATCTGCCCAGTGGCCAGTGACTGCCCTGCCCAGCAGATTCAGGAGTCCAAAGTTCAAGCCAGCTCTGGCCTGTGCAGCTTGTATGGAGACGACTTAGT  1689
GAGTCAGGGCAAGCCTGGGGGCTCCTGGTAGCAGGAAGAGACTCAAGCTGGAAACTTCCCGTACCCTAACCCCTCCCCCCAGAGGCTCTGCCCACGCCGTCCTTGGACACCAGAGTCATG  1809
TCCTACTCCCCTCCAGCCCGCGGCACTCTAGATCAGCCACTCTGTCGAGGGAGGGTACTGTTGACACCAGTTGTGGCCAATGGTCACACTGTCCTGAGGGGTGGCTGGGAGCAGAGTCCG  1929
GGTCCCATCGATCAGCCCCAGGCAGAGGATGTGAGACCGGAAGGAAGCCCAGATTGTGCCCAGAGTGGTCTGCCTCAGGTAGGGTCAAAAGCAAATCCATGTGACCCCCTTGTCCCTGCC  2049
AGCAGCTTTTCTCACCCTTCCCATCAGGGCCCTGGTAGGAAAAAAAAAAAAAGTCATGCCTGAACCATCAGGGACAAAAGCCCATGTCCCCTTACCTCTGCTCCCCACTTCCTCATCAAGT  2169
ACCTAGTCCTTGCTGATGCCCATGGGCCGTAGCCCGTGCTGGAGTCAAGGGCAAGGAACATCCGTCTAACAAGTCCAGGGCATAATTTGCACTCCCTTCAGCTAGATACACAAACTTAGC  2289
AATAAACCTTTGCGTCAGGC-----3'
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described previously[39]. Using 34 μg of poly(A)$^+$ RNA, 805 ng of cDNA was obtained. The screening was carried out by incubation with a mixture of two monoclonal antibodies to the porcine transducin α-subunit (IgG2b isotype), then with ^{125}I-protein A (NEN); the monoclonal antibodies were prepared by the hybridoma technique[40]. Clone pTα108 was thus isolated. For the isolation of clone pTα13, a cDNA library was constructed by the method of Okayama and Berg[6], using 7.8 μg of poly(A)$^+$ RNA and 4.2 μg of the vector-primer DNA . The procedures of transformation and screening were as described previously[39]; the *Ava*II(−4)/*Ava*II(565) fragment from pTα108, labelled by nick-translation[41] with [α-^{32}P]dCTP, was used as hybridization probe.

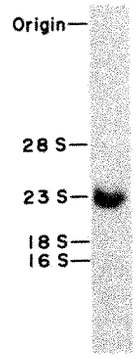


Fig. 2 Autoradiogram of blot hybridization analysis of bovine retinal RNA with a cDNA probe. Poly(A)$^+$ RNA (20 μg) was analysed by the procedure of Thomas[9]. The hybridization probe used was the *Eco*RI/*Sal*I-excised cDNA insert of clone pTα108, labelled by nick-translation[41] with [α-^{32}P]dCTP. The size markers used were bovine and *Escherichia coli* ribosomal RNA[45]. A hybridization-positive band of the same size was observed when the *Ava*II(−4)/*Ava*II(565) fragment derived from pTα13 was used as probe.

second ATG triplet encodes amino acid 49, which is preceded by one of the peptide sequences determined. A translation termination codon (TGA) occurs in-frame after the 350th codon specifying phenylalanine. Thus, we conclude that the bovine transducin α-subunit is composed of 350 amino-acid residues (including the initiating methionine) and has a calculated relative molecular mass of 39,964, which agrees with the reported value[2,8]. Blot hybridization analysis[9] of bovine retinal poly(A)$^+$ RNA with cDNA probes showed a hybridization-positive RNA species of ~2,900 nucleotides (Fig. 2).

The *ras* gene products, like the α-subunits of transducin and related proteins, are membrane-associated proteins that bind guanine nucleotides and have GTPase activity[10–13]. Comparison of the amino-acid sequences of the transducin α-subunit and the *ras* protein family reveals several homologous regions (Fig. 4*a*–*f*). Interestingly, regions *a*, *b* and *d* of *ras* p21 proteins correspond to the regions considered to be involved in guanine nucleotide binding. Region *a* of p21 corresponds to the sequence surrounding Gly 12; this sequence exhibits homology with the nucleotide binding region of the β-subunit of ATP synthase[14] as well as of several dinucleotide binding enzymes[15]. Region *b* of some p21 proteins contains a threonine at position 59 which is autophosphorylated by GTP (refs 11, 16). Furthermore, such proteins show a lower GTP binding affinity than do proteins with alanine at position 59 (ref. 13). Region *d* constitutes part of the homologous segment found in G-proteins[17] which has been suggested to lie adjacent to the nucleotide binding site in elongation factor-Tu[18]. Thus, regions *a*, *b* and *d* of the transducin α-subunit may also contribute to guanine nucleotide binding. Furthermore, mutations causing amino-acid substitutions at positions 12, 13, 59, 61 or 63 of p21 are known to enhance the transforming potential of *ras* genes[19–28] and p21-associated GTPase activity is selectively impaired by an amino-acid substitution at position 12 (ref. 13). Thus, region *a* of the transducin α-subunit, like that of *ras* proteins, may be involved in GTPase activity. Region *c* of the transducin α-subunit contains the tetrapeptide sequence Ser-Arg-Val-Lys, which has been identified as the site that is ADP-ribosylated by cholera toxin[29]. Region *f* represents the carboxy-terminal tetrapeptide sequence of both the transducin α-subunit and the *ras* proteins (except for the YP2 product) and in the transducin α-subunit, constitutes part of the peptide carrying the site that is ADP-ribosylated by islet-activating protein[30]. It has been proposed that the conserved cysteine in this region is involved in the post-translational events leading to the biologically active membrane-associated form of

p21 (ref. 31). Thus, the regions conserved between the transducin α-subunit and the *ras* proteins seem to correspond to the functional regions of the two groups of G-proteins.

Analysis of the amino-acid sequence of the transducin α-subunit reveals two highly hydrophobic regions with predicted secondary structure[32,33] which corresponds to continuous stretches of uncharged amino acids, including many nonpolar residues (residues 65–81 and 213–226). These regions may be involved in hydrophobic interaction with the other transducin subunits or with photolysed rhodopsin or the phosphodiesterase. Alternatively, they may contribute to the association of the protein with the plasma membrane.

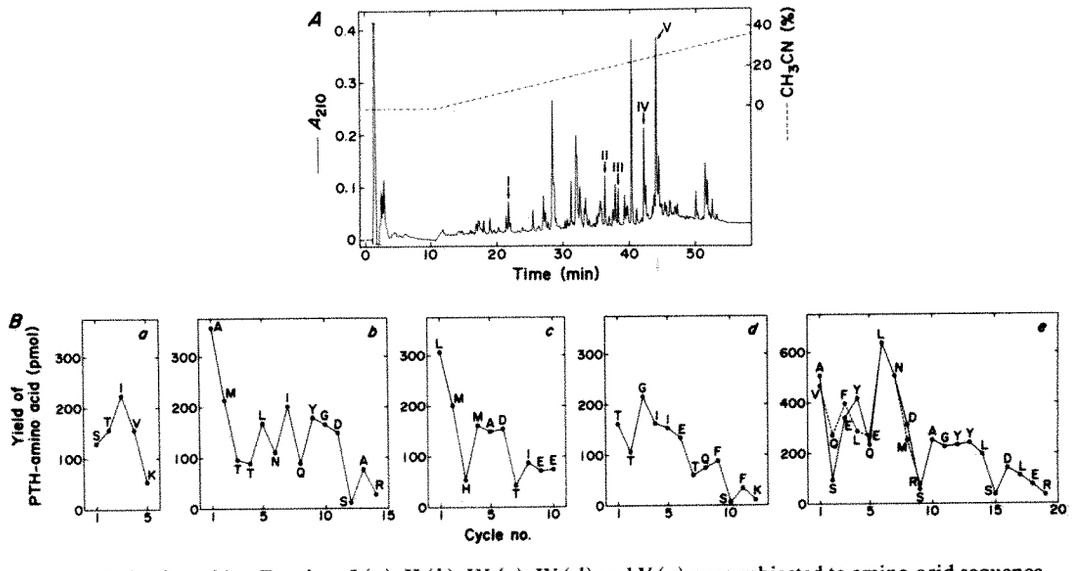


Fig. 3 Partial amino-acid sequence analysis of the α-subunit of bovine transducin. ***A***, Reverse-phase HPLC of tryptic peptides. The tryptic digest was loaded directly onto a Chemcosorb 3μ C_{18}-H column (8 × 75 mm; Chemco) equilibrated with 0.1% trifluoroacetic acid, and a linear gradient of 0–60% acetonitrile was applied over 80 min at a flow rate of 2 ml min^{-1}; a Hitachi 635 HPLC system with a solvent programmer was used. The eluate absorbance at 210 nm was recorded with a Hitachi 41 ultraviolet monitor. Five fractions (I–V) corresponding to absorbance peaks were collected. ***B***, Sequence analysis of peptides. Fractions I (*a*), II (*b*), III (*c*), IV (*d*) and V (*e*) were subjected to amino-acid sequence analysis with a gas-phase sequencer (model 470A, Applied Biosystems). Phenylthiohydantoin (PTH)-amino acids were analysed by HPLC at 50 °C using an Ultrasphere ODS column (4.6 × 250 mm; Altex) with a three-solvent gradient (acetonitrile, water and 40 mM sodium acetate buffer, *p*H 5.6) at a flow rate of 1 ml min^{-1}. The yield of PTH-amino acids at each cycle of Edman degradation is shown; the one-letter amino-acid notation is used. Fraction V was a mixture of two peptides, whose sequences were assigned as indicated, assuming that leucine and asparagine are shared by the two peptides at the sixth and seventh amino-acid positions, respectively.

Methods. Transducin was partially purified from rod outer segments, prepared from frozen bovine retinas, according to the procedure of Baehr *et al.*[8]. Approximately 20 mg of the partially purified transducin derived from about 1,000 retinas was subjected to electrophoresis on SDS-polyacrylamide gels[43] to purify each subunit; electroelution[44] from the gels yielded 620 μg, 780 μg and 300 μg of the α-, β- and γ-subunits, respectively. Approximately 120 μg of the purified α-subunit was dialysed against water, lyophilized and dissolved in 830 μl of 20 mM Tris-acetate buffer, *p*H 7.8, containing 2 mM $CaCl_2$. TPCK-treated trypsin (Worthington) was added at a transducin α-subunit/trypsin ratio of 30 (w/w). After incubation at 37 °C for 12 h, a further identical aliquot of TPCK-treated trypsin was added, and the incubation was continued for an additional 12 h.

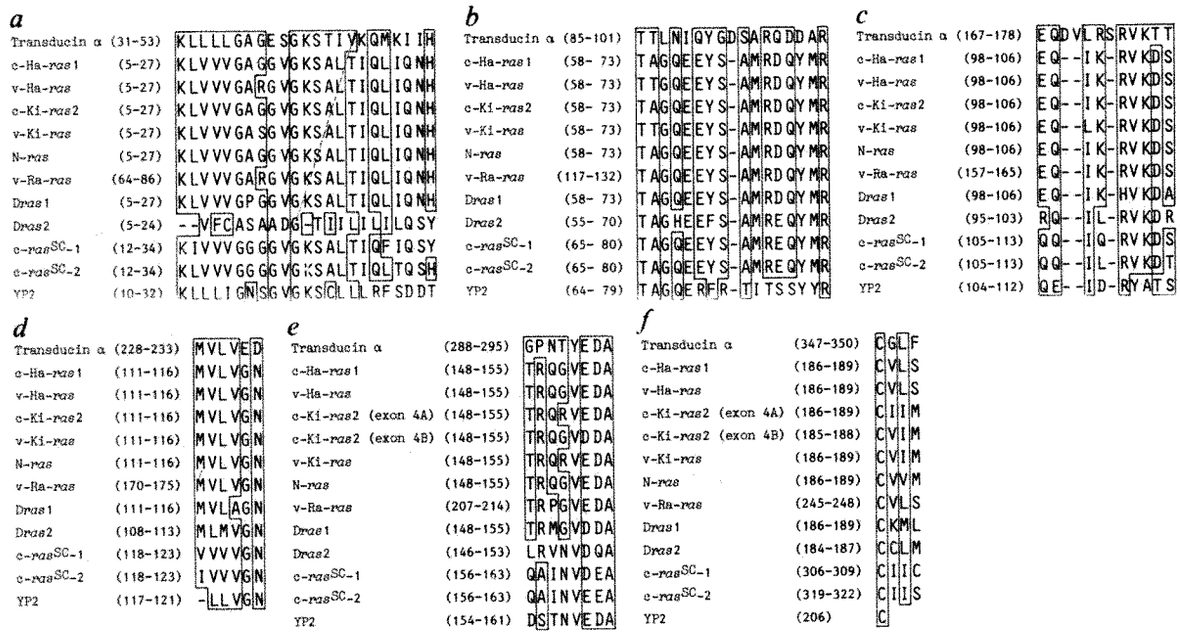


Fig. 4 Amino-acid sequence homology in different regions (*a*–*f*) between the transducin α-subunit and *ras* proteins. The sequences of the *ras* gene products have been taken from the literature: c-Ha-*ras*1 (ref. 46); v-Ha-*ras* (ref. 47); c-Ki-*ras*2 (ref. 25); v-Ki-*ras* (ref. 48); N-*ras* (ref. 26); v-Ra-*ras* (ref. 49); D*ras*1 and D*ras*2 (ref. 50); c-ras^{SC}-1 (refs 51, 52); c-ras^{SC}-2 (ref. 52); YP2 (ref. 53). The one-letter amino-acid notation is used. Amino-acid residues are numbered beginning with the initiating methionine of the respective polypeptides, and the residues constituting each aligned sequence are indicated by their numbers. Identities or favoured substitutions, as compared with the residues of the transducin α-subunit, are boxed. Favoured substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W (ref. 54). Gaps (-) have been inserted to achieve maximum homology. In *e* and *f*, both the sequences encoded by exon 4A and exon 4B of c-Ki-*ras*2 are given.

Partial amino-acid sequences of the bovine transducin α-subunit reported recently by Hurley *et al.*[34] correspond, with some undetermined residues, to residues 19-51, 205-226 and 311-350 of our deduced amino-acid sequence. These authors have also noted the sequence homology between the transducin α-subunit and *ras* proteins in the region surrounding Gly 12 of p21.

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Translocation of vesicles from squid axoplasm on flagellar microtubules

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Directed intracellular particle movement is a fundamental process characteristic of all cells. During fast axonal transport, membranous organelles move at rapid rates, from 1 to 5 $\mu m\ s^{-1}$, in either the orthograde or retrograde direction along the neurone and can traverse distances as long as 1 m (for reviews, see refs 1–3). Recent studies indicate that this extreme example of intracellular motility can occur along single microtubules[4], but the molecules generating the motile force have not been identified or localized. It is not known whether the force-transducing 'motor' is associated with the moving particle or with the microtubule lattice. To distinguish between these hypotheses and to characterize the membrane–cytoskeletal interactions that occur during vesicle translocations, we have developed a reconstituted model for microtubule-based motility. We isolated axoplasmic vesicles from the giant axon of the squid *Loligo pealei* as described previously[5]. The vesicles (35–475 nm in diameter) were then added to axonemes of *Arbacia punctulata* spermatozoa that served as a source of microtubules. Axonemes were used because the tubulin subunit lattice of the A-subfibre of a given outer doublet is the same as the subunit lattice of neuronal microtubules along which motility occurs[6,7]. Moreover, all the microtubules of a single axoneme show the same structural polarity[8–10], indicating that the axoneme represents an oriented microtubule substrate. Here we demonstrate that vesicle motility is ATP-dependent, that it is not mediated by the flagellar force-transducing molecule dynein and that the direction of movement is not specified by microtubule polarity.

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When axoplasmic vesicles are added to flagellar microtubules in the presence of ATP, the vesicles move along the axonemal microtubules in either direction on a single axoneme. Figure 1*a–c* shows a vesicle (arrowhead) that has moved to the end of the axoneme and has remained attached on reaching the end. Figure 1*d–f* shows another vesicle that became associated with the same axoneme 8 minutes later, and the vesicle travelled in the opposite direction to that shown in *a–c*. The axonemes used in Fig. 1 were prepared from *Arbacia* spermatozoa by dialysis against low ionic strength Tris-EDTA buffer to remove the dynein arms[11]. SDS-polyacrylamide gel electrophoresis (Fig. 2) demonstrates that the dynein has been removed from the axonemes shown in Fig. 1. (Figure 2*d* shows the polypeptide profile of the axonemes before Tris-EDTA dialysis; lane *e* shows these axonemes after the dialysis, whereas lane *f* shows the proteins removed from the axonemes by the dialysis treatment.) These data demonstrate that the axonemes supporting vesicle motility lack dynein, the flagellar force-transducing molecule.

To examine the vesicle–microtubule interaction illustrated in Fig. 1 at higher resolution, a sample of axonemes and associated vesicles was fixed and prepared for thin-section electron microscopy (Fig. 3). The vesicle in Fig. 3*a* is in association with an axoneme; *b* illustrates a representative field of axonemes and a vesicle attached to a microtubule. Figure 3*c* shows that the vesicle membrane contacts the microtubule by crossbridging filaments. The distance between the crosslinks varies from 14 to 20 nm. Those segments of microtubules that are not in association with a vesicle lack these filamentous crosslinks. The other projections seen on the axonemal microtubules have the periodicity and appearance of radial spokes[12] and are unaffected by the low ionic strength dialysis that removes the dynein arms. Both the crossbridging filaments and the axonemal projections lack the tilt and periodicity (24 nm) expected for dynein. The sample used to generate Fig. 3 was obtained from the same axoneme/vesicle preparation as in Fig. 1. For electron microscopy, however, axonemes and vesicles were combined and fixed in solution. Thus, although Fig. 3 shows apparent inter-

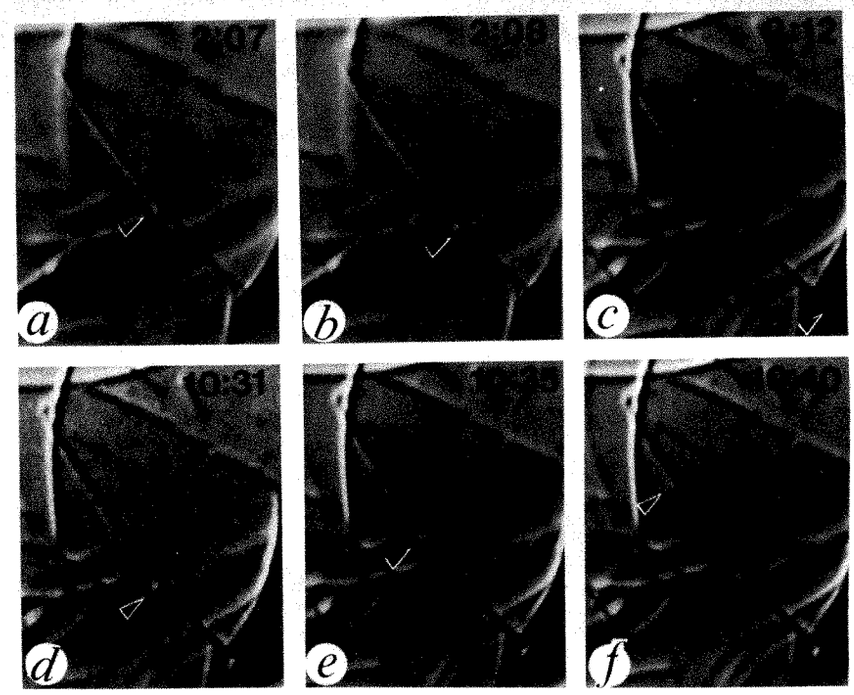


Fig. 1 Video micrograph of two vesicles moving on flagella axonemal microtubules in the absence of flagellar dynein. The sequence in *a–c* demonstrates a vesicle (arrowhead) that became attached to the axoneme at ~2 min after the vesicles were added to the axonemal microtubules. The sequence in *d–f* shows another vesicle (arrowhead) that became attached to the same axoneme 8 min later and moved in the opposite direction. The time (min:s) of each photograph of the video record is indicated. Scale bar, 1.2 μm.

Methods. Axonemal microtubules were prepared from *Arbacia* spermatozoa and dialysed against 1 mM Tris-HCl *p*H 8.0, 0.1 mM EDTA, 0.1% 2-mercaptoethanol to remove the dynein[11]. The axonemes were collected by centrifugation and washed 2–3 times in HEPES buffer (10 mM HEPES *p*H 7.0, 0.1 M KCl, 5 mM $MgSO_4$, 0.5 mM EDTA, 1 mM dithiothreitol). Washed axonemes were allowed to settle on a no. 0 coverslip and any unattached axonemes were removed by washing with the HEPES buffer. The preparation was covered with a second coverslip and transferred to the microscope for observation. Axoplasmic vesicles were isolated from the giant axon of the squid *Loligo pealei* using discontinuous sucrose gradient centrifugation as described previously[5]. These vesicles in buffer (0.3 M sucrose, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EGTA, 1 mM ATP, 1 mM phenylmethylsulphonyl fluoride, 10 mM imidazole, *p*H 7.4) were added at the edge of the coverslip 'sandwich' containing attached axonemes and washed across the preparation by absorbing buffer with filter paper at the opposite side of the coverslip. Observations of the vesicle addition and of the vesicle movement were made by video-enhanced contrast differential interference contrast microscopy[18].

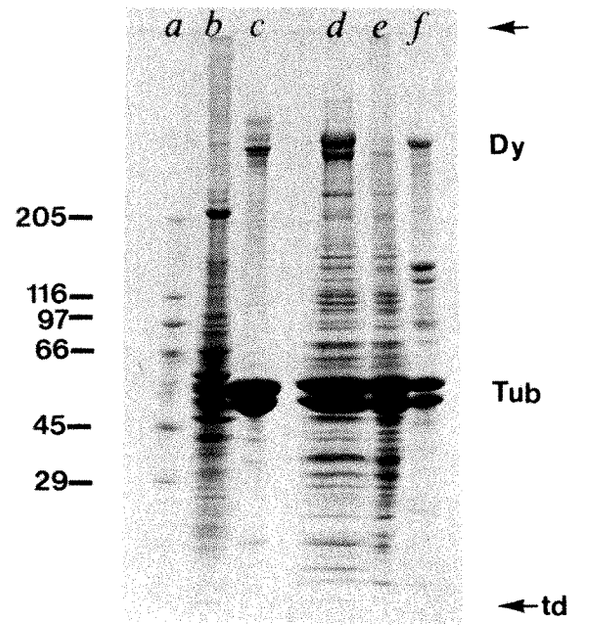


Fig. 2 SDS-polyacrylamide gel electrophoretic analysis of the protein composition of the axonemal microtubule preparation. Lanes *a*, *b* and *c* show various standards: *a*, relative molecular mass markers ($\times 10^{-3}$); *b*, squid axoplasm; *c*, purified mammalian brain microtubules. Lane *d* demonstrates the polypeptide composition of the axoneme preparation before the Tris-EDTA dialysis; lane *e*, the axonemes after dialysis and centrifugation, showing the loss of dynein (Dy) from the axonemal microtubules. (This sample (lane *e*) is from the same preparation as that used as the source of axonemes in Fig. 1.) Lane *f* shows the proteins removed from the axonemes by the dialysis step. Arrows indicate the top and bottom of the 3–15% acrylamide/12–48% urea linear gradient slab gel, using the buffer formulation of Laemmli[19] and stained for protein with Coomassie brilliant blue. Tub, tubulin; td, tracking dye.

action between a moving vesicle and an axoneme, we did not determine whether this vesicle was moving at the time of fixation.

We have found that axoplasmic vesicles move along: (1) outer doublets; (2) outer doublets treated with 0.5 M KCl to remove the outer dynein arms[13]; and (3) axonemes dialysed against Tris-EDTA to remove both the outer and inner dynein arms[11], but they do not move along intact flagella (that is, axonemes with the flagellar membrane present) or on glass fibres. Moreover, neither large unilamellar liposomes (100–400 nm in diameter; phosphatidylserine/phosphatidylcholine, 2:1) nor microspheres (200 and 400 nm in diameter) move along axonemes in the buffer that supports vesicle movement. Axonemes without added vesicles never demonstrate particle movement in the presence of ATP.

Rates of vesicle movement on axonemes subjected to various treatments were quantitated from video records[5]. Figure 4 illustrates similar rates of vesicle movement in three different axonemal preparations. The rates in *a* describe vesicles moving on outer doublets. The outer doublets were prepared by treating axonemes with trypsin to hydrolyse the nexin links, followed by reactivation with ATP to cause the microtubule outer doublets to slide relative to one another[14,15]. This results in the loss of the axonemal 9+2 configuration, but the dynein arms remain intact. The axonemal preparation in Fig. 4*b* was obtained by treating outer doublets (isolated as described for *a*) with 0.5 M KCl to remove the outer dynein arms[13]. The axonemes of Fig. 4*c* were dialysed against low ionic strength Tris-EDTA to remove both the outer and inner dynein arms[11].

Vesicles were observed to move in both directions on a single axoneme (Fig. 1). These observations indicate that the polarity of the microtubule did not dictate the directionality of vesicle movement because all the microtubules of a single axoneme have the same polarity[8–10]. Most vesicles do not change their direction of travel once in association with the axoneme, although we observed one vesicle that stopped and changed direction of translocation along the axoneme and some vesicles that changed from one axoneme to another and continued moving. However, the rates of movement were the same both for a vesicle that moved on two different axonemes and for several vesicles on the same axoneme. Furthermore, experiments

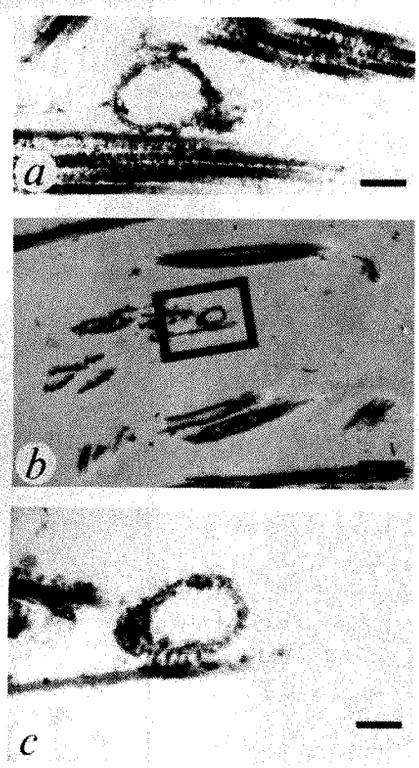


Fig. 3 Transmission electron micrograph illustrating vesicle association with axonemal microtubules. *a*, The vesicle (230 × 346 nm) illustrated is in contact with an axoneme. *b*, A typical field of axonemes with one vesicle in association with an axonemal microtubule. *c*, Higher magnification of the area enclosed in the rectangle in *b*. The vesicle (180 × 309 nm) is in association with an axonemal microtubule (24 nm) and seems to be attached by cross-bridging filaments; the spacing between the filaments is 14–20 nm. **Methods.** Vesicles and axonemes were prepared as described in Fig. 1 legend. Vesicles in buffer were added to axonemes and incubated for 5 min to allow vesicles to associate with axonemal microtubules. The preparation was fixed in 2% glutaraldehyde, 1% tannic acid, 1 mM $MgSO_4$, 0.1 M sodium phosphate buffer, *p*H 6.8 at 4 °C and post-fixed in 1% osmium tetroxide at 4 °C. The sample was dehydrated in ethanol, transferred through three changes of propylene oxide and embedded in LX-112 (Ladd Research Industries, Burlington, Vermont). Thin sections (60–70 nm) were stained with saturated aqueous uranyl acetate followed by lead citrate[20] and observed with a JEOL 100CX electron microscope at an accelerating voltage of 60 kV. *a*, Scale bar 150 nm; *b*, scale bar 500 nm; *c*, scale bar 100 nm.

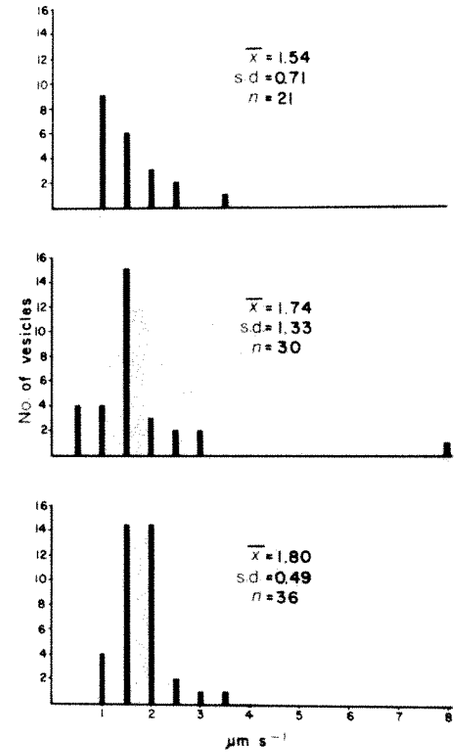


Fig. 4 Rates of vesicle movement on axonemal microtubules. *a*, Rates of vesicle movement occurring on axonemal outer doublet microtubules obtained by treating axonemes with trypsin followed by soybean trypsin inhibitor and reactivation with ATP. This treatment caused the outer doublet microtubules to slide apart out of the normal 9 + 2 configuration of the axoneme[14]. *b*, Rates of movement of vesicles on outer doublets obtained as in *a*, followed by treatment with 0.5 M KCl to remove the outer dynein arms[13]. *c*, Rates of movement of vesicles on intact axonemes dialysed against Tris-EDTA to remove the outer and inner dynein arms[11]. The three mean rates ($\bar{x}$) are not significantly different at a *P* of 0.05 as determined by either Student's *t*-test or Mann–Whitney *U* test. $\bar{x}$, Mean; s.d., standard deviation; *n*, sample size.

designed to test the competency of axonemes to support vesicle motility demonstrate that all axonemes tested support vesicle movement.

The results presented here demonstrate clearly that vesicles from squid axoplasm move on flagellar axonemal microtubules at the same rate in the presence or absence of flagellar dynein. Thus, vesicle motility is not mediated by the axonemal force-generating molecule dynein. In addition, the vesicle motility seems to be a different phenomenon from that studied by Bloodgood *et al.* in which polystyrene microspheres move on *Chlamydomonas* flagella[16,17]. The ATPase mediating the motility of microspheres on *Chlamydomonas* flagella demonstrates a strict dependence on both the flagellar membrane and Ca^{2+}. In contrast, axoplasmic vesicles do not move on intact flagella (that is, axonemes with membrane in place); furthermore, vesicle motility on axonemal microtubules occurs in the presence of 1 mM EGTA (that is, in the absence of calcium). The results presented here, when taken together with those of Gilbert and Sloboda[5], suggest that key vesicle surface proteins interact with the microtubule to effect vesicle movement. However, it is possible that there is an as yet uncharacterized ATPase associated with the axoneme that remains bound under conditions that remove flagellar dynein from axonemal microtubules. Alternatively, a soluble axoplasmic ATPase not associated with the membrane may co-purify with the vesicles and mediate vesicle movement. The results reported here do not distinguish between these possibilities, but rather present a rapid *in vitro* motility assay that may be used to evaluate microtubule-dependent motility. This model system will allow vesicle characteristics to be manipulated experimentally to localize and characterize the force-transducing molecules involved in fast axonal transport.

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A gelsolin-like Ca^{2+}-dependent actin-binding domain in villin

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Villin is an actin-binding protein of relative molecular mass (M_r) 95,000 found in the core bundle of microfilaments in brush border microvilli from intestine[1-3]. In physiological calcium concentrations (<1 μM), villin crosslinks actin filaments into bundles. However, in free calcium concentrations (>1 μM), villin severs actin filaments into short pieces[3-5]. To understand how villin can sever and bundle actin filaments, we are studying the molecular basis of villin–actin binding interactions by identifying important actin-binding domains in villin. Here, we report the purification and preliminary characterization of a 44,000-M_r fragment of villin which contains a calcium-dependent actin-severing activity. In addition, the partial amino-acid sequence from the amino terminus of this fragment reveals homology with a 16-residue region near the amino terminus[6] of gelsolin, an actin-severing protein found in many cells[7-9] and sera[10-12]. The sequence homology suggests a common structural basis for the calcium-regulated actin-severing properties shared by villin and gelsolin.

Glenney and co-workers[13,14] used V-8 protease to cleave villin into a 90,000-M_r amino-terminal fragment (villin core) and an 8,500-M_r carboxy-terminal fragment (villin headpiece) (Fig. 4*c*). Using electron microscopy, viscometry and pelleting assays, they showed that villin core retained the calcium-dependent actin-severing activity but lost the bundling activity of the intact villin molecule. In comparison, villin headpiece, independent of calcium, could bind but not bundle or sever actin filaments[13-15]. More recently, Hesterberg and Weber[16,17] identified one non-exchangeable and two exchangeable (with micromolar dissociation constants) calcium-binding sites in villin. One of the exchangeable sites was located in villin headpiece and the other two sites in villin core. We have used trypsin and other proteases to determine whether the calcium-dependent severing activity of villin can be localized to a fragment smaller than villin core. Trypsin cleaved villin into two fragments[18] of M_r 44,000 and 51,000 (named 44T and 51T, respectively, Fig. 1*a*). The time course of appearance of 44T and 51T (Fig. 1*b*) shows that both fragments are produced at similar rates and suggests that trypsin cleaves villin near the middle of the molecule. Digests for longer times or at higher trypsin/villin ratios caused further degradation of 51T while 44T remained stable to proteolysis. The instability of 51T relative to 44T explains the lower accumulation of 51T during proteolysis.

Villin fragments which retain calcium-regulated actin-binding activity were identified using the ^{125}I-actin gel overlay assay devised by Snabes and co-workers[19,20]. (They used this assay to identify gelsolin-like proteins in a variety of other cells and tissues.) In our experiments (Fig. 2), the ^{125}I-actin probe bound villin and 44T only in the presence of calcium; 51T did not bind actin in either calcium or EGTA. Thus, a calcium-regulated

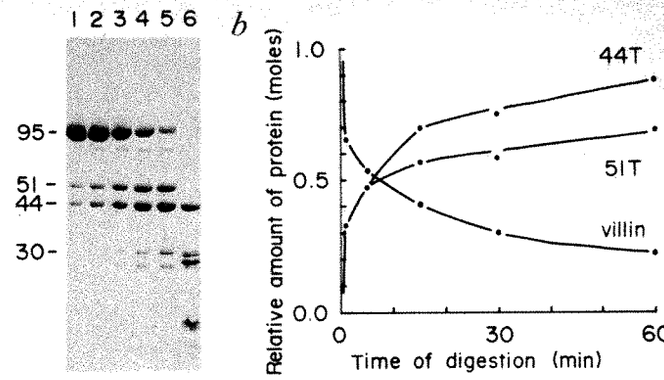


Fig. 1 Trypsin cleavage of villin. ***a***, Trypsin cleaves villin, M_r 95,000, into two major peptides of M_r 44,000 and 51,000. Lanes 1-6, digestion after 1, 5, 15, 30, 60 and 135 min, respectively. Trypsin, 5 mg ml^{-1} in 0.1 M Tris *p*H 7.5, 20 mM $CaCl_2$, was purified by affinity chromatography to soybean trypsin inhibitor coupled to Sepharose 4B. The column was washed with tryptamine (5 mg ml^{-1} in the Tris-$CaCl_2$ buffer) to remove chymotrypsin. Trypsin was eluted from the column with 3 mM HCl, 10 mM $CaCl_2$ and stored at −20 °C. Villin (5 mg ml^{-1}), purified using the method of Bretscher and Weber[3], in digest buffer (50 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EGTA, 10 mM PIPES *p*H 7.0), was cleaved by trypsin at room temperature at a 1:5,000 (wt/wt) ratio of trypsin/villin. The digest was quenched with phenylmethylsulphonyl fluoride (PMSF) to 0.1 mM after 1, 5, 15, 30 and 60 min (lanes 1-5). Another aliquot of villin was digested at 1 : 500 for 135 min (lane 6). The digests were mixed with SDS sample buffer and electrophoresed[31] on 7-25% microslab gels[32]. ***b***, The amount of the two villin tryptic fragments increases with time at similar rates. The gels of the trypsin digest were measured by densitometry and the peaks cut out and weighed. The weights of the peaks were converted to moles of peptide assuming equal dye binding. The amount of peptide present was plotted as a function of time of digestion.

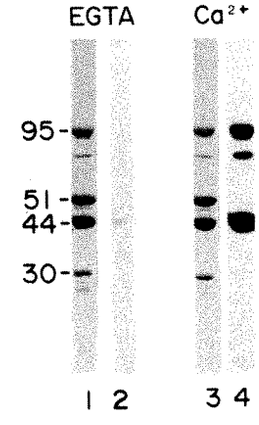


Fig. 2 Identification of calcium-dependent actin-binding fragments by a ^{125}I-actin gel overlay assay[19]. SDS gels of incomplete villin digests were incubated with ^{125}I-actin in 1 mM EGTA (lane 1) or 0.5 mM $CaCl_2$ (lane 3). Their corresponding autoradiograms (lanes 2 and 4) show only binding in calcium to villin, 44T and an 80,000-M_r contaminant.
Methods. An incomplete tryptic digest of villin was electrophoresed on SDS microslab gels and processed for actin binding. Duplicate samples were electrophoresed on one gel. The gels were fixed in 50% methanol, 10% acetic acid for 15 min, incubated in 10% ethanol for 2 h and then in phosphate-buffered saline (PBS) for 2 h, all at room temperature. After incubating overnight at 4 °C or for 2 h at room temperature with ^{125}I-actin (10,000 c.p.m. ml^{-1}, 100,000 c.p.m. total) in either 0.5 mM $CaCl_2$ or 1 mM EGTA in PBS, the gels were washed, stained, destained and dried. The dried gels were then autoradiographed for 8-36 h at −70 °C in cassettes fitted with Cronex intensifying screens.

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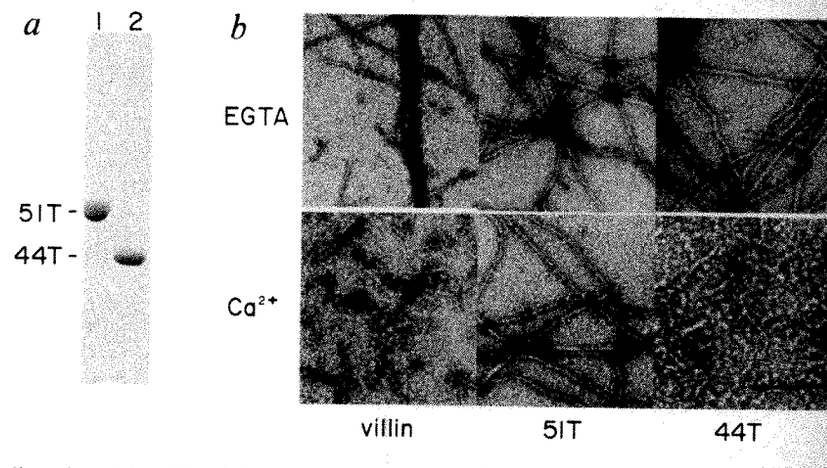


Fig. 3 Identification of the 44T and 51T actin-binding activities. *a*, The gel of the 51T (lane 1) and 44T (lane 2) fragments after purification over DEAE and Affi-Gel Blue chromatography. *b*, Electron microscopy of F-actin mixed with villin, 44T or 51T fragments. In EGTA (top panel), villin bundles filaments while 44T and 51T have no effect on actin filament organization. In calcium (bottom panel), 44T and villin both sever filaments into short pieces. In these conditions, 51T has no effect on actin filaments. Scale bar, 100 nm.

Methods. *a*, 44T and 51T purification. The villin digest was loaded onto DEAE-Sephacel (10 ml bed volume) previously equilibrated in digest buffer. The bound protein was eluted by a 25–250 mM NaCl gradient. The major peak eluted at 125 mM NaCl and contained 51T. The 51T fractions were made 10 mM with 2-[*N*-morpholino]ethane sulphonic acid (MES), the *p*H adjusted to 5.0 and loaded onto an Affi-Gel Blue (Bio-Rad, 10 ml bed volume) column equilibrated with 10 mM MES *p*H 5.0. The column was developed with a 0–0.5 M NaCl gradient in 10 mM MES. The major peak contained only 51T and was concentrated against solid sucrose, dialysed against 50% glycerol, 100 mM NaCl, 0.1 mM EGTA, 10 mM imidazole and then stored at −20 °C. 44T does not bind DEAE-Sephacel in digest buffer. The flow-through fraction from the DEAE column was collected and loaded immediately onto Affi-Gel Blue (10 ml bed volume) which had been previously equilibrated with digest buffer. The column was eluted with a 0–1 M NaCl gradient. The major peak which contained 44T was concentrated with sucrose and stored as described above. All steps were performed at 4 °C. Protein concentrations were measured using a modified Lowry method[33]. The purified fragments were electrophoresed on SDS gels to check for purity. A typical preparation yielded 20 mg of 44T and 7 mg of 51T from 50 mg of villin. *b*, The purified fragments were mixed with rabbit skeletal muscle F-actin (2 μM final concentration) at a 1:20 molar ratio in 150 mM NaCl, 1 mM $MgCl_2$, 10 mM PIPES *p*H 7.0 and with either 0.1 mM $CaCl_2$ or 0.1 mM EGTA for 30 min at room temperature. The samples were applied directly to carbon films on 400-mesh grids, rinsed with buffer, negatively stained with 2% uranyl acetate and then examined in the electron microscope. The micrographs were printed to the same final magnification.

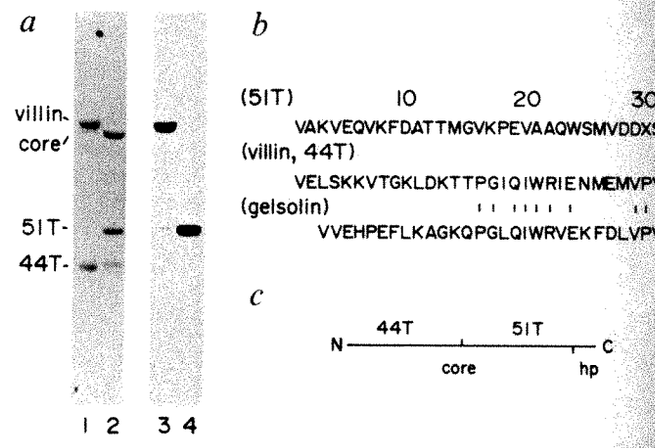


Fig. 4 Localization of the 44T and 51T fragments in villin. *a*, Purified villin, 44T (lane 1), villin core and 51T (lane 2) were electrophoresed on 10% microslab gels and then immunoblotted with a rabbit polyclonal antiserum (R200.2) raised against purified villin headpiece (lanes 3, 4). The blot shows that the headpiece antiserum recognizes villin (lane 3), villin headpiece (not shown) and 51T (lane 4), but not villin core (lane 4) or 44T (lane 3). *b*, Amino-acid sequence of the amino termini of villin, 44T and 51T compared with the amino terminus from rabbit macrophage gelsolin. Identical residues are indicated by vertical lines. *c*, Diagram of the locations of the 44T and 51T fragments relative to villin core and headpiece (hp).

Methods. *a*, Villin headpiece was purified from a V-8 digest of villin by a new method. Villin (5 mg ml^{-1} in digest buffer) was cleaved with *Staphylococcus aureus* protease (1:2,000 (w/w) enzyme/protein, 45 min, room temperature) and then applied to a DEAE-Sephacel column previously equilibrated in digest buffer. Villin headpiece does not bind to the column. The flow-through fraction was collected and applied to Affi-Gel Blue equilibrated with digest buffer. The column was eluted with a 0–1 M NaCl gradient in digest buffer. The headpiece-containing peak was pooled and concentrated as above. Villin core was eluted from the DEAE column with a 50–250 mM NaCl gradient in digest buffer. The peak containing villin core was pooled, concentrated against sucrose and then dialysed against 50% glycerol. Villin core was then stored at −20 °C. The identity of the headpiece fragment was checked by manually sequencing[34] the first four residues from the amino terminus of the fragment. Antisera were raised in rabbits by injecting 80 μg in complete Freund's adjuvent at multiple sites in the back, followed by subsequent injections at 2-week intervals in incomplete Freund's adjuvant. The rabbits were bled out at week 8. The titres of the antisera were quantified by enzyme-linked immunosorbent assay (ELISA). As judged by immunoblots and quantitative ELISA, the preimmune serum contained no detectable cross-reactivity against villin or its proteolytic fragments (not shown). The antiserum was characterized by immunoblotting[35] against villin, villin core, 44T and 51T after electrophoresis through microslab gels and electrophoretic transfer to nitrocellulose filters. The filters were blocked with 2% bovine serum albumin, incubated with antiserum at a 1:2,000 dilution and then with swine anti-rabbit IgG coupled to peroxidase (Dako) at a 1:400 dilution. The filters were developed with 4-chloro-1-naphthol. *b*, Purified villin, villin core, 44T and 51T (20 nmol each) were dialysed extensively against 5% formic acid, freeze-dried and subjected to the Edman degradation procedure using a modified Beckman 890B automated sequencer[36]. The phenylthiohydantoin (PTH) amino acids were analysed and identified by HPLC. The position and identity of each residue were determined at least twice by sequencing different preparations of protein. The villin sequences were checked for homology with protein sequences in the Doolittle data bank[37].

actin-binding domain seems to lie within a 44,000-M_r region of villin. The calcium dependence of binding rules out nonspecific binding as a possible explanation of our results. A minor contaminant (M_r 80,000) in the preparation also bound actin in a calcium-dependent manner, but its presence varied between experiments and was probably caused by a contaminating protease.

The actin-binding properties of 44T and 51T were then examined more directly by electron microscopy. Fragments were purified by ion-exchange and Affi-Gel Blue column chromatography. At neutral *p*H, 51T but not 44T binds to DEAE-Sephacel and thus the fragments can be easily separated. Each fragment separately can then be purified from minor contaminants by chromatography over Affi-Gel Blue columns. The purified fragments (Fig. 3*a*) were mixed with rabbit skeletal muscle actin at a 1:20 molar ratio in conditions[3–5] in which the intact villin molecule can bundle or fragment actin filaments (Fig. 3*b*). In EGTA, only villin bundled the actin filaments; the 44T and 51T

fragments had no effect on actin filament organization. In calcium, both villin and 44T shortened actin filaments to small pieces while 51T had no effect on actin filament integrity. Thus, electron microscopy directly confirms that the gel overlay assay has correctly identified 44T as a calcium-regulated actin-binding fragment of villin. Although we have not used more quantitative methods[15,21,22] to test whether 44T caps, nucleates and severs actin filaments in an identical manner to villin, the electron microscopic results suggest that severing can occur.

We surmised that 44T and 51T represent different regions of villin from the kinetics of their appearance during proteolysis. We then used antisera and protein sequence information to identify the location of these fragments more precisely within the villin molecule. We raised a polyclonal antiserum against the villin headpiece, which retains the carboxy terminus of villin[13,14]. Immunoblots of the villin proteolytic fragments showed that the antiserum recognized villin and 51T but not villin core or 44T (Fig. 4*a*), suggesting that 51T is associated with the carboxy-terminal half of villin while 44T could originate from the amino-terminal half. This was confirmed by sequencing the amino termini of villin, 51T and 44T (Fig. 4*b*). The first 30 residues in their sequences showed that villin and 44T share the same amino terminus and, therefore, 44T comprises the amino-terminal half of villin.

In addition, comparison of the villin amino-terminal sequence with the published amino-terminal sequence of rabbit macrophage gelsolin[6] revealed a short stretch (residues 16–31) of homology. This region shares 10 identical residues and 6 conserved replacements (Fig. 3*b*). Of these six replacements, five are consistent with a single base change. Although previous studies[1,9,20,23] have stressed that villin and gelsolin have different locations in cells, produce different peptide maps and elicit antisera which do not cross-react, our sequence information provides structural evidence that explains the similar actin-severing activities of these two proteins.

Our results (summarized in Fig. 4*c*) document a calcium-regulated actin-binding region which lies within the amino-terminal 44,000-M_r fragment of villin. As seen by electron microscopy, this fragment, like villin, villin core and gelsolin, seems to sever actin filaments into short pieces. The similarity between villin and gelsolin in their actin-severing activity as well as in the sequences of their amino termini suggests that villin could be a member of a related family of actin-severing proteins. This family could also include other proteins having M_rs and calcium-regulated actin-binding activities similar to the villin 44T domain, for example, severin from amoeba[24,25], fragmin and cap proteins from slime moulds[26–29], and an actin-binding protein from sea urchin eggs[30]. We speculate that these proteins could have sequence homology in regions corresponding to their actin- and calcium-binding sites.

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Local protein–DNA interactions may determine nucleosome positions on yeast plasmids

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The structure of the nucleosome core particle, the basic structural subunit of chromatin, is well known[1]. Although nucleosomes often appear to be positioned randomly with respect to DNA sequences, in some cases they seem to occupy precisely defined positions on the DNA[2–8]. The yeast plasmid TRP1ARS1 contains three precisely positioned, stable nucleosomes, I, II and III, which are flanked by nuclease-sensitive regions.[5] Our aim in the present study was to determine whether the positions of these three nucleosomes relate to (1) protein–DNA interactions; (2) the limited space between nuclease-sensitive regions, which is just long enough to accommodate three yeast nucleosomes (that is, boundary conditions[9]); or (3) proximity to the putative origin of replication in one of the nuclease-sensitive regions[5,10]. We have tested these alternatives by analysing the positions of nucleosomes after insertion of various lengths of DNA into this region and assembly of chromatin *in vivo*. Our results suggest that specific protein–DNA interactions are the most likely determinants of these nucleosome positions.

We chose as the DNA to be inserted segments of a sea urchin 5S sequence which forms a positioned nucleosome *in vitro*[11]. An appropriate site at which to insert DNA fragments lies between the strong micrococcal nuclease cutting site in chromatin at position 1,022 and the strongly protected cutting site at 1,082 (Fig. 1). A shift of nucleosome I would prevent cutting at 1,022 and/or would expose the DNA cutting site at position 913. A shift of nucleosomes II and III would expose the cutting site at 1,341 or even that at 1,082. To obtain a suitable cloning site, we introduced a 10-base pair (bp) *Bam*HI linker into the *Nae*I site at position 1,069. Insertions of 75, 155 or 300 bp derived from the 5S gene were made (see Fig. 2). The plasmids derived in this way were transformed into yeast for assembly into chromatin. All constructs gave similar transformation efficiencies and yields in plasmid chromatin preparations; the inserts do not grossly interfere with either *TRP1* or *ARS1* function.

The plasmids were partially purified[5] and their structures analysed by micrococcal nuclease digestion. All plasmids had DNA packed in nucleosomes (not shown). To determine the locations of the nucleosomes, cutting sites were mapped using the indirect end-labelling method[4–6]. Mapping of the micrococcal nuclease cutting sites within the *TRP1* gene region showed

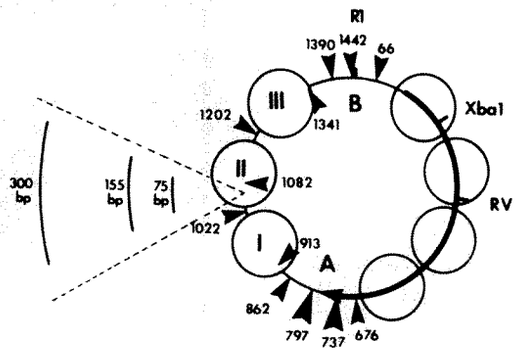


Fig. 1 Chromatin structure of the TRP1ARS1 plasmid: location of the inserts used to study nucleosome positioning. The detailed structure of TRP1ARS1 chromatin is shown[5], with the open reading frame for the *TRP1* gene indicated by the heavy arrow. Two nucleosome-containing regions are separated by two nuclease-sensitive regions A and B. A is located at the 3′ end of the *TRP1* gene and the putative origin of replication. B is located 5′ to the gene. The four nucleosomes on the *TRP1* are imprecisely positioned or unstable. Three stable nucleosomes (I, II and III) are precisely positioned on a sequence with unknown function (UNF). The cutting sites for micrococcal nuclease which are protected in TRP1ARS1 chromatin (913, 1,082 and 1,341) are shown inside the circle; cutting sites that are exposed in chromatin (676, 737, 797, 862, 1,022, 1,202, 1,390, 1,442 and 66) are shown on the outside. DNA fragments of 75, 155 or 300 bp were inserted between positions 1,069 and 1,070, as indicated. Nucleosomes I, II and III are characterized by protection of cutting sites at 913 and 1,082 and partial protection of the site at 1,341. Restriction enzymes used and their cutting sites are: *Eco*RI/1 (RI), *Xba*I/186 (XbaI), *Eco*RV/385 (RV).

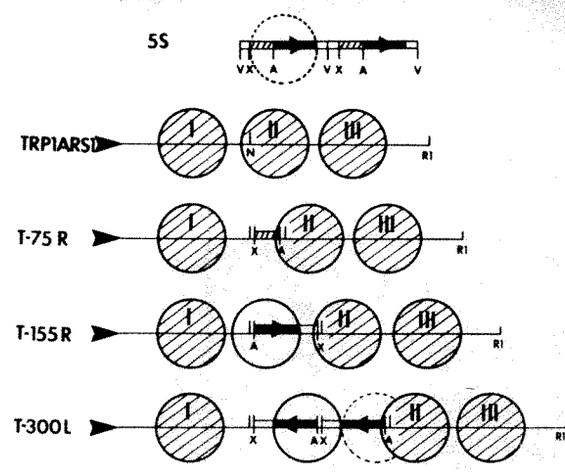


Fig. 2 5S rRNA gene region insertions in the TRP1ARS1 plasmid and nucleosome positions after reassembly in yeast. 5S: Two (of 18) tandemly repeated 190-bp fragments containing 5S sequences are shown. The position of the nucleosome assembled *in vitro*[11] is indicated by a dashed circle (from nucleotide 20 to 165 of the published sequence). TRP1ARS1: The positions of the three stable nucleosomes (I, II, III) on the TRP1ARS1 UNF region are shown as hatched circles. T-75R: Using two 10-bp *Bam*HI linkers, the 55 bp-long *Xmn*I/*Alu*I fragment of the 5S DNA (hatched box) was inserted in the same orientation as the *TRP1* gene. T-155R: Using two 10-bp *Bam*HI linkers, the 135 bp-long *Alu*I/*Xmn*I fragment of 5S DNA was inserted in the same orientation as the *TRP1* gene. T-300L: Using three *Bam*HI linkers, two 135 bp-long *Alu*I/*Xmn*I fragments of 5S DNA were integrated in tandem in the opposite orientation to the *TRP1* gene. The *Bam*HI linkers are shown as vertical ticks. Restriction sites are: V, *Ava*I; X, *Xmn*I; A, *Alu*I; RI, *Eco*RI; and N, *Nae*I. The large arrowhead on the left indicates the end of the *TRP1* gene.

Methods. The 1,453 bp-long TRP1ARS1 *Eco*RI fragment was inserted into the *Eco*RI site of a pBR322 derivative, from which the *Bam*HI site and all *Nae*I sites were deleted. A *Bam*HI linker was inserted into the *Nae*I site of the TRP1ARS1 sequence between nucleotides 1,069 and 1,070 to give plasmid pBRAT2. The insertions in the *Bam*HI site were derived from a segment of *Lytechinus variegatus* DNA containing the 5S rRNA gene[11]. A plasmid was constructed containing 18 tandemly repeated copies of a segment consisting of the DNA from nucleotide 1 to 178 of the published sequence[11], flanked by *Eco*RI and *Ava*I sites; the overall repeat length of the insert is 190 bp. The 55 bp-long *Xmn*I/*Alu*I fragment and the 135 bp-long *Alu*I/*Xmn*I fragment were gel-purified, attached to *Bam*HI linkers and inserted into pBRAT2. The modified TRP1ARS1 *Eco*RI fragments were gel-purified, circularized and used to transform yeast SC-3 (*mata ura3 his3 gal2 gal10 trp1*) obtained from L. W. Bergman (University of Maryland).

that all constructs had unstable nucleosomes similar to those of unmodified TRP1ARS1 (results not shown; see ref. 5). Figure 3 shows the mapping of the micrococcal nuclease cutting sites on chromatin and DNA, from the *Eco*RV site (position 385) counterclockwise using a 199-bp *Xba*I/*Eco*RV fragment as a probe. All the plasmids are strongly cut near the 3′ end of *TRP1*, around the putative origin of replication (arrowhead). Further, chromatin from all the plasmids is frequently cut between positions 1,341 and 66. We conclude that insertion of DNA affected neither the chromatin structure of *TRP1* nor the two nuclease-sensitive regions.

In chromatin incorporating plasmid T-75R, strong cutting was observed around position 1,022, accompanied by strong cutting in the inserted DNA region (open bracket in Fig. 3). Cutting sites in this region are weak in naked DNA controls and therefore exposed in chromatin. In the regions of chromatin from position 862 to 1,022, from the DNA insert to 1,202 and from 1,202 to 1,390, prominent naked DNA cutting sites (around 913, 1,082 and 1,341) are protected in a manner similar to that in TRP1ARS1 chromatin. We conclude that nucleosomes are formed at the same positions in the modified plasmid as in TRP1ARS1. The inserted DNA appears to be present as an exposed, long linker between two positioned nucleosomes, I and II (Fig. 2).

In T-155R chromatin, the nucleosomes at positions 862 to 1,022 and 1,202 to 1,390 again occupy identical locations in the derivative and parent plasmids (Fig. 3). Cutting of naked DNA at position 1,082 and at two positions in the insert is strong (relative to cutting at 1,202 and 1,022) while cutting at these three sites in chromatin is weak. As there are ~335 bp between cutting sites 1,022 and 1,202 (including the 155-bp insert), we suggest that there are two tightly packed nucleosomes positioned in this region (Fig. 2).

In T-300L chromatin, protection against micrococcal nuclase cutting between position 862 and 1,022, from the insert to 1,202, and from 1,202 to 1,390 indicates that nucleosomes I, II and III originally resident on the yeast sequences are positioned on the same sequences. The inserted DNA, however, appears to be cut through a nuclease-sensitive region of ~50 bp at one end and 90 bp at the other end. Strong cutting sites present on naked DNA in the insert are protected in chromatin, suggesting the presence of a single nucleosome on the 300-bp insert. To localize precisely this nucleosome on the inserted DNA, we have mapped micrococcal nuclease cutting sites clockwise from the *Xba*I site and counterclockwise from the *Eco*RV site at three levels of digestion. Cleavage sites at the boundaries of the linker DNAs are at positions 1,032 ± 15 and 1,122 ± 12 on one side and 1,266 ± 9 and 1,315 ± 15 on the other side (note that positions here refer to sites in T-300L, not TRP1ARS1). The protected region covers 144 bp between positions 1,122 and 1,266. With respect to the sea urchin 5S sequence, the nucleosome provides protection from nucleoside 162 on one repeat to 163 on the other (Fig. 2); the additional nucleosome assembled *in vivo* occupies a position on the 5S sequence which is similar to the position obtained in the *in vitro* reconstitution experiments[11].

The *ARS* function in the TRP1ARS1 fragment has been localized between positions 615 and 1,453 (ref. 10). We have

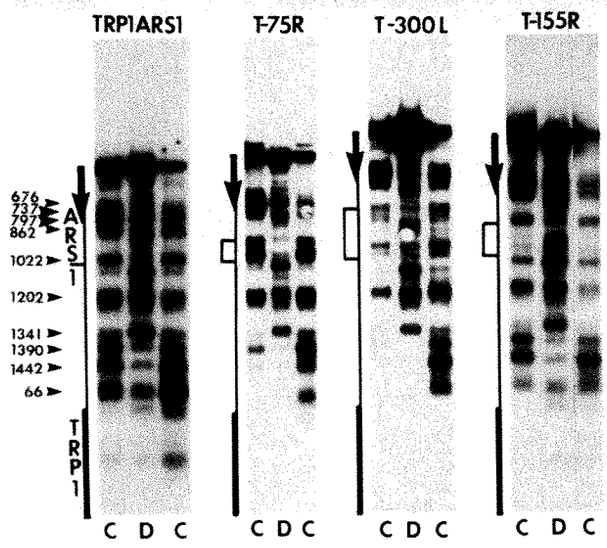


Fig. 3 Mapping of the micrococcal nuclease cutting sites on chromatin (C) and DNA (D) counterclockwise from the *Eco*RV site. TRP1ARS1: Mapping data and the mean cutting sites, indicated on the left-hand side, are taken from Thoma *et al.*[5], and serve as a standard. The position of the insertions is marked by a tick. T-75R, T-300L, T-155R: The positions of the inserted sequences are indicated by open boxes. The vertical arrow indicates the open reading frame of *TRP1*.

Methods. Preparation of plasmid chromatin and control DNA, digestion with micrococcal nuclease and mapping of the cutting sites were done in a similar way to that described by Thoma *et al.*[5]. Yeast cells were grown at 30 °C in minimal medium supplemented with uracil and histidine (0.67% yeast nitrogen base without amino acids (Difco), 2% dextrose, 20 g uracil per l, 20 g histidine per l) to an absorbance of 1-4 at 600 nm, washed twice with water and converted to spheroplasts using Zyolyase 60,000 (Kirin Breweries, Takasaki, Japan). The spheroplasts were washed in yeast wash buffer (40 mM K-phosphate *p*H 7.5, 1 M sorbitol, 1 mM phenylmethylsulphonyl fluoride (PMSF)) and lysed in buffer A (20 mM Tris-HCl *p*H 8, 150 mM NaCl, 5 mM KCl, 1 mM EDTA, 1 mM PMSF) containing 0.2% Triton X-100. The genomic chromatin was pelleted at 27,000 *g* at 0 °C for 30 min and the small contaminating material was removed by gel chromatography on Sephacryl S300 in buffer A. The plasmid chromatin eluted in the void volume and was pooled. DNA from one half of the chromatin pool was extracted using phenol, phenol/chloroform, and chloroform; RNA was digested with RNase A, and DNA was re-extracted as above and precipitated twice. Micrococcal nuclease digestions were done in buffer A supplemented with 5 mM $CaCl_2$ for 5 min at 37 °C using four different enzyme concentrations. To map the micrococcal nuclease cutting sites, DNA was extracted as above, cut with *Eco*RV, separated on 1% agarose gels in Tris-borate buffer containing ethidium bromide, blotted onto nitrocellulose, and hybridized to radioactively labelled *Xba*I/*Eco*RV fragments. The radioactive bands were detected using Kodak XAR-5 film.

shown that different fragments of DNA can be inserted at position 1,069 without disrupting *ARS* function. As the nucleosome between 862 and 1,022 in TRP1ARS1 is highly stable[5], we suggest that the functional *ARS* sequence lies in the nuclease-sensitive region A between 676 and 862, in analogy to simian virus 40, where the origin of replication is known to be in a nuclease-sensitive region[12].

We next consider the possible determinants of nucleosome positions. Insertion of 75–300 bp of DNA had no effect on the positions of nucleosomes I, II and III. Surprisingly, yeast prefers to assemble long linkers (75, 49, 90 bp), although on average the yeast micrococcal nuclease repeat length is ~160 bp[13], consistent with 145-bp nucleosome cores connected by ~15-bp core-to-core linkers.

One may ask whether nucleosome formation near the replication fork aligns or phases nucleosomes in positions which are determined by the location of the origin of replication. Although the precise location of the origin of replication and the nature of the replication process are unknown, the existence of variable-length linkers in T-75R and T-300L excludes the possibility that nucleosome positioning is a simple function of replication.

The existence of long linker DNA demonstrates that the yeast did not take the opportunity to rearrange or randomize its nucleosome positions; this observation excludes a simple boundary mechanism in which the positions of nucleosomes are determined by a statistical arrangement within limited space between two boundaries, regions which interact with other proteins to preclude nucleosome formation[9]. Although we must consider the possibility that the inserted DNA itself (particularly the DNA which exists as long linkers) may represent a boundary, this seems unlikely for the linkers in T-75R and T-300L. Thus, these sequences can associate with histones to form nucleosomes, that is, the X-A fragment in T-75R formed *in vitro*[11] and the sequences represented by both the open and black boxes in T-300L in the additional nucleosome formed *in vivo* (Fig. 2). As we have excluded the simple boundary mechanism and a replication-related mechanism, we conclude that local protein-DNA interactions, involving either histones *per se* or positioning proteins[14], probably determine the positions of these yeast nucleosomes.

The nucleosome formed on the 300-bp insert appears to occupy a position identical to that of the core particle assembled *in vitro*[11]. As only 90 bp of sequence are shared in the nucleosomes assembled *in vitro* and *in vivo*, we suggest that this sequence (*Alu*I site to position 162) must contain a nucleosome positioning signal. A similar conclusion has been reached in studies of the *in vitro* assembly of modified 5S fragments with histones, although *in vitro*, sequences to the left of the *Alu*I site serve a discriminatory function in phasing (P. FitzGerald and R.T.S., unpublished observations). As only histone-DNA interactions were necessary *in vitro* to position the nucleosome, we infer that, in yeast, histone-DNA interactions may be the dominant mechanism for positioning of this particular nucleosome *in vivo*. It is interesting that chicken erythrocyte histones were used for the *in vitro* experiments[11], therefore the positioning signal may be universal.

Whatever mechanisms are responsible for positioning of these nucleosomes, the fact that only one of the two positioning signals on the 5S DNA was used in T-300L (formation of the nucleosome indicated by the dashed circle in Fig. 2 did not occur), together with the observation that nucleosome II did not shift in T-75R or T-300L, suggests that a hierarchical array of positioning signals must exist. The signal for nucleosome II must be stronger than that for the 5S sequence.

We have shown that yeast and yeast plasmids can be used as an *in vivo* chromatin reassembly system for addressing questions relating to structure and function of nucleosomes *in vivo*. Although the present experiments have analysed structural changes only in circular plasmids, homologous recombination in yeast will allow the extension of such studies to the chromosome itself.

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Determination of surface topography of biological specimens at high resolution by scanning tunnelling microscopy

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Although techniques are available for the determination of the three-dimensional structure of biological specimens, for example scanning electron microscopy, they all have some serious drawback, such as low resolution, the requirement for crystals or for the sample to be analysed in a high vacuum. In an attempt to develop a technique for high-resolution three-dimensional structure analysis of non-crystalline biological material, we have tested the applicability of scanning tunnelling microscopy (STM), a method that has been used successfully in the analysis of metal and semiconductor surface structures[1–3]. We report here that scanning tunnelling electron microscopy can be used to determine the surface topography of biological specimens at atmospheric pressure and room temperature, giving a vertical resolution of the order of 1 Å. Our results show that quantum mechanical tunnelling of electrons through biological material is possible provided that the specimen is deposited on a conducting surface.

We have used a STM microscope similar to the one developed at the IBM Zürich Laboratory[1], but operated at air atmospheric pressure. The microscope works by scanning a metal tip of very small radius at a distance ~5–10 Å above the surface by means of three mutually perpendicular piezoelectric ceramics. In the constant-current mode, the tip–surface distance remains roughly constant during the scanning because of the extreme sensitivity of the tunnel current with respect to the tip–surface separation[4].

To proceed with the STM of biological samples, a flat conducting and structureless substrate was needed. This condition was satisfied by pyrolitic graphite (Fig. 1*a*), where a surface which is atomically flat on a ~2,000-Å range displays a step of monoatomic height. From this image, we could estimate the stability and resolution of our microscope to be ~1 Å of vertical resolution and ~5–10 Å of lateral resolution. These values were obtained at atmospheric pressure and room temperature.

We have analysed two types of of biological samples, both derived from bacteriophage ϕ29 particles. This small bacteriophage is composed of a prolate icosahedral head (415 × 315 Å) and a complex tail (410 Å long)[5,6]. Phage particles release their DNA and part of their neck–tail complexes when treated with EDTA[6]. The purified phage samples were diluted in 0.1% glycerol for examination by STM. A control experiment to check the influence of the 0.1% glycerol solution on the graphite surface demonstrates a noise level of the surface lines only slightly higher than that of the graphite substrate (Fig. 1*b*).

The phage-derived structures were allowed to air dry over an electron microscope grid and then stained to detect the degree of destruction provided by this drastic drying procedure. Figure 2*a* shows that phage particles (either with or without DNA) retained their characteristic gross morphology[5,6]. A parallel sample was then adhered to a surface of freshly cleaved graphite by drop deposition, allowed to air dry and then examined by STM.

Over a flat background (similar to that shown in Fig. 1*b*), a series of reproducible scans allow us to define objects (Fig. 2*b–d*). A comparison of these surface topographies with the electron micrographs of the phage-derived structures suggested that the STM topographic profiles could be correlated qualitatively with the general shape and dimensions of DNA-filled phage, empty head and empty phage particles. Flattening of the

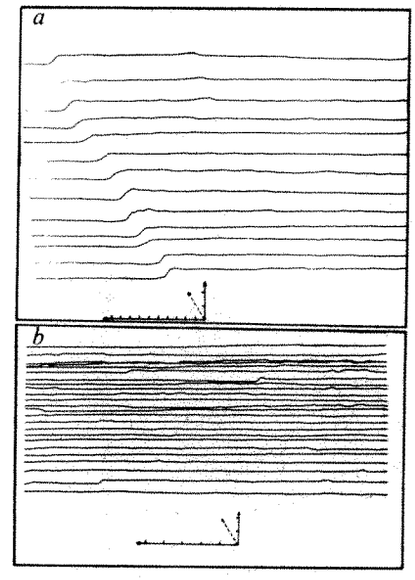


Fig. 1 *a*, STM topographic curves of a pyrolitic graphite surface cleaved in air, obtained at ambient pressure. Divisions on the axes are 10 Å apart. The scale along the *y*-axis (broken line) is not well determined. A step of height 7 Å corresponding to the lattice constant of graphite (6.708 Å) is clearly visible. *b*, STM topographic curves of a graphite surface covered with 0.2 M ammonium bicarbonate containing 0.1% glycerol used to deposit the bacteriophage-derived structures. Divisions on the axes are 50 Å. Note that the substrate is flat over the range ~1,000 Å.

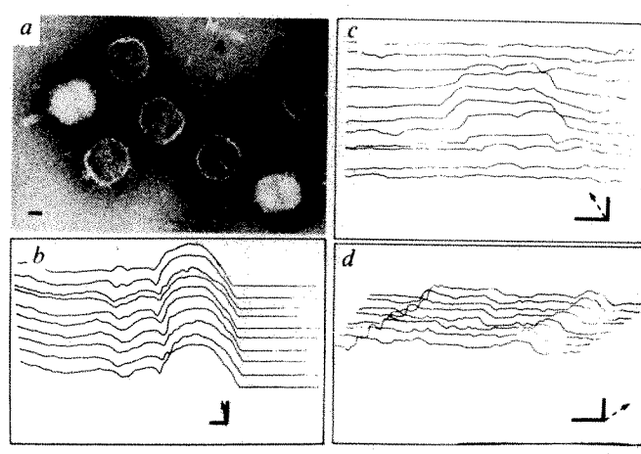


Fig. 2 *a*, Electron micrograph of various structures derived from bacteriophage ϕ29. Phage particles treated with EDTA[6] released their DNA and part of their neck–tail complexes. After partial purification of the empty head fraction, the material was diluted 10 times in 0.1% glycerol and allowed to air dry over a collodion–carbon-coated electron microscope grid. After drying, the sample was negatively stained with 2% uranyl acetate and observed by transmission electron microscopy. Numbers 1, 2 and 3 correspond, respectively, to DNA-filled phage particle, empty head and empty head with tail. Neck–tail complexes (number 4) can also be seen. Scale bars, 100 Å. *b–d*, STM surface profiles from a parallel sample dried over graphite. Profiles in *b* probably correspond to scans along the head–tail axis of a DNA-containing phage particle. Profiles in *c* can be correlated with the expected flattened structure obtained after air drying an empty capsid without tail. Profiles in *d* can be interpreted as scans along the head–tail axis of an empty phage particle with tail complex. Note that the height of the flattened head in *c* and *d* (~70 Å) is much smaller than in the case of the DNA-containing head shown in *b* (~200 Å) whereas the height of the tails in *b* and *d* is very similar (~75 Å).

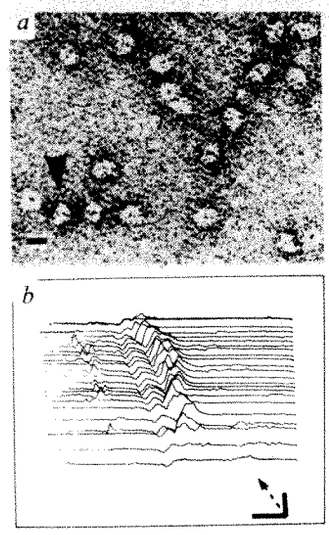


Fig. 3 *a*, Electron micrographs of the upper collar of bacteriophage ϕ29. The upper collar protein of phage ϕ29 was purified[8,9] and dialysed against 0.2 M ammonium bicarbonate containing 0.1% glycerol to prevent destruction during the air-drying procedure. This sample was negatively stained with 2% uranyl acetate and observed by transmission electron microscopy. The collars appear as circular structures with a hole in the centre. Occasional side views show a dumbbell shape with a hole running through the centre of the neck (arrowhead). Scale bar, 100 Å. *b*, STM surface topography curves obtained from a collar-containing sample deposited over graphite and air-dried. The scans show profiles of a structure (100 Å in diameter along the *x*-axis and 80–90 Å in height along the *z*-axis) that correlates with the collar structure inferred from the images shown in *a* (ref. 9).

air-dried specimens, commonly observed in all electron microscope methods[7], is also seen in the present experiments for empty viral heads (Fig. 2*c*, *d*). Repeated scans over the same area always showed very similar topography, implying that STM does not drastically alter the biological surface structure.

The second sample studied by STM was the oligomeric protein structure that forms the upper collar of ϕ29 (refs 8, 9). Transmission electron micrographs of this oligomer showed a circular front view (135 Å diameter) with a hole in the centre (Fig. 3*a*). Occasional side views showed that the oligomer has a dumbbell shape (125 Å on height). The collar sample was dialysed against 0.2 M ammonium bicarbonate (containing 0.1% glycerol) to remove salts and low-relative molecular mass components and then analysed by STM.

Over a general flat surface, some series of scans were observed that could be correlated with the presence of the phage collars (Fig. 3*b*): the more characteristic feature is a structure ~100 Å in diameter (*x*-axis) and 80–90 Å in height (*z*-axis) showing a hole in the centre, which is in good agreement with the structure of this collar observed by transmission electron microscopy[9] (Fig. 3*a*).

Most of the pictures presented were scanned insufficiently in the *y*-direction, which prevents the observation of the entire object. This limitation resulted from the difficulty in controlling the piezo movement in the slow direction (*y*-axis), because both piezo relaxation and external scanning may have a similar value.

We stress that we have obtained real corrugated structures in the STM of as much as 250 Å for an applied voltage of 0.4–0.8 eV, giving a tunnel current of 10 nA. Assuming an average tunnel area of 300 Å^2, as indicated by the experimental lateral resolution[4], an effective tunnel resistivity of ~1 Ω cm^{-1} is obtained. This resistivity is much too small for a single tunnel process over ~200 Å. We propose a transport mechanism to explain the effective conductivity of the biological material analysed by assuming the existence of regions where the electrons tunnel (conduction levels higher than the kinetic energy of the electrons) and regions where they propagate (conduction levels lower than the kinetic energy of the electrons). If such regions of tunnelling and propagation of electrons exist in the specimen, electrons can easily move by percolating in the propagating regions, whereas resonant tunnelling in the tunnel regions can substantially enhance the conductivity over single tunnel processes. Calculations on one-dimensional disordered systems show an enhancement of the conductivity[10]. We believe that both type of regions are provided by the different bonds and radicals existing in the protein of the biological material. More information will be provided in a forthcoming paper.

In conclusion, our results show that STM can be used to determine the surface topography of air-dried untreated biological specimens at atmospheric pressure and room temperature without altering the material. Real-space three-dimensional topographic profiles giving a vertical resolution of the order of 1 Å are obtained, which can be correlated in terms of size and shape with biological objects. In addition, STM can be used to determine the surface microstructure of other materials such as metals, semiconductors, thin films and polymers. The operation of the microscope at atmospheric pressure opens the possibility for investigation of the technological applications of these materials in such fields as metallurgy, microelectronics and catalysis.

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Corrigendum

High-affinity binding site for a specific nuclear protein in the human IgM gene

L. Hennighausen, U. Siebenlist, D. Danner, P. Leder, D. Rawlins, P. Rosenfeld & T. Kelly Jr
Nature **314**, 289–292 (1985)

Lines 8 and 9 of Fig. 3 should read "in the absence (lane 4) or presence (lane 3) of the".

Erratum

Functional modifications of cytotoxic T-lymphocyte T200 glycoprotein recognized by monoclonal antibodies

L. Lefrançois & M. J. Bevan
Nature **314**, 449–452 (1985)

ON page 450, column 2, line 6 of the first paragraph should read "... precipitated proteins with apparent M_rs of 240,000 (240K), 220K ...".

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Health or Hoax? The Truth about Health Foods and Diets.
By Arnold E. Bender.
Elvendon Press, The Old Surgery, High Street, Goring-on-Thames, Reading, Berkshire, UK: 1985. Pp.184. £8.50.
The Penguin Encyclopaedia of Nutrition.
By John Yudkin.
Viking: 1985. Pp.431. £14.95, $20.
Diet-Related Diseases: The Modern Epidemic.
By Stephen Seely, David L.J. Freed, Gerald A. Silverstone and Vicky Rippere.
Croom Helm/Avi: 1985. Pp. 272. Hbk £19.95, $35; pbk £9.95.

THE dilemma of nutrition is not merely caught in these three books; it is writ large, as six authors wrestle with what nutritionists do and do not know, and, more importantly perhaps, with what almost everyone else seems so certain about. Each book has a different aim, and a very different style, but they share a common constituency of the layman, the undergraduate and the professional in search of diversion.

Professor Bender's book, a swingeing, shotgun attack on health foods and their purveyors, will clearly attract the greatest attention (an extract has already appeared in the *Daily Mail*). Although I have some reservations about it, a detailed dissection of health food mythology is long overdue and Bender provides this in a most entertaining style. The message of the book is neatly encapsulated in his opening contention, that there are no such things as health foods, there is merely an industry which sells them. Bender not only shows that the claims made for health food products have little or no scientific basis, he casts doubt on the motives, practices and business standards of the industry, concluding briefly with his own recommendations for a good diet. All this is done with gusto, employing a mixture of sarcasm, wit and his encyclopaedic knowledge of nutrition. But while I finished the book with my appetite whetted it was not satisfied: at times I was left wishing for more, or fuller, answers to some of the issues he raises, hoping that he might return to the attack at greater length after each skirmish; at others, in flat disagreement with some of his scientific assertions.

One issue touched on tantalizingly, but not fully explored, is the nature of the health food movement itself. With apparent relish, Bender quotes the views of a colleague who describes it as compounded of both cranks and swindlers "bent on the commercial exploitation of the frightened and helpless", and certainly he cites enough cases to demonstrate the existence of both. But there is clearly more to be said, as when he remarks that "many of the [health-food] magazines are owned by the same organisations that make and sell the health foods". This is indeed "more than interesting" because through such magazines manufacturers can freely make claims that even the feeble British regulations would forbid them to publish in advertisements. The implication that the followers of the health food movement are encouraged in their views by a gospel of arrant nonsense, surreptitiously funded by manufacturers, is a worrying one. Here is a can of wholemeal worms that should have been opened wider.

Though manipulation of information may in part be the reason for the rapid growth of the health food industry (turnover has increased by 400% in Britain in the past decade, for example), it is not enough to account fully for its success. Bender briefly mentions other factors, such as the general hypochondriasis about food in our society, and the pervasive Western wish to believe in a Golden Age of dietetic innocence, when, guided by nature, we unerringly chose the diet of untarnished pastoral perfection. It is, I think, a pity that he did not examine more fully the reasons why we are so prone to these obsessions, and particularly the role that his (and my) profession has played in encouraging everyone to believe that eating should carry a government health warning.

This is part of a general weakness in the book. In pointing out the error of the health fooders' ways at such length, Professor Bender necessarily asserts continually that the nutritionist knows best. This may be true more often than not, but sometimes he is unduly soft on scientists and complacent about what they have shown or done. When he states that artificial sweeteners are safe because tests have not shown them to be harmful in human beings, or that "apart from premature babies, extra Vitamin E does not have any value", there is a real danger of confusing particularly the lay reader not only about the nature of scientific proof but about the extent of a professional consensus on these issues. On the other side of the coin he sometimes quotes relatively weak scientific evidence as demonstrating a *proven* danger of health food products, as, for example, in his listing of toxic effects of high doses of Vitamins C, B_6 and B_1, where the professional view (as evidenced by the other two books discussed here) would be that there is no firm evidence as to the adverse effects of overdoses of these compounds. And while he pronounces long and loud about even putative cases of vitamin poisoning attributable to the advocacy of health food freaks, he says nothing about the crop of deaths of children in Britain 30 years ago which resulted from professional nutritionists advocating too high a level of supplementation of infant foods with Vitamin D: a compound that, as he says, is more poisonous than cyanide.

These remarks are not intended as a defence of the health food business or to detract from Professor Bender's well-justified attack on it. It is just that I regret what I see as unnecessary certainty in his tone because it lessens the force of his criticisms. It is particularly unfortunate in a book which ends with a chapter giving advice on a healthy diet. For what Professor Bender recommends is the currently fashionable recipe of low fat, low saturated fat, less sugar, less salt and more fibre. The sort of advice, in short, which not so long ago nutritionists were wont to ridicule when health food cranks proposed it.

Such errors are inevitable in a science whose discoveries can be implemented so easily, and which has a public avid to hear of each whisper of a miracle. But that makes it necessary for its practitioners to consider carefully everything they say, particularly when writing for the general public. To some Professor John Yudkin would seem an arch-offender against this precept, an eloquent voice who has put his views on the adverse effects of dietary sucrose to the public over the heads of his colleagues. In fact Yudkin has devoted very little of his working life to attacking sucrose. Far more of it was spent in devising the first undergraduate degree in nutrition, a pioneering attempt to provide in a single course a perspective on the diverse disciplines — from cooking to physiology, economic history to chemistry — of which a nutritionist should be aware. In doing that he also fostered neglected areas of the subject, most notably the social and historical, that have suffered because many scientists (and, in Britain, the Research Councils) see nutrition simply as applied biochemistry.

The Penguin Encyclopaedia of Nutrition is a reflection of this side of Yudkin's activities. It is a large book, but neither exhaustive nor exhausting, and an amalgam of some truly nutritional entries and some plucked from the ancillary disciplines that Yudkin has always argued a would-be nutritionist must master. The entry on health foods jostles one on *p*H, gout is discussed, so is osmotic pressure, nutrient balance and locust beans. Necessarily much is missed out and it seems that Yudkin has frequently chosen to concentrate upon what interests him. There is a long entry

on sucrose, of course, but much more on the history of nutrition, notably the succinct biographies of many of the founders of nutritional science and the accounts of the food habits and nutritional impact of the Neolithic revolution. The air of relaxed authority emerges in entries such as that on "Food", which he calls "a word universally used and presumably universally understood" but not susceptible of a "sufficient and necessary definition" before going on gently to refute the slick definitions found in dictionaries. This a reference work to browse through and ruminate upon. It will be particularly useful to those who feel impelled to pronounce upon nutritional matters, among whom one might include the authors of the third book reviewed here.

Diet-Related Diseases, a collaborative effort by an epidemiologist, an immunologist, a chemist and a clinical psychologist, is a refreshing book but suffers from three things. The first is the unevenness in style, predictable in a multiple-author volume. The second is the cover: a clichéd photograph of a collection of junk foods that fails to reflect the thrust of the book and suggests that the publishers did not fully appreciate what their authors have tried to do. For in no sense is this book clichéd, nor is it particularly concerned to attack or defend junk food. What the authors do is set out what they see as possible roles for diet in many of the diseases of the affluent society, and to present both conventional and novel (and in parts highly idiosyncratic) analyses of this case. The core is a collection of chapters by Stephen Seely which set out the evidence for a dietary role in arterial diseases and various cancers; on the periphery are chapters by his colleagues on food toxicants, food allergies, diet in mental illness, and diet and ageing.

Seely is the author of a provocative series of papers in *Medical Hypotheses* which have tried to cast new light on the well-trodden field of diet and degenerative diseases chiefly by attempting a more thorough analysis of data on national diets and the prevalence data on diseases. While the evidence is set down in the book with commendable clarity, it is the very clarity which makes apparent the occasional, but important, misconception about the quality of the nutritional, and other, assumptions which underpin the arguments.

Here lies the book's third and most serious fault. For example, Seely's analyses make great use of estimates of per capita availability of foodstuffs in various countries, which he calls "food consumption data". They are not: the two may differ by some hundreds of calories and the discrepancy is a notorious source of error. He asserts axiomatically that "a correlation coefficient of 0.9 denotes a degree of positive correlation when causal connection is probable". He suggests that the longevity of the human species is explained by our low level of energy consumption for our size. This is an arguable contention, but it is presented in a way that suggests Seely is unaware of Rubner's work of a century ago which showed that human beings live four times longer than a comparative study of energy turnover would suggest, or of Flourens's (1856) "law of longevity" that lifespan of different species is about five times as long as the duration of the growth period, a generalization that does fit the human species.

Such errors might yield to a more thorough training in nutrition and a respect for its historical background such as is to be found in Yudkin's *Encyclopaedia*. But though Seely's theories would be better for greater nutritional sophistication, this failing does not destroy the book. What particularly recommends itself is not the correctness or otherwise of the authors' specific views, it is an overall air of caution, reasonableness and balance in their tone. Even when the writers are expounding their own pet theories they sound more like judges than prosecutors. I hope they produce more books, because dispassionate commentators will be in demand as nutrition increasingly becomes a public health issue which is seen to require missionary zeal on all sides. □

John Rivers is a Senior Lecturer in the Department of Human Nutrition at the London School of Hygiene and Tropical Medicine, University of London.

Biology at work

Norman Carey

Biotechnology and Genetic Engineering Reviews, Vols 1 and 2.
Executive editor Gordon E. Russell.
Intercept, PO Box 2, Ponteland, Newcastle upon Tyne/Scholium, 265 Great Neck Road, Great Neck, New York: 1983/1984. Each volume pp. 450, £50, $79.50.

AT THE moment that these volumes arrived on my desk for review I was in conversation with one of my colleagues. "Oh no", he said, "not another one. There's too much being written about biotechnology these days." I suppose the comment could mean either that the topics have already been well covered elsewhere or that the speaker does not have time to read all that appears on the subject. Whether this series really is "too much" depends on the objectives and whether they are achieved.

The term "biotechnology" was coined at the time of the revolution in thinking which was brought about by the arrival of recombinant DNA techniques. To many people it has come to mean the industrial application of recombinant DNA. Indeed, it does to some of the authors of articles in these volumes, as is evidenced by the use of the word (Vol. 1, p.231). Of course, although a new (or at least newly publicized) term, by any definition biotechnology covers a multitude of highly profitable industrial activities which long pre-date the recombinant revolution.

In his preface, the editor refers to this fact. He says that the articles are intended to cover biotechnology "in the widest sense of that word". He implies, however, as does the title of the books, that it is the application of recombinant DNA which is the unifying feature of the articles. If that were the intention, then I feel there would be some value in a series such as this. One is forced to conclude, however, that this cannot be the aim, since many of the contributions scarcely mention *in vitro* recombination. The first article in Vol. 1, for example, "Applications of Biocatalysts to Biotechnology" (a few years ago this would have been called "Industrial Applications of Enzymes"), mentions genetics and protein engineering once, in the penultimate paragraph of the conclusions.

Perhaps, then, I have read too much into the title and preface. Maybe the series is intended to be a set of reviews on biotechnology and the title just has a high buzz-word quotient. In that case, the volumes should be judged by the quality of the articles and need for a review of each field. While there are a number of similar review series without a recombinant slant, this kind of duplication has not been a disadvantage in any other field.

Most of the contributions are informative and well written. However, there is an unnecessary degree of superficiality and repetition which a firm editorial hand should have dealt with. For example, three of the articles in Vol. 2 contain accounts of how to do cDNA and genomic cloning which are at once too slight to be of value to anyone who wishes to learn the techniques and too familiar to anyone with a degree in biochemistry. Such descriptions are now in the textbooks and do not belong in a series of "up to the minute in-depth reviews", to quote the preface again.

Librarians in industrial laboratories may subscribe to this series. But I think it likely that they will consider that scientists in their companies already know more about the topics in their own fields than these articles contain, and that they would have no reason to refer to such a wide variety of other subjects. However, libraries serving university departments of biochemistry and microbiology should have it. As general reading, most of the articles would be valuable to advanced and even first degree students; moreover, they do provide an excellent picture of the breadth of application of their disciplines. This series shows biotechnology both as high-quality science and as an important and valuable industrial activity. □

Norman Carey is Director of Research and Development at Celltech Ltd, Slough, Berkshire.

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This treatise is aimed at assembling seminal contribution in the field of bacterial immunology centred on monoclonal antibodies The contributors are pioneers, and the contributions themselves are, for the most part, first attempts at reviewing data and publication in their particular restricted areas. One of the main purposes of the book is to provide in a single reference work as much fundamental data as possible on generation, characterization, and utilization of monoclonal antibodies against bacteria, and other monoclonal products (e.g., lymphokines) relevant to antibacterial immune responses. As such, this treatise should be regarded as a milestone, a place where some of those who were the first to generate and to work with monclonal antibodies against bacteria paused for a while and reviewed their original observations.

Volume II

Late 1985, In Preparation, ISBN. 0 12.463002.2

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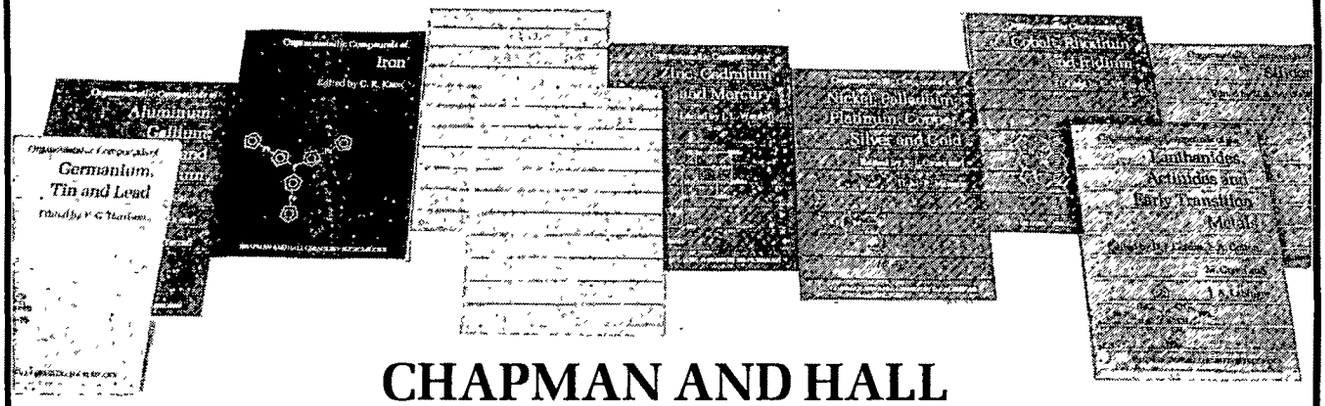
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Building for the scientist

Michael Brawne

Design, Construction and Refurbishment of Laboratories.
Edited by R. Lees and A. F. Smith.
Ellis Horwood/Halsted: 1984. Pp.375. £37.50, $79.95.

LABORATORIES are among the most expensive and least permanent of buildings. Any attempt to increase awareness of the difficulties in designing them must be welcomed, and must be seen as a contribution towards more satisfactory solution of an especially intractable building problem. Economics, of course, are often a decisive factor and it is at the same time especially important to keep the apparently high capital cost in perspective: as W.P. Horswell, one of the contributors to this volume, points out, the capital cost of a science department at a British university, calculated as an annual mortgage payment, is only 14 per cent of the running cost — 86 per cent goes to recurrent costs — and is only 7 per cent if buildings and land alone are counted. This, however, is a relationship that rarely influences the decisions of universities and other institutions funding laboratory buildings.

It was the aim of the editors, two scientists at the Laboratory of the Government Chemist in London, that the work of the 40 contributors should build up into a kind of mosaic to cover a wide range of issues and enable the designer of a new or a refurbished laboratory to avoid the major pitfalls. The foreword, moreover, suggests that the information should be widely relevant in both developed and developing countries.

As might be expected, the 36 chapters which make up the book differ both in intention and quality. Many of them contain useful information, clearly set out; some deal with generalities, without in the short space available going beyond these; and a few do no more than to set out checklists, which may be helpful reminders to those who already know the answers but do little to transmit any knowledge. Few say anything original, but there are some surprises. For example, in his paper on safety, N.H. Pearce of Bristol University suggests that the accepted wisdom on fire escapes, enshrined in legislation and enforced by fire officers, may actually make conditions much worse by trapping smoke rather than allowing it to disperse.

Nor is there necessarily unanimity among the contributors. As in science itself there will always be different views of what is most appropriate in a laboratory building. Not only are the final decisions invariably the upshot of a set of value judgements made by the designer, but laboratories are continually changing to reflect advances in scientific and technological research and to house the new equipment best suited to that research. Developments of the recent past have resulted in a drift towards opposite ends of the scale; towards very large equipment, as in radioastronomy or nuclear research institutions such as CERN, or towards miniaturization. A common factor, however, is that many controlling, measuring and recording instruments are in some way dependent upon computer technology, yet little of that impact is described in the book. At an elementary level, for example, the use of visual display units has serious implications for lighting but no mention of this is made in the pertinent chapter.

A number of the issues covered — on flexibility and adaptability, on safety, on the choice between conversion of an old building or construction of a new one, on reliability principles or building appraisal in use — have clear application to other building types and are thus of general interest. Some of the more particular discussions also have relevance beyond laboratory design since a number of other types of building — for the manufacture of electronic components or the processing of food — may well need much the same conditions. Thus, for all its failings, the book should have appeal beyond those principally concerned with laboratories.

It should never be forgotten that a laboratory is not only a place of work but is a kind of home for a large number of people. In the conversations I have had with scientists in both the physical and the life sciences for whom I have designed laboratories, the final and most searching questions were always about "what will it be like to be there?". It is assumed that there will be enough fume cupboards or adequate electrical screening; what is difficult to pin down and capture is that elusive sense of place. The book says nothing about this. Perhaps the editors felt that it was unimportant or outside their mandate; so, unfortunately, have too many others who have been responsible for laboratory buildings. □

Michael Brawne is Professor of Architecture at the University of Bath.

Ways to deal with discrete data

P.W. Hawkes

Digital Image Processing Techniques.
Edited by Michael P. Ekstrom.
Academic: 1984. Pp.372. $49.50, £40.
Applications of Walsh and Related Functions: With an Introduction to Sequency Theory.
By K.G. Beauchamp.
Academic: 1985. Pp.308. $55, £33.50.

MANY books have now been published on image processing, ranging from full-scale treatises to multi-author texts on certain specialized topics. Ekstrom's volume repeats much material already presented in more detail elsewhere, but it aims to meet a need not yet specifically catered for: how can an outsider know which of the many branches of image processing he ought to study in depth to solve his particular problem, and what hardware and software is already available? This is indeed a problem, for the introductory volumes are thick and the advanced ones incomprehensible to the uninitiated.

Ekstrom has invited a distinguished team to contribute short accounts of most of the main themes of image processing, from which the reader can rapidly grasp what the methods are capable of doing and whether they might be of use to him. The chapters cover enhancement, restoration, detection and estimation, 3-D reconstruction, coding, spectral estimation and analysis, and a longer concluding contribution surveys architecture, display hardware, software and the various systems. This should therefore be regarded as a guidebook rather than a textbook, and as such it is extremely successful.

Image processing is the subject of one of the chapters in Beauchamp's new book on discrete functions, which conveniently brings together much scattered material, but which, owing no doubt to this diversity, is very uneven. The opening chapters on the functions and transforms themselves are clear and full, including many interesting details about such important questions as the problem of shift-variance and the conversion from one transform to another.

The second half of the book, in which applications as various as seismology, medical signal processing, image enhancement and restoration, non-sinusoidal electromagnetic radiation and minimization of logic functions are discussed in 120 pages, is much less satisfactory. In some cases the text provides a good introduction to the more detailed literature, but in others the treatment is so inadequate that omission of the topic would have been the better course. Section 6.3.3 on image restoration occupies barely one page, for example, and, still in Section 6.3, it is not much use to be told that "optical .. images [are] always positive quantities and therefore do not obey completely normal statistical operations". Conversely, the four pages on nonlinear applications of signal processing do succeed in conveying the originality of Walsh functions. This, then, is a useful book, but one which could have been much better. □

P.W. Hawkes is Maître de Recherches at the Laboratoire d'Optique Electronique du CNRS, Toulouse.

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Vision as a guide to action

Stuart Sutherland

Visual Perception: Physiology, Psychology and Ecology.
By Vicki Bruce and Patrick Green.
Lawrence Erlbaum: 1985. Pp.369. Hbk £26.95, $39.95; pbk £8.95, $14.95.

IN THE edifice of visual psychology are many mansions. Most textbooks on the subject describe only the more conventional ones — colour vision, the visual illusions, visual after-effects, flicker, perspective, adaptation to wearing prisms and so on. In *Visual Perception* Vicki Bruce and Patrick Green ignore these hoary topics and present instead some of the exciting new developments that have taken place over the past 20 years. Their book is unusual: it provides almost the first elementary account of the use of computer simulation to further our understanding of vision (rightly relying mainly on the work of David Marr); it devotes many pages to vision in insects; and rather than concentrating on how visual sensations are produced or visual recognition achieved, it examines in some detail how vision can guide action.

For good measure, the authors throw in a useful and up-to-date account of the neurophysiology of the visual system including a description of X, Y and W cells, of whose elusive properties nobody has yet succeeded in making sense. They write and argue clearly, and, as a supplement to existing textbooks or as an introduction to an advanced course on vision, the book will be invaluable; it could also be helpful to workers in other scientific disciplines who want to learn about recent developments in vision.

There are, however, a few faults, some of which arise because topics are not dealt with in sufficient breadth. For example, in describing how lines and edges are extracted from the image to achieve Marr's "primal sketch", Bruce and Green should have attempted to motivate the reader by giving the reasons why this is, rather surprisingly, a very difficult thing to do; or again, they describe Marr and Nishihara's method of representing objects for the purpose of recognition without attempting to evaluate carefully how far it fits in with our current knowledge of the visual system; and their account of scene analysis is so condensed that the uninformed reader will have difficulty understanding what it is all about. Moreover, several of their diagrams are completely wrong (for example those on pages 76, 131 and 264) and will puzzle or mislead the unsophisticated.

A more debatable feature is the attention paid to the ideas of J.J. Gibson, who believed that the dynamic visual image contained invariants that specified what was present in the environment, and that these invariants were "picked up" directly by the eye with no underlying computational or inferential processes. The view is so silly that it is not worth taking seriously. Gibson was of course correct in believing that the starting point for what we see is the visual image, but who has ever doubted that? One example of an invariant is horizontal disparity between corresponding parts of the image on the left and the right eye: this disparity yields stereoscopic depth. But when we examine how disparity is actually detected and used, it turns out to be extremely complicated; despite much computational work on stereopsis over the past few years, there is still no theory that is completely adequate.

The same is true of most other invariants. Nor does one have to have read Gibson to understand that the time at which an object approaching at a constant speed will collide with someone's head can be derived from the expression dt/ds, where s is the angle subtended at the eye by any two points on the object, and t is the time of observation. As the authors point out, a more elaborate expression yields the hit-time for an object that is accelerating at a constant rate. Both the insect and the human brain almost certainly make use of formulae like these, the former for landing, the latter, among other things, for driving across busy intersections without collision. The use that organisms make of such invariants is fascinating and is spelt out in detail in the present book, but the formulations owe little or nothing to Gibson's ideas (which, after wasting much space on them, the authors are in any case eventually led to reject).

This misplaced emphasis on Gibson apart, Bruce and Green have written an interesting and pleasingly idiosyncratic book. If you want to know why sheep dogs can be trained to round up sheep, you will find the answer here, just as you will find answers to many other rarely discussed questions. And it is salutary to have a textbook that stresses that vision is a guide to action and not merely a device for providing a picture of the world in one's head. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

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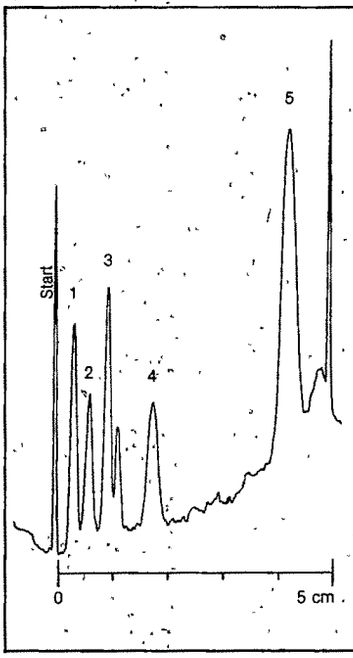
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THE FORMAT of the conference will provide ample opportunity for discussion—each formal presentation will be followed by a question period, and there will also be appropriate round-table discussions.

PARTICIPANTS in the conference will be provided in advance with a selection of reading matter intended to provide background to particular talks.

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CONFERENCE PROGRAMME

Day 1 Tuesday, June 25th

MORNING

Enzymes, immunogens
Dr D. Thomas, Université de Technologie de Compiègne.
Dr. G Winter, MRC Laboratory of Molecular Biology, Cambridge.
Dr. A. Klug, MRC Laboratory of Molecular Biology, Cambridge.

AFTERNOON

Antibodies
Dr. J Birch, Celltech Ltd., Slough.
Dr. M. Neuberger, MRC Laboratory of Molecular Biology, Cambridge.
ROUND TABLE DISCUSSION

Day 2 Wednesday, June 26th

Biosensors
Dr M. Flanagan, University College, London
Professor I.J. Higgins/Dr. A. Turner, Cranfield/Leicester:
Micro-organisms
Dr. S. Emtage, Celltech Ltd., Slough.

Micro-organisms continued
Professor D. Hopwood, John Innes Institute, Norwich.
Dr. B. Carter, Searle R&D, High Wycombe
ROUND TABLE DISCUSSION

Day 3 Thursday, June 27th

Mammalian systems
Professor P. Tiollais, Institut Pasteur, Paris.
Dr. A. Levinson, Genentech, San Francisco.
Dr. R. L. Brinster, University of Pennsylvania

Plants
Professor M. Fowler, Wolfson Institute, Sheffield.
Professor J. Schell, Max Planck Institut, Köln
ROUND TABLE DISCUSSION or FINAL TALK

POSTER SESSIONS will be held each day and will be open to those registering for the conference Please send a title and a brief synopsis to Dr Peter Newmark, Deputy Editor, Nature, 4 Little Essex Street, London WC2R 3LF, UK

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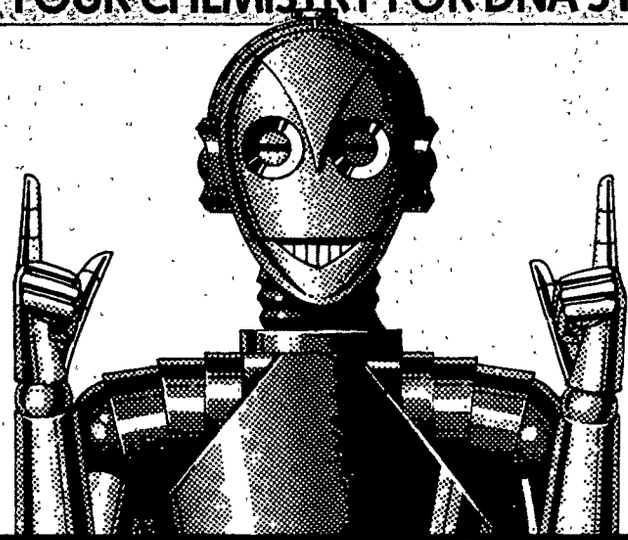
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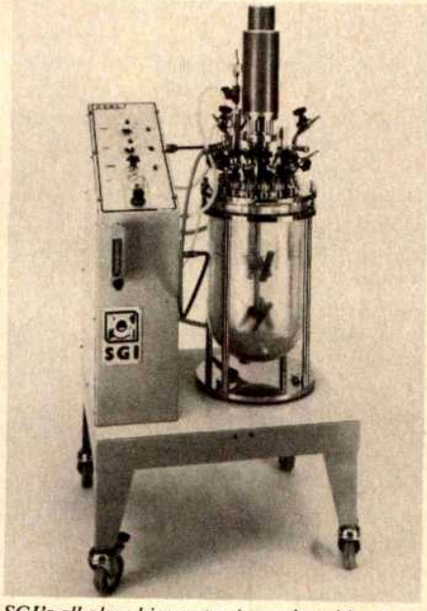
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Putting the technology into biology

Recent entrants onto the expanding 'biotechnology market' include sophisticated computerized systems for monitoring fermentations.

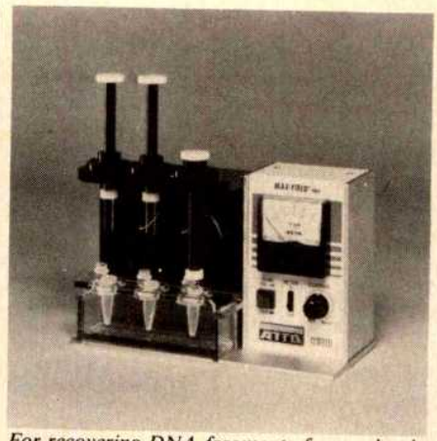
● A new all-glass bioreactor from Astra Scientific has been designed specifically for cell culture applications. Working volume is 10 litres (20 litres total), and the vessel has a hemispherical bottom and is dimensioned to provide the optimum 1:1 height to depth ratio required in this application. The vessel is completely jacketed up to its maximum working volume capacity for accurate temperature control. Two impellers provide gentle agitation, and accurate speed is assured through the use of a tachometer and feedback control loop.
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SGI's all-glass bioreactor is marketed by Astra Scientific in the United States.

● Monoclonal antibody scale-up is among the services offered to the biotechnologist by Celltech. Celltech specializes in bulk antibody production having the facilities to produce monoclonals in kilogram quantities. Celltech is also active in the business information field — Abstracts in BioCommerce is a commercial news index available as an on-line computer database.
Reader Service No. 101.

● The new Dako catalogue for 1985 permits easy location of any one of over 400 antisera, purified proteins and immunohistochemical staining kits — including monoclonal and polyclonal antibodies. Recent additions include a new line of fluorescein- and peroxidase-conjugated $F(ab')_2$ fragments of antibodies. A FITC-conjugated $F(ab')_2$ rabbit antibody against mouse immunoglobulins is useful in screening for immunoglobulin production in hybridoma cultures.
Reader Service No. 102.



For recovering DNA fragments from gels, the DNA-3 Max-Yield.

● The DNA-3 Max-Yield system, now available from Genetic Research Instrumentation, has been designed specifically to recover DNA fragments from electrophoresis gels quickly with high yields and no further nicking of the fragments. Up to 98% of each band fragment may be recovered and the system may be used as a purification procedure or for DNA recovery for further experimental assays. The DNA-3 will process up to three samples simultaneously and is complete with its own power supply, tube rack and microelectrodes. Gel fragments are placed within a special holder which in turn is placed within a 1.5 ml microcentrifuge tube. Between 200 and 500 μl of buffer is added to each tube and ice is added to the tube rack in which the tubes are resting. A pair of microelectrodes is lowered into each tube, one either side of the gel fragment, and voltage is applied. At a voltage setting of 20 volts, DNA will be removed from the fragments within 15 to 20 minutes.
Reader Service No. 103.

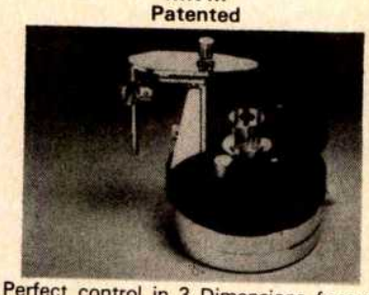
● A four-page bulletin describing Pall Biodyne ImmunoAffinity Membrane, a solid support for immobilization of antibodies and antigens, with particular application for use in immunodiagnostic test procedures, is available from the Biotechnology Division of Pall Ultrafine Filtration Corporation. Suggested protocols are provided for immobilization and binding of monoclonal or polyclonal antibodies and antigens to the membrane by wetting, immersion or perfusion. Protocols for the blocking of nonspecific protein binding to Biodyne ImmunoAffinity membranes are also discussed. The membrane is available in precut disks, squares, cartridges and roll form. It is offered in two standard pore sizes: 0.1 and 0.2 μm, but other pore sizes can be ordered.
Reader Service No. 104.

● Bacteriophage T7 gene 6 exonuclease, a 5′-3′ exonuclease with a strong preference for double-stranded DNA, is now available from United States Biochemical Corporation. Its catalytic mechanism allows it to be used for controlled digestion of DNA in both analytical and preparative experiments. The exonuclease is available in packages of 2,000 or 10,000 units; each unit is enough enzyme to release 1 nmol of acid-soluble nucleotide in 15 min at 37°C.
Reader Service No. 105.

● Cross-reactivity with both human and mouse immunoglobulins has been virtually eliminated in Tago's expanded line of goat antibodies to rat IgG (H and L chains). Tago conjugated anti-rat IgG products are designed for use as a second antibody with rat monoclonal IgG in indirect immunoassays and histochemical staining. They are especially suitable for ELISA and flow cytometry. The range includes conjugates with fluorescein, lissamine rhodamine, horseradish peroxidase, alkaline phosphatase, β-galactosidase, or biotin.
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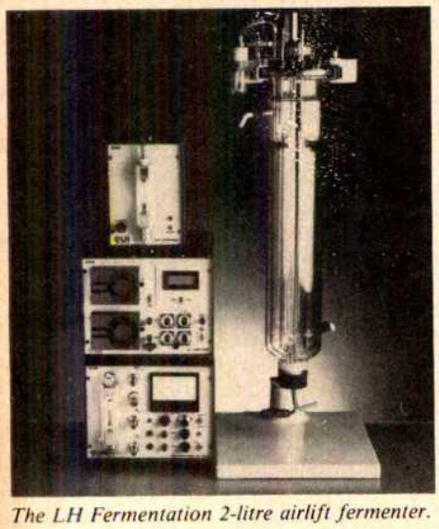
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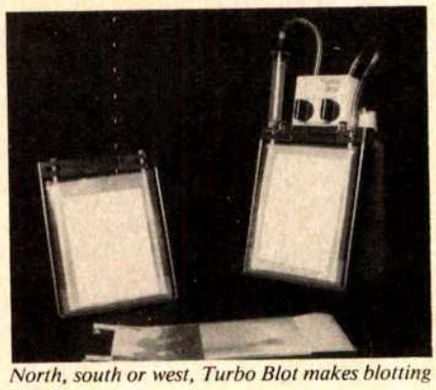


The LH Fermentation 2-litre airlift fermenter.

● A new 2-litre airlift fermenter that will be ideal for monoclonal antibody production, media optimization and other similar applications is available from LH Fermentation. Based on a 2-litre total volume glass vessel, the fermenter has a working volume of 1,700ml with a removable glass draught tube and a stainless-steel top plate. This has ports for oxygen and carbon dioxide electrodes and other sensors. Temperature control is via thermostatically controlled water circulated around a jacket on the glass vessel; agitation is achieved on the standard unit by the "air-lift" principle with a glass sinter providing the gas dispersion. Also from LH Fermentation is a new microprocessor-based fermentation control package which can monitor eight parameters in single or multiple fermentation.
Reader Service No. 107.

● Four new restriction enzymes have been added to Boehringer Mannheim's line of premium enzymes — *Asp*700, *Ban*I, *Dde*I, and *Nco*I. *Asp*700 is an isoschizomer of *Xmn*I. Another recent introduction from Boehringer is a ribonuclease inhibitor prepared to high quality for molecular biology. The preparation is supplied with a volume activity of 10–50 units per μl. It is checked for the absence of endonucleases and nicking activity.
Reader Service No. 108.

● Promega Biotec's Protoclone GT system allows the cloning and expression of a DNA insert in one day. Protoclone GT is a lambda gtll cloning and expression system which is particularly useful in the construction of cDNA libraries. It achieves high cloning efficiencies and a high percentage of insert-containing clones. And for RNA probe synthesis, Promega Biotec has introduced Riboprobe Gemini, a system allowing selective synthesis of RNA from either DNA strand following a single plasmid construction. Riboprobe Gemini produces useful plasmids for probe synthesis regardless of the insert orientation. The kit consists of: (1) two specially prepared RNA polymerases, bacteriophage SP6 polymerase and T7 polymerase; (2) two plasmids, each containing SP6 and T7 promoters flanking a multiple cloning site; (3) positive control transcription template.
Reader Service No. 109.



North, south or west, Turbo Blot makes blotting simpler.

● The American Bionuclear Turbo Blot filter processing system is the first device of its kind to handle the set-up and washing of Southern, Northern and Western blots. Hybridization and antibody binding reactions are quickly and safely performed, without need of a heat sealer, via a disposable pouch system. Probe reagents are conveniently added and removed from the pouch by means of a syringe. Blots are washed in minutes in a vacuum-wash system providing backgrounds typically lower than with manual processing.
Reader Service No. 110.

● New Plant Products, the first trading activity of Agricultural Genetics Company, has published a new leaflet describing their range of rhizobium inoculants for legumes. NPP produces a high quality product based on sedge peat containing strains of rhizobia specially selected for their nitrogen fixing ability from the extensive collection of rhizobia held at Rothamsted Experimental Station. The basic range covers all the legume crops grown in Europe and NPP can also supply inoculants for use in the tropics, and for the more unusual legumes used for reclamation work. Agricultural Genetics Company was set up to make use of the fruits of the Agricultural and Food Research Council's plant biotechnology research programme.
Reader Service No. 111.

● New from Systec Inc. is 1/2 μm phosphoramidite synthesis software for the Microsyn-1450A automated DNA synthesizer. The Microsyn-1450A syringe delivery system permits the addition of just 7.8 mg of phosphoramidite base per synthesis cycle. The quantity of CPG support is also halved in this new synthesis program (approximately 20 mg per synthesis). The 1/2 μm software does not compromise either coupling reaction efficiency (98.5–99.5%) or cycle time (6.25–7.5 minutes). It is available for use with either *O*-methyl di-isopropyl or β-cyanoethyl di-isopropyl phosphoramidites.
Reader Service No. 112.

● Dianorm's Liposomat achieves the reproducible preparation of uniformly sized unilamellar liposomes (formed within just 10 minutes) with very high encapsulation efficiency for both hydrophilic and lipophilic agents. The liposome size is selectable between 25 and 600 nm diameter, with a lipid loss of less than 1%. Sample volume is normally 5-10 ml but can be up to 200 ml if the Liposomat is used with other apparatus. The Liposomat is especially suitable for studying drug/protein/cell—liposome interactions *in vitro* and *in vivo*, for reconstitution experiments, and for the transfer of genetic material in gene- and biotechnology.
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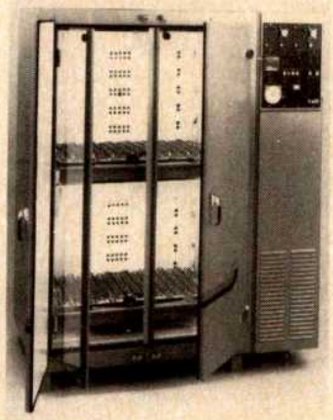


Monoclonals in cartoon form.

● A layman's explanation of monoclonal antibodies is now available on videocassette from Hybritech Europe. The video, entitled "The Monoclonal Antibody Revolution", presents in cartoon form (1) The fundamental principles of the immune reaction; (2) how antibodies can be used for medical purposes; (3) the difficulties encountered with "polyclonal antibodies"; (4) the breakthrough represented by monoclonal antibodies; (5) immediate medical applications; (6) near-term future potential.
Reader Service No. 114.

● Two new monoclonal antibody purification systems (MAPS) utilizing affinity chromatography and hydroxylapatite (HPHT) have been introduced by Bio-Rad. Affi-Gel protein A MAPS makes use of a column matrix of protein A crosslinked to agarose beads. When used with specially optimized MAPS binding, elution and regeneration buffers, Affi-Gel protein A has a binding capacity for mouse IgG_1 from ascites fluid that it is 8-10 times higher than that attainable by published methods. Affi-Gel protein A is also ideal for purifying human immunoglobulins, with a binding capacity of 20 mg of purified IgG per ml of gel. HPHT-MAPS can be used in a dedicated hydroxylapatite HPLC system for separating monoclonal antibodies from usual contaminants, such as albumin and transferrin, as well as other contaminating immunoglobulins. Using the HPHT-MAPS system, mouse monoclonal antibodies can be ultra-purified regardless of class or sub-class.
Reader Service No. 115.

● Several new hybridization probe DNAs have been added to the Oncor range. These highly purified, sequence specific DNA fragments are designed for use in nick-translation reactions and for the detection of oncogenic sequences by nucleic acid hybridization. The new probes include DNAs to detect the following sequences: *abl*, *Alu*, *erb-1*, *fes*, *sis* and *src*. Also available are antibodies to *src* and epidermal growth factor receptors. The probes are supplied in a kit containing control DNAs. Technical data sheets are available on request from the UK distributors, Stratech Scientific Ltd in London (tel. 01 354 2601).
Reader Service No. 116.



Four individually-controlled shakers make up the Bioengineering Associates environmental control cabinet.

● Bioengineering Associates Inc. has introduced an environmental control cabinet containing four individually controlled shaking units. The flow of air guarantees uniform distribution of temperature, gaseous mixtures and humidity. Shaker heights are adjustable, allowing different shakers to be run with various size flasks; as well as a variety of speeds between 10 and 400 r.p.m. The temperature range is approximately 15°C below room temperature to 80°C with ± 0.2°C accuracy. Magnetic drive ensures silent vibration-free running, without the need to anchor the four units.
Reader Service No. 117.

● Seraclone fetal calf serum is specially selected for its ability to support the growth of mouse hybridoma and/or myeloma cells in limiting dilution cloning in microtitre plates at 1 cell per well. Only those fetal calf sera which support cloning efficiencies of ± 100% as predicted by Poisson distribution frequencies are given the label Seraclone. Available from Sera-Lab of Crawley Down in Sussex, this new grade of fetal calf serum is suitable for all phases of monoclonal antibody production.
Reader Service No. 118.

● The latest edition of the Serotec catalogue of monoclonal and polyclonal reagents has many new additions including sheep anti-human antisera to complement proteins, thyroid hormones, coagulation factors, serum proteases and inhibitors, conjugated to FITC, TRITC, peroxidase and alkaline phosphatase; purified monoclonal antibodies to immunoglobulins, serum components, MHC antigens and leukocytes; and mouse and rat monoclonal antibodies to MHC antigens and immunoglobulins. Serotec has established a network of distributors in Australia, Denmark, Greece, Ireland, Italy, Israel, Japan, New Zealand, Portugal, Spain, Sweden, Switzerland, Taiwan, The Netherlands, West Germany, Korea and Canada and is in final negotiations with companies in the United States.
Reader Service No. 119.

● Clontech now has cDNA libraries available for researchers. These libraries are prepared from human, rat and mouse tissues, from various cell lines, and there is also a yeast genomic library. The vectors are lambda gt10, gt11 and pBR322.
Reader Service No. 120.

● MicroGenie from Beckman Instruments is a software program which can collect, analyse and compare nucleic acid and protein sequences. MicroGenie can be used by the computer novice, guided with simple menus. It can analyse sequences of up to 60,000 nucleotides, enough for bacteriophage lambda or adenovirus. The memory stores up to a million nucleotides.
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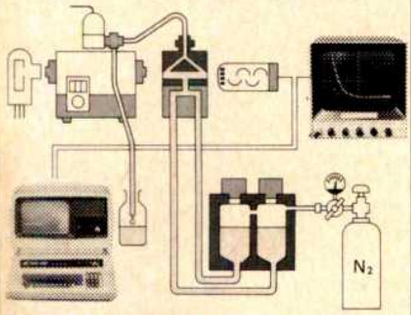
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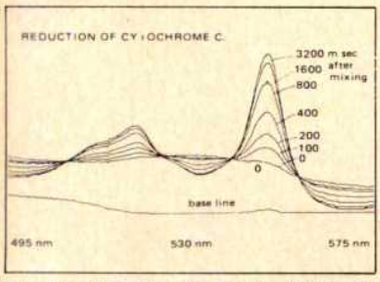
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Reader Service No. 15



Computer-directed DNA synthesis — Autogen 5500.

● Genetic Design of Watertown Massachusetts has developed the AutoGen 6500, the first automated DNA synthesizer to incorporate computer-directed variable-cycle control of the synthesizing process. The new system has the capacity for automatic synthesis of as many as three sequential 100-base fragments with no operator intervention. The AutoGen 6500 uses an Apple IIe computer (128KB, dual disks, CRT, voice synthesizer, Okidata 92 printer) for all control functions. Software is user-programmable by simple menu-driven commands. In addition, numerous utilities are provided for cycle and function parameterization defined by the user. A modem and printer are included with each system.
Reader Service No. 122.

● Now available in the UK from P & S Biochemicals of Liverpool, Promega Biotec's Packagene system is a single-extract *in vitro* lambda DNA packaging system. By combining all the necessary components and buffers in one tube, the system enables the user to conduct a packaging reaction simply by adding a sample of DNA to a volume of Packagene and incubating. Packaging efficiencies of over 10^8 PFU per μg DNA are guaranteed, allowing effective and successful construction of DNA libraries.
Reader Service No. 123.



A new medium for hybridomas.

● Ventrex Laboratories have formulated a new serum-free cell culture medium, HL-1, that is ideal for hybridomas and lymphoid cells. HL-1 will facilitate the *in vitro* scale-up of monoclonal antibody production and simplifies or eliminates purification steps. In the United Kingdom, HL-1 is available from Tissue Culture Services of Slough.
Reader Service No. 124.

● A powerful new data acquisition and logging package has been introduced by the Laboratory Computer Systems Division of Milequip, the Gloucestershire based computer systems house. The package, known as Logger, is ideal for use with medium- or long-term biotechnological and process biochemical experiments and by technicians with no previous computer experience. The program runs in real time with a variable scanning interval. Following each scan, readings are displayed with an average facility for data reduction of a large number of scans, if required. Readings outside of preselected parameters sound an audible alarm, which may be deactivated if required.
Reader Service No. 125.

● The LSI mammalian cell transfection kit has been developed to make the transfer of stably expressing genes into mammalian cells a simple routine operation. Each kit includes: (1) All the pre-tested solutions for calcium phosphate transfecting up to 100 100-mm plates of cells; (2) a detailed protocol; (3) sterile 0.2-μm syringe filters; (4) sterile storage vials; (5) test results for each lot. This new LSI kit is available from Vanguard International of Neptune, New Jersey.
Reader Service No. 126.

● Mentor is a new computer process control and data logging system for fermentation, developed by Life Science Laboratories Ltd of Luton. The software is optimized so that the Mentor system can be installed on an existing as well as a new fermentation plant without delay. Mentor 1 hardware is IBM PC based and utilizes a colour VDU, floppy disk drive, Winchester disk and six-pen colour plotter. The system is completely menu-driven with all aspects of configuration and operation being available to the keyboard user through a high-level language. Control sequences for operation of valves and loops can be specified by the user such that routines like sterilization, inoculation and transfers can be carried out automatically.
Reader Service No. 127.

● Bethesda Research Laboratories' new murine reverse transcriptase, Cloned M-MLV RT, synthesizes a higher percentage of full-length cDNA from mRNA than does avian reverse transcriptase. Murine RT is free of many of the contaminants common in avian RT preparations. BRL has cloned the reverse transcriptase coding sequence from M-MLV (Moloney murine leukaemia virus), ensuring reproducibly high performance. Full-length cDNAs can be synthesized more efficiently using Cloned M-MLV RT because it does not contain the DNA endonuclease activity associated with AMV RT. Purity is greater than 95% and there is no detectable DNase or RNase.
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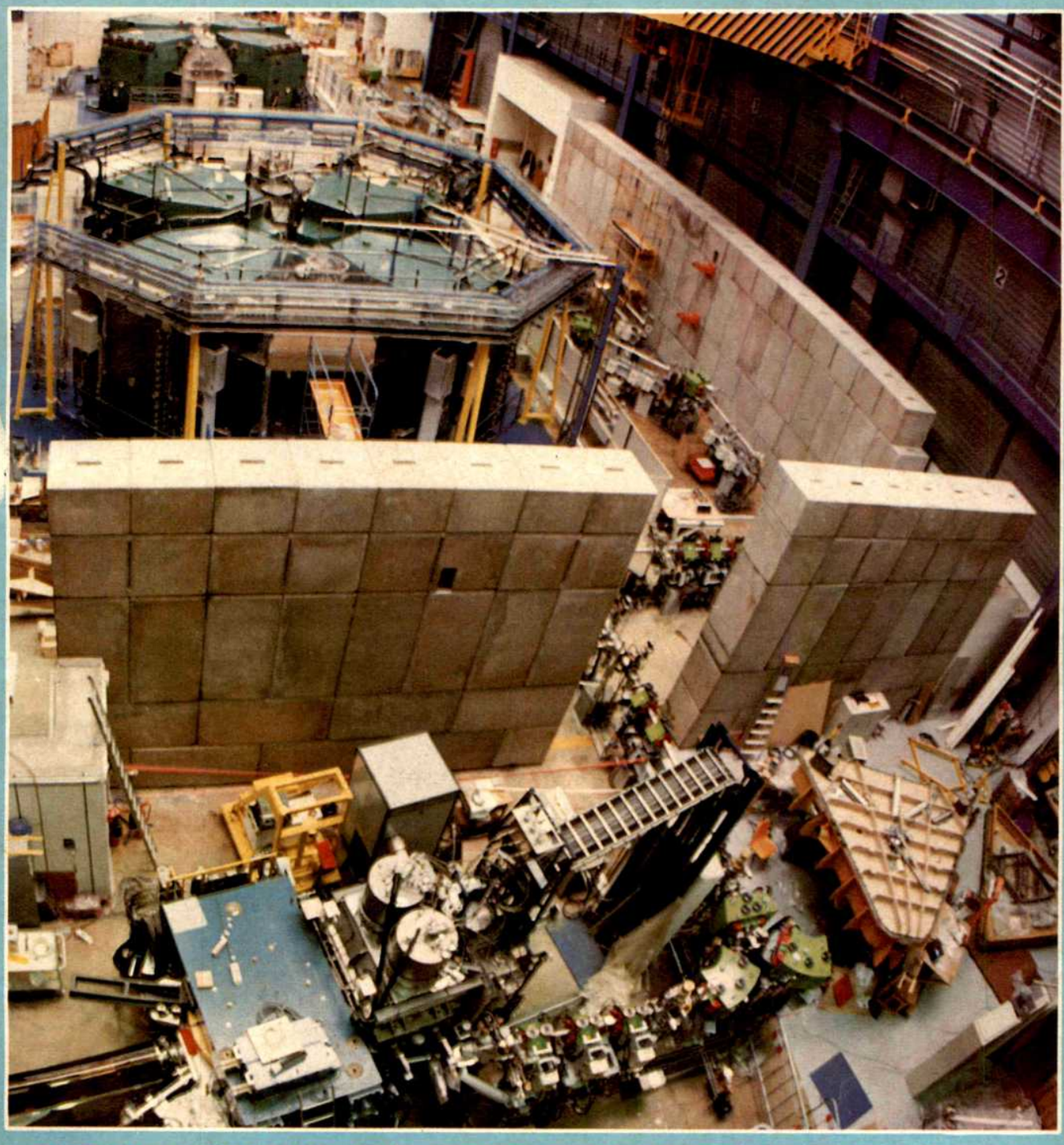
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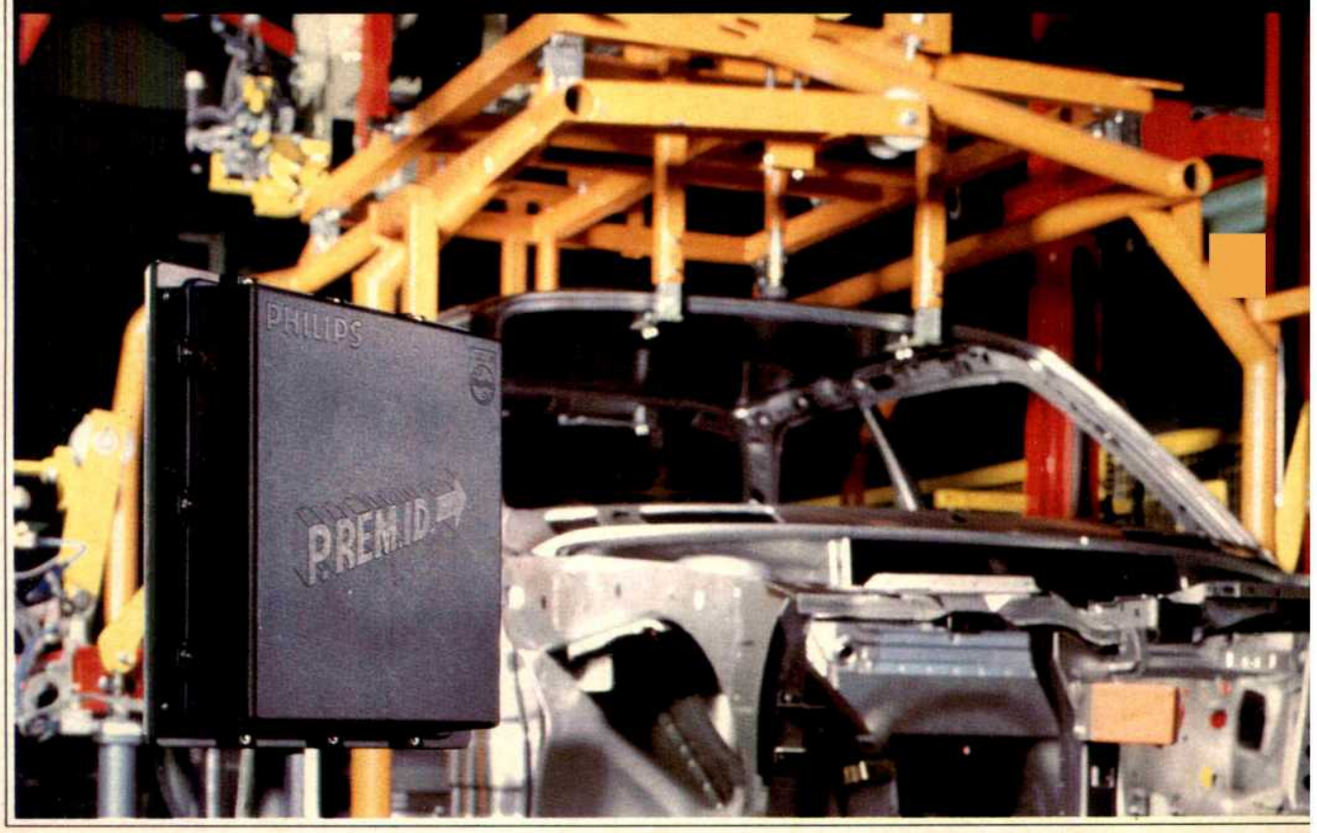
Wuhan's Number 2 Steel Works, in the Peoples' Republic of China, has recently made use of Philips expertise in industrial automation to improve its efficiency, quality and use of raw materials.

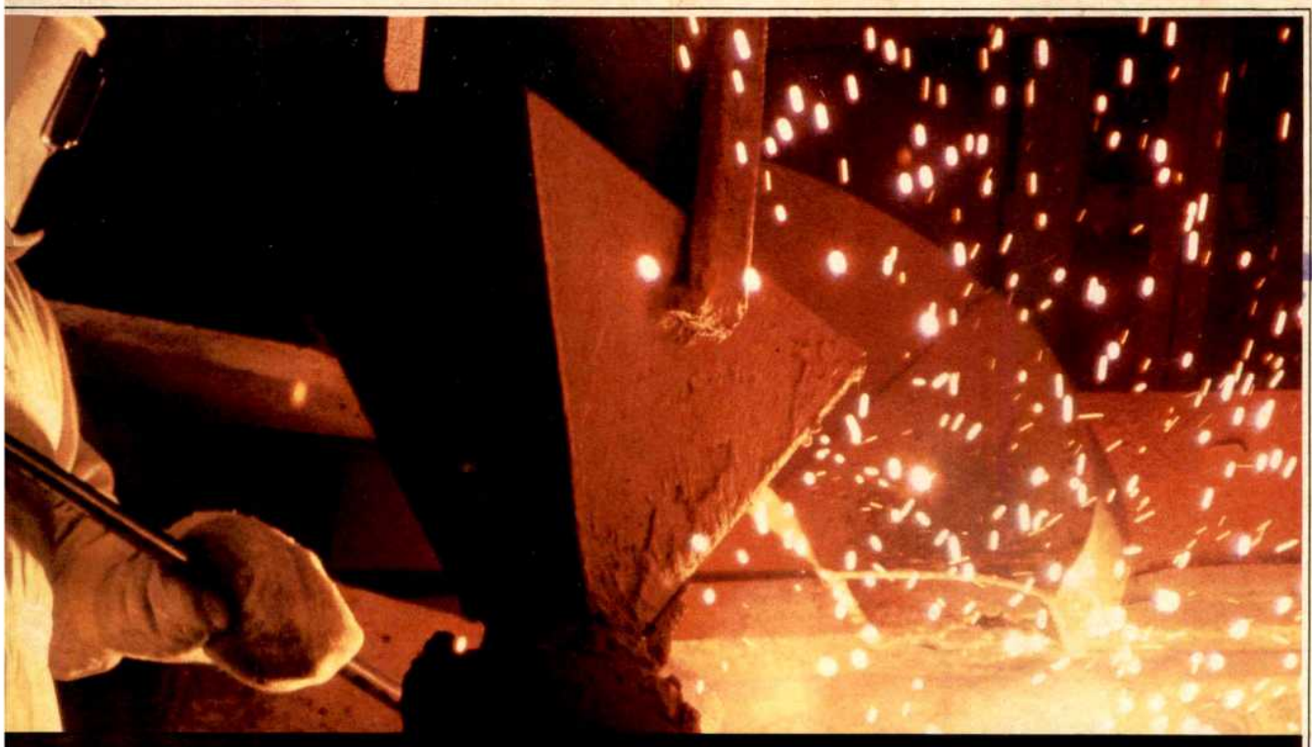
The Philips electronic weighing and batching system, which now forms the sophisticated heart of the factory, controls three LD converters, each equipped with six weighing hoppers for additives, two for intermediate bunkers, and one for ladle additive. Individual compact batching systems are connected to a supervisory micro-computer controller.

In addition, electronic weighing techniques are employed at various other stages of the process. For example, when scrap is charged into the converter, it is weighed on a specially designed platform with built-in protective features to prevent damage.

In Germany the problem was how to ensure that production ran smoothly, while still offering customers "made-to-measure" cars.

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The Philips P.REM.ID. system (Programmable REMote IDentification) has been introduced with great success for the production of BMWs. The system includes "recognition" modules, which are programmed through a microwave signal. These signals determine which particular item is required for which individual car.

In this way, a personalised car – with a special gear box, transmission system, interior, and so on – can be included in the normal assembly line. The system allows a high degree of flexibility, while retaining a high output level on the line.

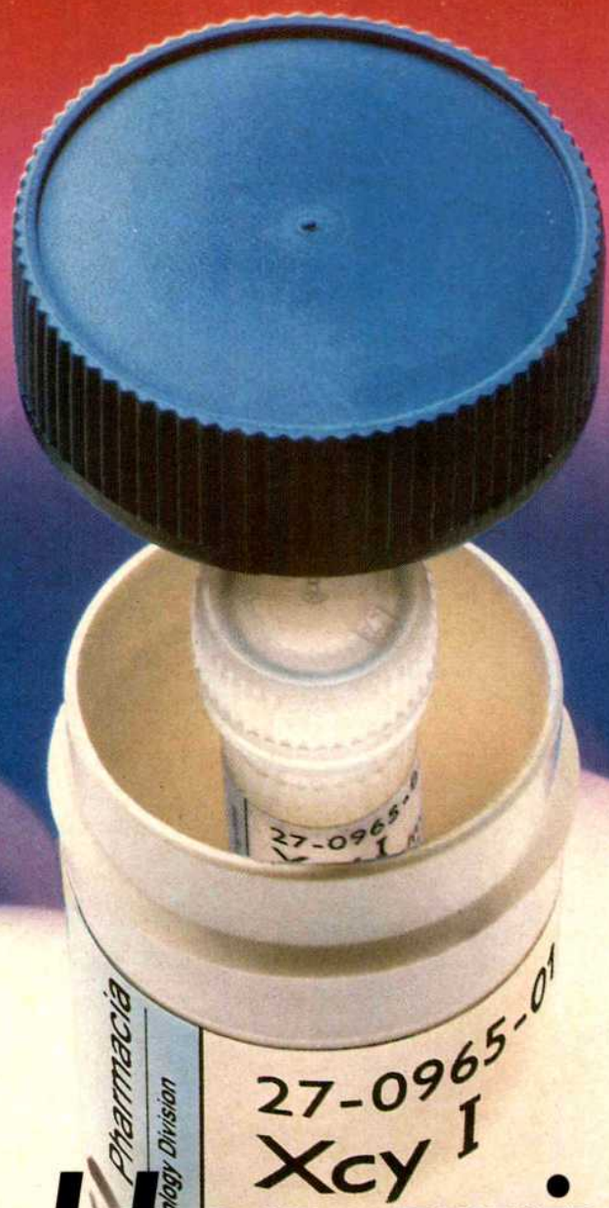
These are but two examples of how Philips expertise can be put at the service of industry – throughout the world. For more information about Philips expertise world-wide, write to the Philips organization in your country or to Philips C.P.M.S., VOA-0217/N9, Eindhoven, the Netherlands.

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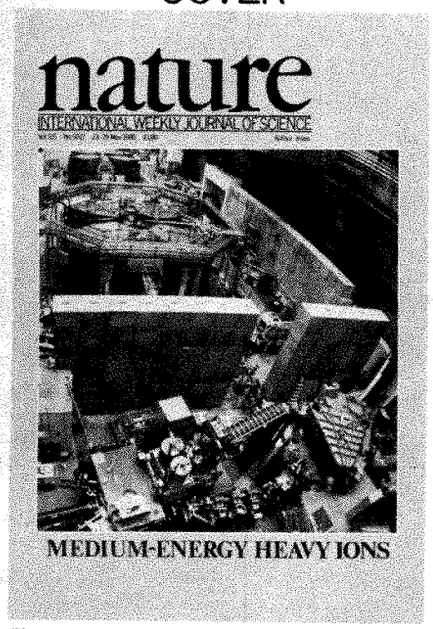
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NATURE VOL. 315 23 MAY 1985

COVER



The French heavy-ion accelerator GANIL (Grand Accelerateur National d'Ions Lourds). GANIL consists of three cyclotrons, linked in series to produce some of the most complex of all nuclear phenomena. These occur when nuclei collide at energies too high for them to behave like drops of liquid yet too low to behave like clouds of independent neutrons and protons. GANIL physics is described by the director of the accelerator, Claude Détraz, on page 291.

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4 Suggested Applications

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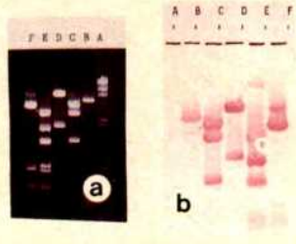


fig. 1

a) Restriction enzyme digest of pBR 328 and pSVK1 (plasmid containing pBR and SV-40 sequences)
b) Blot of restriction enzyme digest hybridized with pSVK1, modified by Orgenics CHEMIPROBE Kit. Detection by double sandwich enzyme immunoassay.

Lanes: A. λ /Hind III marker
B. pBR328/EcoR I
C. pSVK1/Pst I
D. pSVK1/EcoR I
E. pSVK1/Hind III
F. pSVK1/Stu I

2. — Dot hybridization

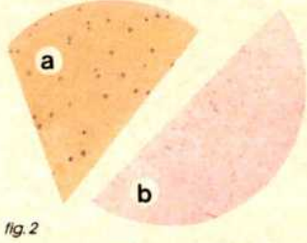


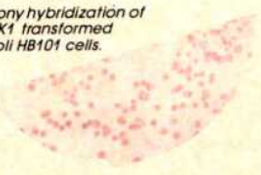
fig. 2

a) Plaques of M13mp8 and M13mp8 with pSVK1/Hind III insert.
b) Blot of plaques hybridized with pSVK1, modified by Orgenics CHEMIPROBE Kit. Detection by double sandwich enzyme immunoassay.

3. — Colony hybridization

fig. 3

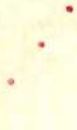
Colony hybridization of pSVK1 transformed E. coli HB101 cells.



4. — Plaque hybridization

fig. 4

Dot hybridization to decreasing amounts of target DNA (pSVK1).



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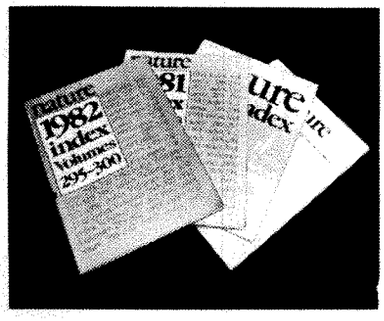
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Enzymes, immunogens
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Dr. G. Winter, MRC Laboratory of Molecular Biology, Cambridge.
Dr. A. Klug, MRC Laboratory of Molecular Biology, Cambridge.

AFTERNOON

Antibodies
Dr. J. Birch, Celltech Ltd., Slough.
Dr. M. Neuberger, MRC Laboratory of Molecular Biology, Cambridge.
ROUND TABLE DISCUSSION

Day 2 Wednesday, June 26th

Biosensors
Dr. M. Flanagan, University College, London
Professor I.J. Higgins/Dr. A. Turner, Cranfield/Leicester.

Micro-organisms
Dr. S. Emtage, Celltech Ltd., Slough.

Micro-organisms continued
Professor D. Hopwood, John Innes Institute, Norwich.
Dr. B. Carter, Searle R&D, High Wycombe.
ROUND TABLE DISCUSSION

Day 3 Thursday, June 27th

Mammalian systems
Professor P. Tiollais, Institut Pasteur, Paris.
Dr. A. Levinson, Genentech, San Francisco.
Dr. R. L. Brinster, University of Pennsylvania

Plants
Professor M. Fowler, Wolfson Institute, Sheffield.
Professor J. Schell, Max Planck Institut, Köln.
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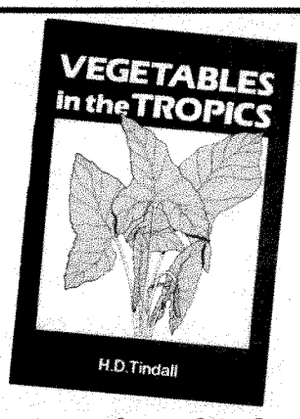
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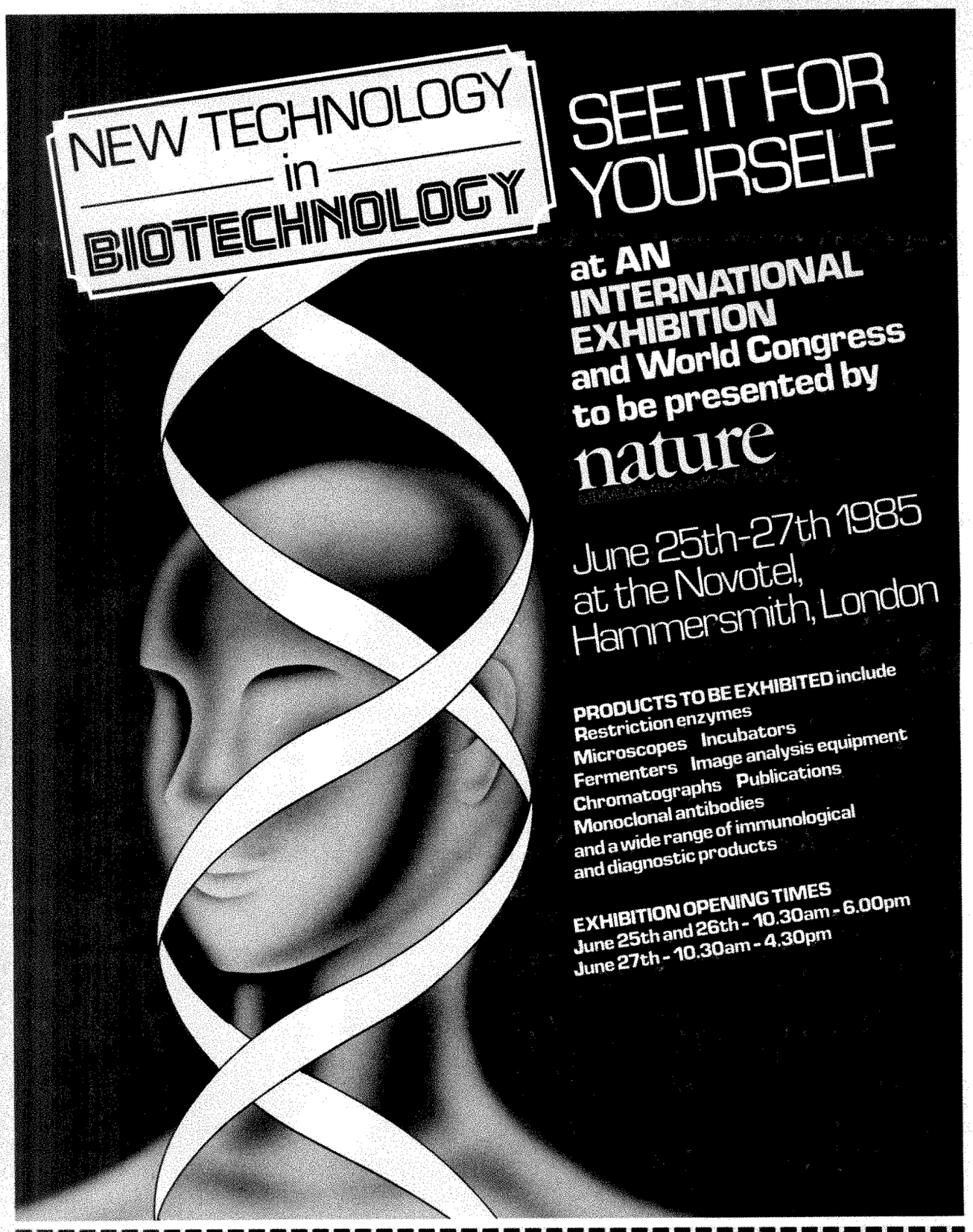
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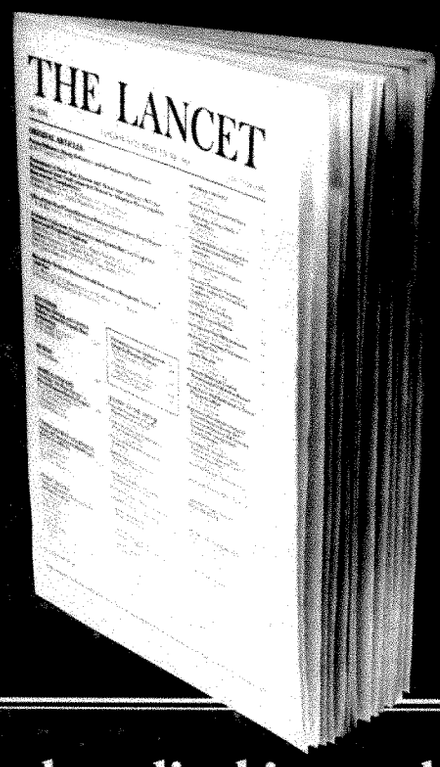
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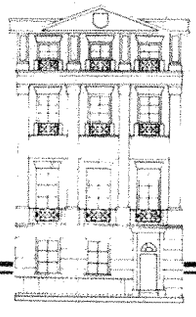
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University paymasters discredited

The British government's latest policy document on higher education is a disgrace. Academics now have nothing to lose but the tenure of their jobs (itself proscribed).

THE British government's latest proposals for higher education (p.270) are most of all distinguished by their vacuity, the quality of being "content-free" in the newest civil service argot. The officials at the Department of Education and Science who have laboured for the past year and more to put flesh on the bones of Sir Keith Joseph's preconceptions about higher education and those who work therein have produced an agenda for a non-discussion, for interested parties to say where they have always stood while the cause they are supposed to share is lost.

Shabby is the best word for this literally appalling document, and calculated indifference is the only way of describing the policy it embodies. Ordinary innocents might suppose that a state paper with "*...higher education into the 1990s*" in its title might have something valuable to say about the function of higher education in contemporary society. Carefully, the draughtsmen of this latest prospectus for British universities and polytechnics give no hostages to fortune. They simply quote the Robbins report of 1963 and launch into a discussion of the role of higher education in "the production of qualified manpower".

That calculated indifference is the British goverment's policy in higher education is now made crystal clear. (The calculation is that if the government does nothing about the problems of higher education, they will solve themselves just as mail that is left unanswered is usually overtaken by events, in this connection the disappearance of a nuisance.) Thus the green paper stolidy dodges the question that higher education has been asking the British government for at least five years, that there should be a clear definition of its function. (The explanation that there can be no longer forecast of the funds that will be available to higher education than for, say, the Ministry of Defence, is an answer to a different question.) Instead, the green paper parades the need that institutions of higher education should look to other sources than the British government to keep themselves in being. Perhaps universities and polytechnics should take the hint and make a dash for freedom). That way, they might be better off.

The plain truth is that the British government has systematically misled British higher education. Although the once-and-for-all reduction of 8.5 per cent of university budgets decreed in 1980, together with the manipulation of the fees of overseas students whose variable effects were to reduce university incomes by an average of 5 per cent, was accompanied by promises that there would be "level funding" once the shock had been absorbed, the fact is that the budgets of the universities as a whole are still falling by between 1.5 per cent and 2 per cent a year in real terms. The polytechnics lie some two years behind on the path to penury, chiefly because of the delay in creating their supervisory National Advisory Body. During the same period, the government has put a ceiling on the numbers of students that universities and polytechnics separately are allowed to teach, has threatened a cumbersome procedure for denying academics the right to tenure of their jobs and has generally exhorted everyone in sight to find other sources of funds than the public purse.

Nobody should deny that the government has problems. In the prosperous early 1960s, it seemed sensible that there should be a public system of higher education to which all qualified would-be students should have access, which principle would determine the scale of the system. It also then seemed financially feasible that the central government should meet the cost to local authorities of maintenance grants for all students winning themselves a place in higher education. Even the present government's belief that British higher education is indolently unconcerned with Britain's chronic economic difficulties is not new, as shown by the decision during the 1964 Wilson government that the polytechnics should be designated as an alternative system of higher education, one more concerned with practical affairs.

Sourly, the present government has also allowed itself to be captivated by the belief that the whole of higher education is somehow corruptly innefficient. The green paper applauds the recent Jarrett inquiry into the management of six universities but ignores the first of its recommendations, that a prerequisite of efficiency is a coherent policy framework for higher education as a whole There may be something in the suspicion that British higher education could be operated more economically, although the most obvious diseconomies in the system now are those created by the government's own edicts of recent years. Preoccupied with these problems, the government has taken to behaving as if it wished that higher education would simply disappear. The green paper offers such perfunctory acknowledgement that the system now in being has merit that its readers may be forgiven for thinking that the government has no heart for the struggle to keep the system going.

The crunch will come quite soon. If real resources continue to decline by between 1.5 per cent and 2 per cent a year, whatever slack there is in the system will soon be exhausted. Then the grants committee and the advisory body will have to tell the government that the drain cannot continue within the present framework without striking some whole institutions off the public payroll. Prudent institutions, universities or polytechnics, will be well advised to get out of the system before that time comes. Reorganizing for independent survival is now the game. The green paper's list of alternative sponsors should (and will) be studied carefully, but there may be others, municipal authorities for example. Only the more depressed among institutions of higher education will ask why should they bother struggling to survive when their present sponsor is so plainly not merely indifferent but ignorant of their role. Some among the others will sense that the claims upon them consist not merely of inputs and outputs ("student demand" and "qualified manpower") but of young people sharing a common conception of what the future might be like; and that knowledge is neither for its own sake nor for the winning of prestige but for a civility that the author and originators of such a banal state document could not comprehend. Groucho Marx is known not to have wanted membership of a club that would let him in. Who, in scholarship and research, dares admit to sponsorship like this? □

Biological manuscripts

Miranda Robertson, Biological Sciences Editor of *Nature*, is now based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees).

High-energy physics

UK committee to push for CERN budget cuts

BRITAIN'S spending on high-energy physics is out of balance with its spending on other science, and must be reduced substantially, according to the committee set up last year to consider continued British membership of CERN (the European Organization for Nuclear Research). The committee, chaired by molecular biologist Sir John Kendrew, will report next week to the chairmen of the Advisory Board for the Research Councils (ABRC) and the Science and Engineering Research Council (SERC) that it has been impressed with the excellence of CERN's work, but that the organization's budget should be kept constant beyond 1990.

According to one figure being canvassed in the committee this week, the CERN budget could come down by 20-25 per cent after 1990 without substantially affecting the quality of CERN research, which by then will depend almost entirely on the running of the large electron-positron collider LEP and its extension to "phase II" 100-GeV operation But whether the final report will actually mention such a figure is uncertain. Committee members are anxious to avoid a conflict with their European partners in CERN, with whom they would like to seek an agreed reduction, and to quote 20-25 per cent might be counterproductive.

Indeed, French officials last week described a cut on such a scale as "likely to kill CERN, given that half their costs are salaries". The West German position is that a 25 per cent cut "could not be done fairly". Nevertheless both countries consider that some cuts are possible. Paris considers that cuts "of a few per cent" would not harm CERN, and Bonn that a 10 per cent by 1990 might be supportable.

Sir John Kendrew and members of his committee met the West German research minister, Heinz Riesenhuber, and the French research minister Hubert Curien, earlier this year, and were encouraged to learn that others might agree that CERN budgets should be trimmed. But the committee did not visit Italy, where the government recently doubled national spending on high-energy and nuclear physics and is in a mood to increase the CERN budget rather than decrease it.

The West Germans told Sir John that the government was more comfortable with the CERN contribution than Britain because certain other projects supported by the research ministry (BMFT) (such as nuclear energy) are decreasing in cost and because, overall, the ministry has been enjoying a roughly constant real budget. He also argued that the expenditure on research and development in most European nations (including Britain) had decreased as a fraction of gross national product over the past decade, so effective cuts had already taken place. A German committee on big projects under Klaus Pinkau had also set a high priority for CERN and for LEP. Nevertheless Germany is looking for savings.

Mandatory scales of contribution of the eleven member states of CERN, 1985-87:

	Percentage
West Germany	24.90
France	20.23
United Kingdom	16.91
Italy	13.56
The Netherlands	5.39
Spain	4.94
Sweden	4.01
Switzerland	3.87
Belgium	3.61
Denmark	1.98
Ireland	0.60

The CERN budget in 1985 amounts to SF 724.5 million (around £220 million).

Cuts could be found, according to Riesenhuber, in reductions of staff and salaries at CERN. (One complaint is that CERN salaries are so high that it is impossible to attract German scientists back to Germany. There is also, according to Riesenhuber, scope for rationalization of CERN management and in postponing in new investments (such as extending LEP to full performance). A 10 per cent cut by 1990 is "realistic", it is believed in Bonn.

At CERN, however, the prospect of cuts is faced with some horror. CERN director-general Professor Herwig Schopper would like to begin the extension of LEP to 100 GeV per beam as soon as technically possible, which with rapid advances in superconducting accelerating cavities means spending sooner than expected — in 1989. This would mean a small increase in CERN spending in the new five-year planning period.

There will also be a pensions problem. Schopper says that CERN council decided four years ago to increase staff pensions in line with those available in Switzerland, which will mean increased expenditure on personnel when the main retirement bulge begins in 1990. But CERN has not yet made the investments into the pension fund either to pay for these increases or to pay the basic pension. "Our actuaries tell us there is a 'technical deficit' ", says Schopper. CERN is indeed planning a reduction in personnel, and to make more use of outside contractors, but even then "it's unavoidable that there will be an increase in our personnel budget" because of the pension commitments. The only light on the horizon is that Spain will become a full member by 1989, increasing its contribution from the present 5 per cent to 7 per cent of the CERN budget, says Schopper.

For the Kendrew committee, the problem is that even a 10 per cent cut in the CERN contribution would not have provided the increased funding for other sciences that the committee believes necessary. Hence the mooted target of 20-25 per cent, and hence, no doubt, a propensity to be sceptical about Professor Schopper's claims.

Indeed, the feeling in the committee goes even further. First, an attempt should certainly be made first to reach agreement with European partners for a large reduction in the CERN budget. But if that fails, and if the hopes of some members are disappointed that collaboration with the United States will be feasible, the only option will be complete withdrawal from CERN. **Robert Walgate**

Caltech at war on star wars

Washington

MARVIN Goldberger, president of California Institute of Technology, has written to Secretary of Defense Caspar Weinberger taking exception to an announcement that Caltech had joined a "consortium" of universities carrying out star wars research. In his letter, Goldberger says that Caltech's only involvement in the $9 million contract announced by the Innovative Science and Technology (IST) division of the Strategic Defense Initiative organization for research on optical signal processing is a single $50,000 subcontract to one Caltech scientist from the University of Dayton Research Institute.

A press release put out by the IST office announced the "formation of a consortium of several institutes," including Carnegie Mellon, Stanford and Massachusetts Institute of Technology.

Caltech has yet to approve the contract. Both Caltech and Stanford have also expressed concern about news reports that the work would be classified. But according to Dr James Ionson, who heads the IST office, all the work would be totally free of classification or other restrictions in accordance with Department of Defense policy on basic research that is conducted on campus.

Ionson defended the use of the term "consortium", saying that the scientists involved had worked together on the proposal, which was submitted by the University of Dayton and the University of Alabama at Huntsville. "The important news is that some of the most prestigious individual scientists are working together for a common goal", he said. As to Goldberger, "It's no secret that he's not an advocate of SDI." Stephen Budiansky

UK laboratory animals

New legislation forecast

THE British government has taken a further step towards tighter legislation on animal experiments in a second White Paper (policy document) on *Scientific procedures on living animals*, published last week by the Home Office, the government department responsible (Cmnd 9521, HMSO, £3.30). The document is said to be the result of collaboration between the government, scientists and the "realistic element of the animal welfare movement". The main changes from the earlier proposals (see *Nature* **303**, 191; 1983) are: tighter controls on the severity of procedures; modification of the project licensing assessment system; improvements in laboratory animal care; higher penalties for some offences.

Under the new proposals, investigators will need two separate licences, both a personal licence betokening general competence and a project licence for each proposed investigation. Applicants for both kinds of licence must provide the Home Secretary with detailed information about themselves and their proposed work, together with the names of persons willing to act as sponsors. For a personal licence, the sponsor will vouch for the applicant's "competence and integrity". For project licences, the sponsor will give a detailed opinion of the scientific merits of the project and techniques to be used. The white paper says that a field trial has shown that such a system would work in practice. But because of the practical difficulties of assessing all project licence applications in this way, the Home Office now proposed to refer only those projects where the scientific quality of the work is in doubt to an independent assessor.

Criteria for obtaining a licence under the proposed regime will be stricter than at present. The Home Secretary must be persuaded that the work is justifiable, that no satisfactory alternative to the use of animals is available, that the minimum number of animals is used and that the least possible suffering is caused.

Considerable emphasis is placed on the severity of pain, which will be balanced against potential benefit when project licence applications are considered. Such a cost-benefit principle has been widely advocated, in particular by Sir Andrew Huxley, president of the Royal Society, who suggested a graded approach to the justification of experiments on animals in his anniversary address to the Royal Society last year.

The new white paper is an excellent compromise in the debate on animal experimentation, Sir Andrew said this week. The licensing system will make clear who is responsible for a project, and the form of permission for project licences will ensure that granting a licence does not become a "rubber stamp procedure".

In practice, the Home Office will issue personal licences which will, in effect, be a certificate of competence to a particular investigator to pursue a particular project. But project licences will be issued only to applicants "who can satisfy the criterion of permissible purpose". The Home Secretary will impose conditions setting "permissible degrees of severity (of pain) for that project . mild, moderate and substantial". Home Office inspectors will have increased power to order humane killings, "action to be taken at the onset of various signs" such as loss of motor function. The new proposals also require painless killing at the end of a predetermined period, administration of analgesic and constant supervision throughout the procedure.

Before a project licence will be granted, the applicant must agree with the Home Secretary on the likely level of severity of pain an notify an inspector if this forecast is likely to be exceeded. The Home Secretary will decide how the categories of pain are to be defined and applied, and will publish guidelines to inform applicants, licensees and the general public of how the proposals are intended to work.

An animal procedures committee is being set up to advise the government on policy, on applications for research causing "special concern" and on the testing of cosmetics. The committee is chaired by Baroness Warnock and its members include scientists, representatives of organizations that protect animals and representatives of pharmaceutical companies.

The composition of the committee, however, emphasizes the government's commitment to regulated experiments on animals. As Sir Andrew pointed out, the situation cannot arise where experiments can be stopped by a block vote of anti-experimentalists on the committee, which answers the genuine worries of many biological and medical scientists.

Reactions to the white paper are mixed. The Association of British Pharmaceutical Industries accepts the need to update the existing (1876) legislation, but is concerned that there will be "too narrow a definition of the scope of the project licensing provisions". The Humane Research Trust and the British Veterinary Association welcome the proposals in the main. The British Union for the Abolition of Vivisection calls them a "confidence trick" and a "vivisectionists' charter".

The number of experiments on animals in the United Kingdom has fallen from 5.6 million in 1971 to 3.6 million in 1983. In 1985 the Humane Research Trust will give grants of £100,000 to nineteen investigators using alternative experimental procedures that do not animals, and the pharmaceutical industries will spend £10-12 million on development of non-animal techniques in drug testing.

Maxine Clarke

US laboratory animals

NIH watchdog committees

Washington

LABORATORY animal research at publicly supported institutions in the United States will from November this year have to be overseen by special "animal use" committees that will include at least one lay representative, according to new guidelines issued by the Department of Health and Human Services. The guidelines charge the animal use committees with responsibility for reviewing research programmes and inspecting laboratories and animal houses at least annually. Research that does not meet the committees' standards can be stopped.

The requirement that each committee should include at least one lay member is welcomed by animal welfare campaigners, but some had hoped for a representative concerned specifically with welfare. Welfare champions are unhappy that the committees are not required to review in detail research protocols for investigations in which non-trivial pain may be caused.

Christine Stephens, president of the Animal Welfare Institute, says that this omission is a "profound disappointment". Earlier proposals had required special approval by the committee for non-routine or harmful invasive procedures, procedures involving prolonged restraint, diseases maintained for long periods or non-standard methods of euthanasia. The new proposals leave it to committee members to decide if special attention is warranted. Ms Stephens is also disturbed that institutions can apparently apply for a retroactive waiver that would release them from their responsibilities under the new guidelines.

The Association of American Universities, in contrast, is relieved that lay members will not be required to review research protocols, a requirement that the association considers "unacceptable".

The guidelines do not have the force of law, although an institution could in theory lose its financial support if deficiencies went uncorrected for long enough. Critics point out, however, that such an occurrence is almost unknown. The National Institutes of Health are still investigating allegations of mistreatment of baboons used in head injury experiments at the University of Pennsylvania; despite the widespread availability of a video tape showing apparent mistreatment, which was last week being shown to members of Congress, the research is still being supported. A bill introduced in the last Congress by Senator Robert Dole and Representative George Brown would have given statutory force to animal use committees: a new version of the bill is likely to be introduced in Congress within the next few weeks.

Tim Beardsley

DNA probes

Type culture a source of gain

Washington

THE role of the American Type Culture Collection (ATCC) as a repository of materials for basic biological rsearch is being threatened, researchers say, by commercial exploitation of cell lines deposited there. At least one molecular biologist has refused to send any more of his samples to the collection after a commercial company obtained an oncogene he had deposited and, without his knowledge, began marketing to researchers DNA probes derived from it.

ATCC, a non-profit organization, makes cell lines and other materials in its collection available to any qualified researcher who requests them, charging a nominal fee to cover its costs. Although primarily a service to basic researchers, the collection has played an increasingly important role in facilitating the patenting of recombinant DNA products and processes.

As a general rule, the Patent Office requires applicants to deposit novel cell lines or plasmids in a collection such as ATCC to satisfy the public disclosure requirements of patents — the traditional written descriptions are often insufficient to allow someone else to reproduce the invention, a fundamental requirement of patent law. In order to satisfy the legal requirements, no restrictions on the distribution of deposited materials can be imposed.

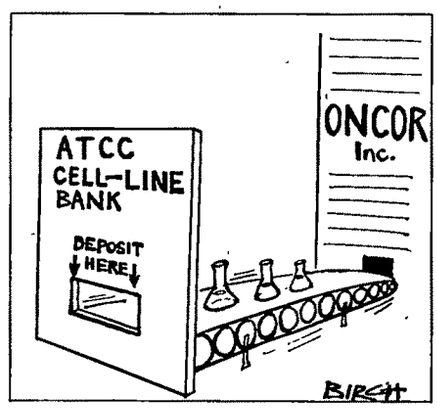
But that totally unrestricted distribution is upsetting some basic researchers. Tom Curran, a molecular biologist at Hoffmann-La Roche, recently deposited a cell line containing the *fos* oncogene, which he had isolated when he was a graduate student at the Imperial Cancer Research Fund Laboratories in London, in collaboration with Inder Verma of the Salk Institute.

Curran says he was subsequently "shocked and distressed" to discover an advertisement in *Nature* from Oncor Inc. for DNA probes for a variety of oncogenes, including *fos*. Curran says Oncor did not even inform him that it had obtained the sample from ATCC or that it was planning to market his product. He says his primary objection is that Oncor is making a profit by selling to basic researchers a product developed by basic research in which the company had no part. Curran says he deposited the oncogene with ATCC so that it would be "freely available to the scientific community".

Some research groups, apparently concerned about the uncontrolled commercial exploitation of cell lines placed in the public domain through ATCC or otherwise, have taken to distributing samples themselves to other basic researchers and requiring a signed agreement that those receiving them will not pass them on to a third party or exploit them commercially without permission. Although there is some dispute over the enforceability of such agreements at law, in at least one well-publicized case, that of David Golde's KG-1 cell line, the originators of the cell line won a sizeable out-of-court settlement from a company that had obtained and was using the material without their permission (see *Nature* **301**, 6; 1983).

Bobbie Brandon of ATCC says that the collection may have to reconsider its policies on free distribution, but that it would not want to be in the position to enforce restrictions against transfers or commercial development. And, although she sympathizes with Curran's complaints, she says Curran had signed a statement agreeing that ATCC could make the oncogene available to anyone requesting it. Brandon says that ATCC distributes about 1,000 oncogene samples each year at a cost of $64 to commercial organizations and $44 to non-profit groups.

Steven Turner, founder and chief executive officer of Oncor, says he sees nothing wrong with the service that his company is providing and that his company's contribution is by no means trivial, as Curran suggests. "We're not just taking the ATCC strain and dropping it into tin cans and selling it", he says. Turner adds that the customers for the products are not basic molecular biologists, but rather clinical researchers who do not have the means to develop the probes themselves from the material available from ATCC. And he says that strains deposited in ATCC are in fact not very attractive for commercial development because they are in the public domain: "I have no protection with what I do with that strain".



Turner, who was the founder of Bethesda Research Laboratories before founding Oncor two years ago, says, "I long ago got used to the hypocrisy of academics saying that their activities are on a higher moral plane than business". While academic researchers refuse to sell their cell lines, they nevertheless demand a *quid pro quo*, such as demanding that the recipients "stick their names on your papers for the next five years". "I've never seen exploitation as I've seen it performed in academic labs."

Stephen Budiansky

Sakharov

Birthday award from US centre

Washington

THE Andrei Sakharov Institute in Washington, founded five years ago to honour the Soviet academician and human rights campaigner, this year added a new feature to the now-traditional Sakharov birthday celebrations — an annual prize for science. The first recipient is Dr Aleksandr Esenin-Volpin, a mathematical logician, for many years a human rights campaigner and a victim of psychiatric repression in the Soviet Union, before he was "persuaded" to emigrate in 1972.

The prize this year is modest, a mere $5,000, and the decision to establish it was taken only two weeks before the event. Vladimir Bukovskii, himself a former human rights campaigner and psychiatric victim, said in announcing the award that the funds were largely collected from Russian emigres in the United States, many of whom are not well-off. But in view of the number and distinction of the universities and institutions from which the "advisory board" of the Sakharov Institute is drawn, and the recent introduction of a congressional bill to grant it a federal charter, there is every hope that a more substantial sum may be available next year.

The prize is not the institute's first educational venture. In addition to its Center for Democracy, which aims at propagating Sakharov's ideas on peace and democracy and publishing in English the works of authors unable to express themselves freely in their own countries, it also maintains an education and research section, which has been adopted as the Washington Campus of Long Island University. This has two major projects in hand, the improvement of the quantity of mathematics and science education in the United States (including the establishment of a National School for Science and Mathematics for Gifted Students) and the upgrading of the quality of Soviet and East European studies.

The announcement of the new prize added an optimistic note to last week's Sakharov "birthday" conference (held a few days before the actual date, 21 May), which otherwise concentrated on current progress (or the lack of it) in international acceptance of Sakharov's views on peace, arms control and human rights.

Inevitably, the issue was raised of plans to renew contacts between the US National Academy of Sciences and the Academy of Sciences of the USSR (see *Nature* 16 May, p.169). Since the National Academy broke off relations with the Soviet Academy in protest at Sakharov's internal exile to Gor'kii, it is not surprising that those gathered to honour him felt that there should be no new approach to the Soviets while Sakharov and his wife remain in exile.

Vera Rich

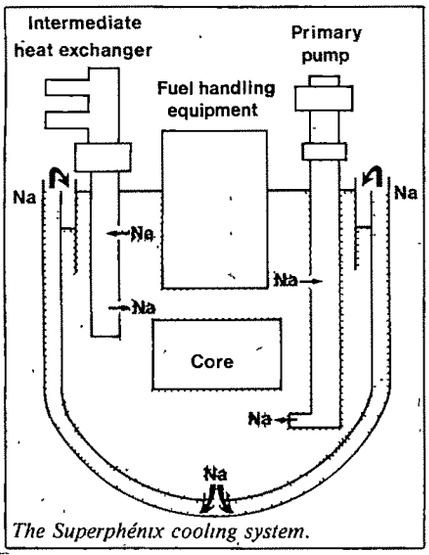
Fast reactors

Superphénix vibrations solved

VIBRATION problems in the primary sodium cooling circuit of the Superphénix European commercial-scale fast breeder reactor "have been solved or nearly so", and will not delay the fuelling of the reactor as had been feared, senior officials at the French Commissariat à l'Energie Atomique (CEA) said last week.

Thus the 1-200 MW(e) Superphénix, which has been built at Creys-Malville on the Rhône by a consortium in which the French and Italians are the major shareholders, with West Germany third, will still be loaded with plutonium at the beginning of September and produce its first fast neutrons soon after, according to CEA.

The problem now considered all but solved has been with an inner jacket of the main reactor vessel, which is little more than a tank full of liquid sodium, topped up with an inert argon atmosphere. As part-



The Superphénix cooling system.

of the cooling circuit, a thin film of sodium passes upwards from the bottom of the main vessel around the outside of the inner jacket, before spilling inwards over the lip of the inner jacket into the core of the reactor. In the first live tests of the cooling system full of heated sodium, the jacket was vibrating with an amplitude of some 12 mm in a diameter of 20 m.

It seems, however, that CEA will now go ahead with the fuel loading without modifying the reactor. Engineers at Creys-Malville have found that a slight adjustment to the sodium flow rate will stop the vibration. The key factor, according to CEA, was the height through which the sodium fell when cascading over the lip of the inner jacket. Initially 100-150 cm, this "sodium fall" has been reduced to 30-60 cm by increasing the volume of sodium in the reactor (by 200 tons) and slightly increasing the flow rate between the inner and outer jackets.

Meanwhile discussions continue in Europe over, first, where to site a planned second fast-breeder reactor, with West Germany and France the main contenders, and second, where to place a single fast fuel reprocessing plant, with the main contenders being France and the United Kingdom. CEA sources believe that a decision on the site and detailed design of a second breeder is necessary by 1987, and that only France could be ready by that date. The potential purchasers, the French national electricity utility Electricité de France, however, which will probably have a large surplus of electricity by the end of the decade, is not thought to be quite so keen. **Robert Walgate**

Belgian research

Autonomy sacrificed to regions

Brussels

REGIONAL tensions in Belgium are eroding the autonomy previously enjoyed by the quasi-governmental institute for the encouragement of scientific research in industry and agriculture (IRSIA). Hitherto, the institute has been free to select areas and projects for applied industrial research, but now its freedom will be further limited by the latest separate decisions of the fiercely competitive Flemish and Walloon regional ministers to increase their influence on its affairs.

Last month, the president of the Flemish regional executive, Gaston Geens, announced that IRSIA would from now on have to submit projects received from Flemish companies for approval to a commission directly under the control of the minister of economics before funds could be granted. It is also required that one-third the of money available should be specifically set aside for small and medium-sized companies, that a certain amount should also be earmarked for projects specified by the regional executive and that all Flemish companies would have to pay back subsidies obtained from IRSIA when the products of research reach the commercialization stage.

Until now IRSIA has always believed in a system of non-repayable subsidies on the basis of a 53 per cent average participation for regular projects and 80 per cent for first ventures, with success and quality governing future eligibility. The old system remains in force in the Walloon and Brussels regions.

The new Flemish system, it is feared, could have the double effect of wilful under-estimation of results by companies to avoid repayment of the loan and could even restrict technological innovation.

Since 1983, when research was defined in terms of the 1980 Belgian regionalization laws, IRSIA has become used to administering separate coffers (the proportional distribution system was revised in August 1984 to try to give each region its fair share). The major industrial sectors of Belgium — glass, textiles, coal, steel and construction — have not been affected by regionalization, and nor have large companies and collective research centres, but IRSIA previously supported small companies not only with money but also with information on research activities elsewhere. Regional separation has meant that research activities are suffering both restrictions and overlap. It has also put IRSIA in a very delicate position: it receives applications and oversees projects from all regions but it has to keep information about research developments in each completely separate.

As well as making extra administrative work for IRSIA, the latest decisions also risk fragmenting the applied research effort in Belgium as a whole. This seems ironic at a time when the European Commission, in the Belgian capital, Brussels, is urging the ten member states of the European Community to make easier the mobility of workers, the exchange of information and cooperation on research. **Anna Lubinska**

Fetal diagnosis trial

CHORION villus sampling, a new technique by which genetic fetal abnormalities can be detected as early as eight weeks of pregnancy, is to be evaluated in a three-year clinical trial run by the British Medical Research Council (MRC). Amniocentesis, the usual method for prenatal diagnosis of genetic disorders, has the disadvantages of being performed later in pregnancy (about 15 weeks) and, because cells have to be grown in the laboratory, the results are generally not available for a further four weeks.

In chorion villus sampling, a sample of placenta containing fetal tissue is removed, allowing immediate diagnosis and much earlier termination. But the technique may be less safe. Preliminary indications (using the 3-400 tests performed so far in the United Kingdom) suggest that the spontaneous abortion rate is 2-5 per cent, compared with 0.5-1.5 per cent in amniocentesis. The randomized trial will include 4,000 women in the United Kingdom and is based on the informed consent of women who elect to join the scheme. Between 10 and 20 UK and European centres will take part; the study will be coordinaed with Canadian and US trials. MRC will also follow up the children born after the prenatal trial to assess for any long-term effects of the technique.

Diseases such as Down's syndrome, anaemias, haemophilias and phenylketonuria can be identified by prenatal sampling; soon muscular dystrophies and cystic fibrosis will be detectable as genetic probes become available. Maxine Clarke

UK higher education

Help business and survive

THE British government's long-promised Green Paper (discussion document) on higher education, published earlier this week (Cmnd 9524, HMSO, £5.80), does nothing to suggest that the pressure on university and polytechnic budgets will be relieved. Indeed, the government says that planning for the impending fall in student numbers, expected on demographic grounds in the 1990s, "will need to begin shortly". The document says that these changes will require "not only rationalization within institutions but the amalgamation or closure of whole institutions".

The document says that there is a need for "means of assessing, on educational and economic grounds", where capacity should be reduced, promises that the chief advisory bodies (the University Grants Committee, UGC, and the National Advisory Body, NAB, which represents the polytechnics), will "have a key role" and adds that "the government will be fully engaged". The government notes the pleas of both bodies as well as of the Jarrett committee on university administration (see *Nature* 4 April, p.392) that institutions should be given longer notice of the public funds at their disposal, but says that its three-year rolling forecasts are the most it can provide, and that higher education institutions should be able to accommodate "change and some inevitable uncertainty".

To most academics, the green paper will be a disappointment, not for what it says or neglects to say but for its tone. The keynote is the statement in the introductory section that "the government believes it is vital for our higher education to contribute more effectively to the improvement of the performance of the economy". Referring to its own initiatives to improve the quality of science teaching in schools, the government says that there "would be no point in continuing to provide more places in science and technology in higher education" unless there are enough able students to fill them, urges that universities and polytechnics should plan for flexibility in the face of change and demands that "higher education establishments" should abandon "anti-business snobbery" and, instead, should foster entrepreneurship, should "go out and develop" links with industry and commerce and should form strong connections with local communities.

With qualifications, the green paper accepts the redefinition by UGC and NAB of the principle that suitably qualified students should have access to higher education (the "Robbins principle") by agreeing that "courses of higher education should be available to all those who can benefit from them and who wish to do so". The qualification is that "so long as taxpayers substantially finance higher education . . . the benefit has to be sufficient to justify the cost". The "test of ability to benefit" should be applied as stringently to those with formal qualifications as to those without.

Inevitably, the green paper rehearses the government's position on the future demand by students for places in higher education, but promises a more considered estimate of demand after a survey now being carried out among young people. Meanwhile, it says, the funds now promised should suffice to allow universities and polytechnics to meet the demands on them in the three years ahead. The document says that UGC and NAB have been asked to recommend how students should be distributed between the universities and the polytechnics, but asks that students should not be admitted to universities if they are more likely to benefit "in terms of personal development and future employment prospects" from a course at a polytechnic.

On research, the green paper rejects the argument of UGC that "research stimulates the teaching of able students" with the statement that "there is no evidence that all academic staff must engage in research". It applauds UGC's plan to distribute research support selectively, hopes that a start will be made in 1986–87 and notes that one consequence may be that "some departments or even whole universities will lose research funding (*sic*) from the UGC". The green paper says that universities will still be free to seek the funds from private sources.

With more enthusiasm, the green paper argues the benefits that would accrue to academics and to "business" from a closer relationship between them, and urges academic institutions to take account of industrial consultancies when assessing candidates for promotion, to encourage academics to "engage in business activity", if necessary on part-time academic contracts, to strike "reasonable deals" with staff over the income earned from innovations and to operate industrial liaison services. (Cautiously, the green paper warns academic institutions of their potential insurance liabilities in these activities.) In the same spirit, the government says it has asked UGC that success in industrial collaboration should be given "due recognition".

The green paper also says the government is not convinced that two-year degree courses are feasible, but would like to see them tried, and that it is dismayed at the growth of the numbers of students now following four-year courses. The paper also says that the government is planning to construct measures of performance for higher education institutions, and that it wishes there to be a uniform system of validation of university courses. On academic tenure, the green paper says that the academic freedom of those who lack this privilege "is not visibly under threat". There is to be an inquiry into the role of UGC.

One of the recurrent themes of the green paper is the right of free speech within universities, and the government promises a reconsideration of the degree to which students' unions should be financed from public funds. Meanwhile, it asks that universities and polytechnics should do what they can to prevent students from suppressing unpopular views. □

Interferon

Agreement on marketing

Washington

HOFFMANN-LaRoche Inc. and Schering-Plough corporation have reached a truce in their battle over marketing rights for human leukocyte (alpha) interferon (see *Nature* 21 March, p.207). Under the terms of agreements reached last week, Schering-Plough will pay royalties to Roche if Schering-Plough's product is sold in the US market, while in Europe each will be free to sell its own product without infringing the other's patent rights. Terms for Japan are expected to be similar to those covering the United States.

Schering-Plough's interferon (trademarked Intron) is produced under licence from Biogen NV of Cambridge, Massachusetts and Geneva, Switzerland. Biogen holds a European product patent for alpha interferon produced by recombinant techniques and expects to receive a similar patent in the United States. Roche, however, holds a very broad US patent that covers all highly pure human leukocyte interferons, and the new agreements recognize that Schering-Plough's product falls within the terms of the Roche patent.

The agreements mean that recombinant interferons will be commercially available while the patent claims are sorted out. Roche has challenged Biogen's European product patent and that challenge will be pursued despite the agreement that both can market their products; the outcome would be important if either company decided voluntarily to withdraw from the market, for example. In the United States, where both Intron and Roche's "Roferon-A" alpha interferon are in clinical trials, the final outcome of the dispute will hang on the scope of the expected Biogen patent for recombinant alpha interferon. The agreements refer only to a specific type of alpha interferon, however; if other variants prove to have valuable properties, there could be more battles to come. In addition, both beta and gamma interferons are in clinical trials and marketing rights for them are likely to be keenly contested.

Tim Beardsley

UK research

Despairing demand for more

THE Oliver Twist of British civil science, the Advisory Board for the Research Councils (ABRC), has this year asked for more in even stronger language. The preface to the annual claim for research funds, agreed by the board at its meeting at the end of April and now on its way to Sir Keith Joseph, the Secretary of State for Education and Science, says that "Parliament, the government and the country as a whole" are "complacent" about the present condition of the dual support system, the means by which support for university research is channelled through the research council and the University Grants Committee.

ABRC says that this complacency is born of a misconception of the function of the research councils and of basic research as such. The board thinks it necessary to point out that it is not true to say that the research councils support only research for its own sake and that academic research is remote from the real world.

As in previous annual applications for funds, ABRC acknowledges that there are limits to what can be afforded for basic research. But it insists that the government which ultimately foots the bill cannot expect that new technologies will emerge unless there is basic science from which they can spring. The board's application also argues that the pace of scientific advance is now such that copying other innovative technology is no longer a recipe for industrial success.

Part of the board's difficulty, this year as previously, is that the government appears on the face of things to have kept the Prime Minister's promise in 1980 that the research budget would be "protected". Using the retail price index as a measure of inflation, the science budget has in fact increased by 6.4 per cent since 1981-82. Even allowing for the decline of the research councils' other income, mostly by way of research commissions from government departments, amounting to roughly 7 per cent on a sixth of the total science budget during the same period, the total income of the research councils has increased by roughly four per cent.

The board says that this simple arithmetic is misleading partly because the retail price index is not a good measure of the increased cost of research. Rather, the board calculates that the real volume of research supported with the funds within its purview has declined by 5 per cent over four years. Matters have been made worse, the argument continues, by the 8 per cent reduction (in real terms) of the budget of the University Grants Committee.

The case is sustained by a harrowing account of the changes which have been brought about within the research council system in the past few years. The total staff of the five research councils has declined by a fifth, by 2,000. But in general, ABRC says, the councils have found that the rate at which they are able to fire or redeploy staff is itself constrained by their shrinking budgets.

The consequences of this state of affairs are catalogued in detail. Thus, the submission to the government is believed to say, the Science and Engineering Research Council is no longer able to make full use of new facilities because it cannot afford the research equipment, is having to pass up opportunities for international collaboration for lack of funds, is unable to meet its commitments (to other government agencies) to encourage information technology and cannot give adequate support to several named areas of research of vital industrial promise.

Other lost opportunities cited by ABRC include the Medical Research Council's incapacity to provide more than half the equipment needed for a new programme of research in protein engineering at the Laboratory of Molecular Laboratory at Cambridge, the Natural Environment Research Council's incapacity to participate in the new ocean drilling programme and the incapacity of the Agricultural and Food Research Council to afford more than a third of the cost of replacing outdated research equipment.

By comparison with previous submissions, the latest request for more research money is terse, almost despairing, as if it had been judged imprudent that an unwilling government should be confused with a plethora of facts. ABRC will protest separately about the way in which the budget of the Agricultural and Food Research Council has been cut by £5 million this year and by twice as much in succeeding years because of a Treasury edict. Meanwhile, ABRC's modest request of government is £15 million for the year beginning next April, twice as much in the succeeding year and £40 million in 1988-89.

On past form, ABRC's submission and the response to it will be published in the autumn. □

UK neuroscience

New lab, old unit in new role

BRITISH neuroscience was given a much-needed fillip last Friday with the inauguration of the new £25-million Merck Sharp & Dohme Neuroscience Research Centre near Harlow, Essex, which is dedicated to investigating chemical transmission processes in the brain. The hope is that one outcome will be the development of new drugs for treating neurological and mental disorders. The centre is at present paying particular attention to senile dementia (Alzheimer's disease) and inhibitory pathways.

The centre is directed by Dr L.L. Iversen, formerly head of the Medical Research Council (MRC) Neurochemical Pharmacology Unit at Cambridge. It will eventually employ about 160 professionals and 50-60 support staff, making it one of the largest brain research centres in the world. It comprises departments of medicinal chemistry, biochemistry and pharmacology, including neurophysiology and animal behaviour, but project teams will be interdisciplinary, involving staff from various departments.

Iversen emphasizes the goal of designing drugs based on a knowledge of the structure and function of the membrane receptor sites for the appropriate transmitter chemicals, instead of the empirical synthesis of chemicals which are then screened for their effects on behaviour. The centre also includes a small clinical research unit to undertake preliminary trials of new drugs. There will be collaboration with universities, particularly under research council schemes for joint direction of research studentships, and with hospitals.

According to Merck Sharp & Dohme, the centre has been established in Britain because of the "pathfinding leadership of British scientists in brain research". Its existence should provide much-needed encouragement to the younger generation of British neuroscientists. The grim job prospects in academic research are resulting in fewer good students going into neurosciences and many that do are leaving Britain after their training. The centre has already attracted several younger scientists back from the United States.

Meanwhile, the Neurochemical Pharmacology Unit at Cambridge, threatened with closure a year ago, has been reborn. After a controversial two-year search to replace Dr Iversen as head of the unit (see *Nature* **309**, 486; 1984), MRC has appointed Professor E. Barnard of the Biochemistry Department, Imperial College, London, as the new director of the unit, now to be known as the Molecular Neurobiology Unit, from 1 October. In the same building as the MRC's Laboratory of Molecular Biology in Cambridge, Barnard's laboratory is intended as the focus in Britain of the use of molecular genetics techniques to study neurotransmitters.

The new unit will have two main lines of research — the development of animal models for the study of muscular dystrophy and motor neurone disease, and the cloning of brain receptors and their genes, in particular in nicotinic acetylcholine receptor and receptors for neuropeptides such as enkephalins and substance P. At full strength the unit will comprise five senior scientists and about 35 visitors, post-doctoral fellows, students and technicians.

Jennifer Altman

More on nuclear winter

SIR — The intrinsic ambiguity of numerical simulations of atmosphere processes guarantees a protracted controversy about the phenomenon popularly called "nuclear winter", as for example described by Turco *et al*[1].

There are, however, ways of injecting large masses of soot into the high atmosphere that are phenomenologically more explicit than those now described. It is necessary only to tell a systems programmer where one guesses the source of carbon should be and to ask him or her please to adjust the computer model accordingly. Consider targeting a coalfield.

The integration of biomass over geological time has endowed millions of square kilometres of the Earth's surface with fossil fuel loadings in the range from 100 to 10,000 g cm^{-2} (ref. 2), compared with the 1 to 50 g cm^{-2} that constitutes the carbon budget of the rural and urban fires in ref. 1. An unknown fraction of this ubiquitous coal, lignite, oil shale and peat is either exposed or lies beneath a thin veneer of overburden[3], and is at risk of interaction with energetic phenomena, including thermonuclear surface bursts and cataclysmic volcanism.

Warheads with yields in excess of 0.5 megatons detonated in such carbonaceous terrain can promptly transform the ground beneath into a mixture of carbonaceous plasma and particulates launched towards the stratosphere by direct entrainment in the rising fireballs. Efficiencies of roughly 10 to 100 megatons of soot per gigaton exploded seem likely (J. Nuckolls and L. Wood, personal communication). It should be noted that NATO (North Atlantic Treaty Organisation) and Warsaw Pact missile fields and fossil fuel fields are partially coextensive, as are the loci of cataclysmic calderas and the massive (trillion ton) hydrocarbon resources that occupy ≥ 10 per cent by area of the western United States.

Every few hundred thousand years, that region is host to a randomly located eruption of 250 to 2,500 cubic kilometres of incandescent tephra. Dr Turco has expressed his willingness to accept the largest of these events as analogues in their climatic impact to the dust baseline of ref. 1. In recent geological history, these eruptions have eviscerated about 5 per cent of the area in question. The worst-case scenarios arising from the interaction of caldera volcanism and fossil fuels involve both carbon and energy budgets that dwarf its inputs of nuclear winter. What were the consequences — local or global, mild or catastrophic?

A survey of the extent of their geological events and of their palaeoecological consequences might serve to illuminate the extent to which the nuclear winter model is a valid predictor of the fate of the Earth. Since a rough calculation yields a mean time of rather less than 10 million years between events capable of producing catastrophic natural carbon injections, the fact that we are not extinct may be germane to the present controversy.

It remains to be seen whether the neologism "nuclear winter" will ever qualify as what philosophers of language call a "rigid designator in the real set", two examples of which are "Carbon County, Wyoming" and "The Yellowstone Caldera". I do not mean to consign "the nuclear winter" to the null set, exemplified by "the canals of Mars" and "the unicorn", but merely wish to point out that a fossil rhinoceros in the hand is more amenable to analysis than two unicorns in the software, and that a diligent search of the fossil record has turned up many a rough beast in the past.

RUSSELL SEITZ
American Security Council Foundation, Boston, Virginia, USA

1 Turco, O R , Toon, P B , Ackerman, T P , Pollack, T P & Sagan, C *Science* **222**, (1983)
2 Seitz, R *Reflections on Nuclear Winter* in Proceedings of the 4th International Seminar on Nuclear War. *Nuclear Winter and the New Defense Systems, Problems and Perspectives*, Erice, 19-29 August 1984 Reidel, in preparation
3 Department of the Interior, *Synfuels Resources* (Washington, 1983)

Nullius in verba

SIR — In your reference to the motto of the Royal Society (*Nature* 2 May, p.9) you make two mistakes. First you misquote the motto itself, and then you give a totally false translation.

The motto is *Nullius in verba*, the word *nullius* being the genitive whereas you give *nullus*, the nominative.

Admittedly, the meaning of the motto is obscure until one knows the rest of the line of Horace (*Epistolae*, I, i, line 14) from which the words are taken. This reads: *Nullius addictus jurare in verba magistri*, which may be translated as "Not committed to swearing by the words of any master". Here Horace declares to his patron Maecenas his own attitude to authority, and it is this rejection of authority as a source of truth that the Royal Society expresses in its motto. Your translation, "nothing in words", is wide of the mark.

ANDREW HUXLEY
The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK

Mass extinctions

SIR — Coming when it did, Michael Benton's article in News and Views (*Nature* 11 April, p.496) must have appeared like manna from heaven to the hundreds of geology and zoology undergraduates throughout the country, and presumably at his institution too, preparing for their final examinations this summer.

It is therefore important to note that Benton chose not to include the single most parsimonious hypothesis underlying the phenomenon of mass extinctions, namely that extinction events are the result of any number of external but unrelated causes which, as a result of simple probability, appear to occur with regularity.

Whether he likes it or not, this single hypothesis, concerned as it is with historic events, will perpetually thwart any attempts to falsify it. The demonstration of cyclicity paralleling these events does not establish a cause/effect relationship. The isolation of a likely cause in one or two cases cannot be extrapolated to the whole. Similarly, the prediction of the cause of the next is not going to help the debate now.

We are left with five explanations for mass extinction cycles. Arguments for and against each view, though futile, will continue. More detailed analyses of the fossil data, and refinements in dating events in the geological past, will not help us to decide which view is correct, though they may enable us to say which is most likely.

GRAHAM B. WILSON
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Future of RGO

SIR — Philip Campbell commented in *Nature* (28 March, p.303) that the staffs of the Royal Greenwich Observatory (RGO) and the Royal Observatory Edinburgh would greet with weary shrugs of resignation the news that the Science and Engineering Research Council (SERC) is to set up yet another committee to decide their future.

Dr Campbell is right in the sense that we have been subjected to so many of these exercises, this will be the fourth since 1983, that much time which should have been spent on observatory projects has been dissipated in justifying our existence.

The staff of this observatory are certainly not, however, facing SERC's latest investigation in a spirit of resignation. On the contrary, we believe that what we have achieved and are continuing to achieve in spite of under-funding and uncertainty is proof that RGO is a viable and efficient base for the support of astronomical research for the United Kingdom and its international partners.

Moreover, the scientific damage likely to be caused by any major disruptions at this stage when we are completing the new observatory on La Palma is too serious to contemplate with anything approaching resignation. The staff at RGO will be entering into discussions on the situation with vigour and determination.

J. DUDLEY
Acting Chairman, RGO Staff Side
Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex BN27 1RP, UK

Trailing AIDS in Central Africa

A meeting in Senegal enabled AIDS researchers to learn more of the possible African origin of the virus that causes AIDS and virus-associated cancers in Africa.

BECAUSE of growing evidence that the virus that causes acquired immune deficiency syndrome (AIDS) originated in Central Africa, AIDS researchers are turning to their atlases. Earlier this month, on 8-10 May, some landed there for the first time to attend a meeting arranged by the French Association pour la Recherche sur le Cancer at the faculty of medicine in Dakar, Senegal, on virus-associated cancers in Africa. What they were forcibly reminded of is that in African terms, AIDS is an insignificant problem, but that most common forms of cancer in Africa are caused in part by viruses.

At times the question of the origin of AIDS has seemed to be no more than a matter of geographical buck-passing — first between the United States and Haiti and then to and within Africa — but the issue is much more serious. At its worst, the argument is that the virus (HTLV-III or LAV) has only recently emerged in virulent form, has been able to do so as a result of changing socio-economic factors in Africa and may be followed by equivalent conversions to virulence of other relatively harmless viruses. More optimistically, the question becomes: can one learn anything from the origin of the virus that would help to contain the disease?

Geographically, the finger of suspicion points at Central Africa, possibly Zaire, as the location from which the virus has spread. The data, however, are sketchy and dependent on what historical and contemporary sera samples are available for analysis, and to whom. From one of the most extensive surveys so far, G.de Thé of the CNRS, Lyon, provisionally concluded that the virus emerged in Kenya only after 1970, that there are still very puzzling differences between adjacent countries, and that there may be evidence of change of viral form in some comparative data.

The evidence of change comes from the comparison of two tests, one of which (an enzyme-linked immunosorbent assay) is less sensitive to change than the other ("Western blotting" of proteins). That the current virus is very prone to change is clear. The sequences of viruses that came directly or indirectly from patients in New York in 1982-83 differ by only a few per cent, whereas the sequences of a virus from a Haitian taken in the same period and one from a resident of San Francisco isolated in 1984 show considerable differences Most variation is in the mid-portion of the gene that encodes the envelope protein of the virus. Up to 25 per cent variation is recorded there, according to W. Haseltine of Harvard Medical School.

By definition, however, all of these isolates and the other hundred or so whose variation is fully documented in only a few cases, are from AIDS patients. All, therefore, have caused immunodeficiency, and no correlation has emerged between variation in viral structure and the precise manifestations of the immunodeficiency syndromes. The pressing question is whether such a correlation can be found and, in particular, whether the African population will yield variants that are less prone to cause AIDS and from which the "American" variants are descended. In other words, do viral differences account for the fact that AIDS in Central Africa seems to be equally distributed between the sexes and transmitted by heterosexual activities rather than largely by homosexual practice and blood or its products? Or are these differences only a temporary reflection of the initial entry of the virus into the industrialized nations through the homosexual community? The signs are more that the latter is the case.

Now the focus of attention has shifted to the African green monkey. Most of some recently collected specimens from the wild in Zaire contain serum proteins characteristic of HTLV-III/LAV and yet are healthy, according to M. Essex from Harvard School of Public Health. A comparative study of the monkey and human virus together with that which caused an outbreak of AIDS-like disease in a captive colony of Macaque monkeys in the New England Primate Center may help to clarify the origin and virulence of HTLV-III/LAV and suggest approaches to vaccination or therapy.

A separate reason for delving deeply into variants is to discover whether sequence changes are responsible for some of the human pathologies other than AIDS with which HTLV-III/LAV is coming to be associated. R. Gallo from the National Cancer Institute in Bethesda, Maryland, pointed in particular to an association with brain abnormalities in children without immune deficiency; evidence exists for the presence of replicating virus in the human brain, particularly in glial cells. Such evidence tends to strengthen the claims of both Gallo and S. Wain-Hobson, of the Institut Pasteur in Paris, that HTLV-III/LAV is related to the lentiviruses, of which the prototype is visna virus, which causes a slowly progressive demyelinating disease of the central nervous system of sheep. Gallo also tentatively suggested a new pathological involvement of the virus that would dispense with the first word of idiopathic thrombocytopaenic purpura.

While AIDS is a growing concern in the industrialized nations, African priorities lie elsewhere. In Senegal, for example, either there is no AIDS or it has not yet been recognized, whereas many die from a liver cancer caused in part by hepatitis B virus. In Africa generally this, and cervical cancer are major types of cancer. A third virus-associated cancer in Africa is Burkitt's lymphoma, an infrequent disease but responsible for many childhood tumours and causally linked to the Epstein-Barr virus.

None of these viruses causes cancer without contributory factors, of which immunodeficiency is the most interesting to AIDS researchers. For Burkitt's lymphoma, immonodeficiency — in the form of lost of T-lymphocyte control of Epstein-Barr virus infected B cells — is induced by malaria, as shown by H. Whittle of the MRC Laboratories, The Gambia. And a mini-epidemic of Burkitt's lymphoma in US AIDS patients suggests that any form of immunosuppression is liable to activate the latent virus.

Hopes, however thin, are pinned on the development of a vaccine against HTLV-III/LAV. For the hepatitis B virus, vaccines are already in trial but for the Epstein-Barr virus the first glimpse of a vaccine was provided in Senegal by M.A. Epstein of the University of Bristol. The vaccine is composed of one of the major proteins of the virus and protects cotton-top tamarins against cancers induced by the virus. The tamarin disease does not altogether resemble the human disease and the frequency of Burkitt's lymphoma may never warrant mass vaccination in terms of cost. In Asian countries where the virus is a part cause of nasopharyngeal cancer, vaccination may be more realistic. Epstein foresees an initial trial of the vaccine against mononucleosis, the relatively mild result of infection by the virus after childhood.

The AIDS researchers who dropped in on Senegal need African collaborators in order, at the least, to carry out extensive trans-African surveys of AIDS, its virus and its origins. The Africans leaving Senegal knew at least that they had increased the awareness of the diseases and priorities in Africa among those who could provide help.

Peter Newmark

Isotope geochemistry

Contamination or source-region heterogeneity?

from Alex Halliday

OVER the past thirty years considerable effort has been invested in using the isotope compositions of certain elements (principally Sr, Pb, Nd and O) to reveal information about the chemistry and history of the source regions of magmas. There are two causes of variation in isotope ratios — radioactive decay (for example, the production of ^{87}Sr from ^{87}Rb) and mass-dependent isotope fractionation during reactions. In the first case, the isotope compositions tell us about the chemical history (that is, the age and parent/daughter elemental ratios) of the source. In the second case we can, in addition, learn about some specific processes that operated in the magma itself. Such data have been fundamental in solving major petrological problems such as the origin of granites and basalts. This has led to a relatively detailed knowledge of the chemical structure and evolution of the Earth's mantle and of the growth of the continental crust.

But the case is not always as clear cut. There is still debate over the interpretation of the isotopic characteristics of rocks believed to have formed in the mantle but to have risen through the continental crust. The argument centres on the relative roles played by contamination by the continental crust and inheritance of features from the sub-continental mantle. The problem is fundamental to the origin of igneous rocks and is made worse by the range and complexity of potential contamination mechanisms, for example, by fluids or partial melts, which produce non-diagnostic effects. One school of thought discounts crustal contamination on the basis of banal assumptions about the probable effects, whereas those from the other school simply ignore the possibility that anomalous mantle is important. Attempts to resolve these views have often resorted to lengthy convoluted arguments.

Fortunately there are some studies which have shed light on this confusion and have provided clear evidence for both open (contaminated) system behaviour and the existence of anomalous mantle. The recent work of Cortini and van Calsteren[1] is refreshing in that they have studied the genesis of lavas from southern Italy by determining the Pb isotope composition in separated phenocrysts (thought to be crystals precipitated from the magma) as well as in whole rocks. The observation of a systematic isotope disequilibrium led to the conclusion that some open system process has operated, which clearly could not have been demonstrated so unambigously if the authors had simply analysed the whole rock samples. Remarkably few such detailed dissections of lavas have been performed previously, although studies of nodule suites[2,3] have provided incontrovertible evidence for anomalous sub-continental mantle and others have proved the presence of crustal components in plutonic rocks[4,5]. Having said this, studying minerals separated from volcanic rocks for signs of open systems behaviour is not new[6-8], and the evidence acquired for significant isotope disequilibrium has been ascribed to a number of possible causes.

These kinds of studies highlight two important features that could provide the key to understanding the genesis of young volcanic suites. First, detailed studies that include mineral separation and analysis of individual mineral grains and glass, although tedious, are of immense potential value in providing clear evidence of open system processes. One might further envisage that with the development of ion-probe techniques detailed spatial resolution of trace-element concentration and oxygen-isotope composition will also be an exceedingly powerful way to proceed. Second, much more information on petrogenetic processes can be obtained by using various isotope techniques to study the samples. The elements in silicate systems vary in their mass-transfer response to different conditions and processes. Hence, it is now simply inadequate to study contamination with a single isotope method. It is also a less efficient use of resources to use different samples when using different techniques. Combined isotope studies optimize information with which to define uniquely a signature for a component or process. As there are many igneous provinces for which the crustal contamination versus enriched mantle debate is still unsettled, it is to be hoped that fresh approaches will provide further rigorous testing for the two hypotheses. □

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Tumour immunology

Molecular basis of tumour spread

from Ian R. Hart

METASTASIS, the spread of tumours throughout the body, is a process of obvious clinical significance. Despite the fundamental importance of the phenomenon, relatively little is known about the molecular and cellular mechanisms of its regulation and control. One way in which disseminating malignant cells may evade host defences is suggested in a report on page 301 of this issue, where transfection of neoplastic murine cells with *H-2* genes is accompanied by nullification of their metastatic capacity[1].

Major histocompatibility complex (MHC) class 1 antigens (termed H-2 K,D and L in mice) are distributed widely on nearly all cell types and play an obligatory role in immunoregulation; cytotoxic T lymphocytes only recognize 'foreign' antigens when they are associated with these class I molecules. Immune regulation of tumour growth and development, then, is not solely a consequence of tumour-cell antigens but also depends on MHC class I antigen expression, as demonstrated experimentally in recent studies using DNA transfection techniques[2,3]. If solid tumour growth can be affected so profoundly by class I antigen expression, how much more likely are metastasizing cells, travelling through the body singly or in small clumps, to be affected by a similarly regulated immune response? The answer, provided by Hammerling and colleagues[1], seems to be that such effects are just as dramatic.

Two clones from the murine T10 sarcoma, one metastatic and one non-metastatic but both lacking expression of H-2 molecules encoded by the K-end of the H-2 complex, were transfected with $H\text{-}2K^b$ or $H\text{-}2K^k$ genes. Expression of these H-2K antigens in the metastatic clone was found to be associated with a profound decrease in metastatic capacity as determined in immunocompetent recipient mice. Metastatic capacity in immunologically depleted animals remained unimpaired, thus demonstrating the central involvement of the immune response in this phenomenon.

Limitation of metastatic spread by an immune response regulated through MHC class 1 antigens not only offers hope for potential therapeutic application (interferons, for example, induce a marked increase in the surface expression of these molecules), but also suggests that an inverse relationship exists between malignancy and expression of MHC class 1 molecules[4].

This phenomenon may account for the marked heterogeneity that exists for the metastatic phenotype in tumour cell populations[5]. Some findings are consistent

with the existence of such an association; melanoma metastases may lack HLA-DR antigen expression[6], and malignant mammary carcinomas may exhibit a deficiency in HLA class 1 antigen expression[7,8]. Certainly, future studies will test the generality of this association, although unfettered enthusiasm for such an all-encompassing theory on how tumour dissemination occurs must even now be tempered by the knowledge that suppression of MHC class 1 gene expression is not a universal characteristic of malignant tumours, nor are specific immune mechanisms the only host defence systems capable of modulating metastatic spread[9]. Indeed, increases in MHC class 1 gene expression may also be associated with metastatic capacity. Earlier studies have shown that expression of the D^k molecule may contribute to the metastatic behaviour of tumour cells[10], and a similar association between metastatic activity and expression of a gene homologous to MHC class 1 antigens has been established in another murine tumour system[11].

Recently the metastatic phenotype has been expressed by Ha-*ras* oncogene-transformed NIH 3T3 mouse fibroblast cells following transfection with genomic DNA isolated from a human metastatic tumour[12]. Acquisition of metastatic capacity correlates with the presence of discrete human DNA fragments, possibly representing a novel gene. Metastatic spread of the Ha-*ras*-transformed cells occurred in immunoincompetent (irradiated) nude mice but not in immunocompetent, histocompatible mice; the metastatic transfectants are capable of metastasizing both in immune depleted and in immunocompetent mice. Presumably the transfected gene confers some protection against the immune system and an attractive possibility is that this gene could turn out to be homologous to the putatively suppressogenic *H-2D*[1,11].

Unfortunately, such attempts to seek a single unifying explanation for the regulation of metastatic spread are probably oversimplifications; the complex multifactorial nature of the phenomenon suggests that answers obtained are likely to be as varied as the models used to address the questions. The true significance of Hammerling *et al.*'s report[1], along with other recent studies, may lie in the fact that these investigations represent the first applications of the techniques of modern molecular biology, such as gene transfer, to the central problem of clinical cancer and to a still major conundrum of tumour biology. □

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It is still unknown whether the recent findings are relevant to scrapie, illustrated here by a Cheviot sheep rubbing compulsively against a fence (photo courtesy of AFRC and MRC Neuropathogenesis Unit, Edinburgh, UK).

Scrapie

Persistently puzzling prions

from Tim Harris

MANY features of scrapie — a degenerative neurological disease of sheep and goats — including pathological similarities to the human degenerative diseases kuru and Creutzfeld-Jacob disease, suggest that it is caused by a 'slow' virus. The inability to find any typical virus particle consisting of nucleic acid and protein, however, coupled with the unusual resistance of infectivity to agents that normally inactivate nucleic acids, has led to some radical thinking about the nature of the scrapie agent. It has been proposed, principally by Stanley Prusiner and his colleagues[1,2], that a new type of proteinaceous infectious particle or 'prion' is involved in scrapie infection (for review, see ref.3). Two recent studies[4,5], one reported on page 331 of this issue[4], have now begun to produce molecular evidence that call this claim into question.

The evidence for the existence of prions stems largely from the fact that scrapie infectivity is proteinase sensitive, and that rod-shaped particles resembling amyloid fibrils are observed to co-migrate with infectivity in sucrose density gradients. SDS polyacrylamide gel electrophoresis of the proteins in this fraction (where infectivity is considerably enriched with respect to protein) has revealed a single polypeptide, PrP (prion protein) 27-30, of relative molecular mass 30,000; no nucleic acid has been found to be associated with it. Several models for the mechanism of prion replication have been put forward[6], the most heretical of which are that the protein is back-translated into nucleic acid (as yet achieved only by computer), or that PrP is a template for its own synthesis.

Recently, PrP has been purified and its partial amino-acid sequence obtained. The availability of this sequence for the design of oligonucleotide probes means that, if there is a messenger RNA for PrP, it would be only a matter of time before the isolation of complementary DNA clones. Given the degree of interest in the elusive scrapie agent, it is not surprising that this has happened sooner rather than later.

These molecular studies[4,5] present the cloning and sequencing of the mRNA for PrP 27-30 and show it to be the product of a single cellular gene. In addition, specific mRNA for PrP 27-30 is found in normal as well as in infected tissue. Can molecular biologists now heave a collective sigh of relief that the central dogma is still intact and that proteins do not self-replicate? For the time being the answer is probably yes, because unless the protein in infected tissue is subtly different to that in normal tissue (as has been found for certain cellular oncogenes), then it seems unlikely that PrP 27-30 is anything other than a normal cellular glycoprotein, the synthesis or degradation of which may be altered as a result of scrapie infection.

Nevertheless, the clarification of the origin of PrP 27-30 still does not explain the nature of the scrapie agent. In a negative way it does lend tacit support to the more conventional argument for a small scrapie-specific nucleic acid, suggested by the existence of different strains of the agent[7]. Whether this nucleic acid is contained in a small virus particle with a low particle-to-infectivity ratio, or is viroid-like and not encapsidated is not clear. But what is clear, now that PrP has been shown to be the product of a cellular gene, is that the hunt for the real scrapie agent is back with a vengeance. □

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Heavy-ion physics

Giant nucleus at Darmstadt?

from Joshua Silver

WHEN an energetic very heavy ion passes close to the nucleus of a heavy atom in a solid target, positrons may be spontaneously produced within the electron shells of the atom. Recently, researchers at the heavy-ion facility at GSI Darmstadt, West Germany, have been examining this phenomenon. One particularly exciting possibility emerging from these experiments is that extremely heavy nuclei, with atomic number and mass of about 180 and 470 respectively, might be produced in heavy-ion scatterings. Although such products would be short lived, they might open up the study of atoms with unprecedented properties.

Consider an atom of variable nuclear charge Z. As Z increases, the binding energy of the innermost electrons, $E(1s)$, becomes more negative. Forgetting for a moment the fact that there are no atoms with $Z > 109$, we may calculate $E(1s)$ using the Dirac equation with a Coulomb potential for arbitrary Z. It is found[1-3] that for a non-zero nuclear charge radius, $E(1s)$ falls below $-2m_ec^2$ for $Z \geqslant 173$, where m_e is the rest mass of the electron and c is the velocity of light. When $E(1s)$ is less than $-2m_ec^2$, the discrete bound electron state becomes degenerate in energy with a three-particle continuum state, consisting of two bound electrons plus an outgoing positron wave.

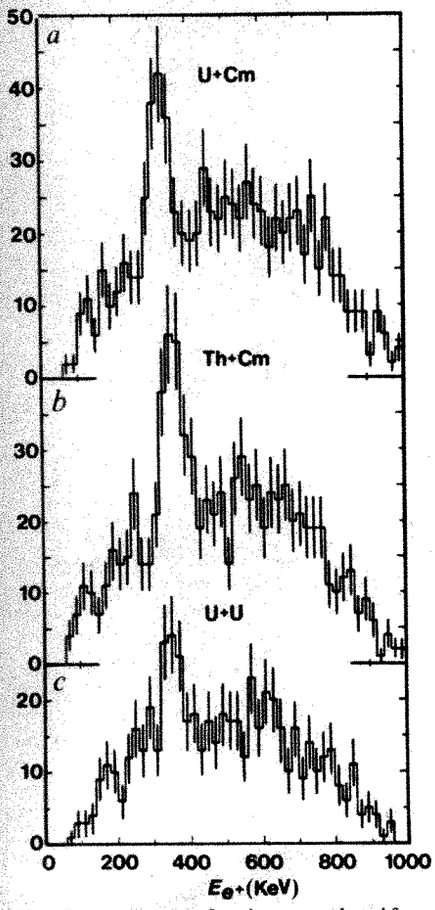


Fig. 1 Energy spectra of positrons produced from collisions of *a*, ^{238}U (Z = 92) on ^{238}Cm (Z = 96); b, ^{232}Th (Z = 90) on ^{248}CM (Z = 96); *c*, ^{238}U (Z = 92). Bombarding energies are ~ 6 MeV A^{-1}, and the angular range (of the order of 100) of the ions scattered in coincidence with the detected positrons gives the most pronounced ~ 300 keV positron 'line' in each case (from ref. 7).

It was first suggested by Gershtein and Zel'dovich[1] that this 'autoionizing' mode of spontaneous positron production might be observable in heavy-ion collisions. The idea is that for a sufficiently close collision of two very heavy ions, it is possible for the 'innermost' 1s electrons of the projectile or target atom to experience a Coulomb field similar to that from a nucleus with $Z \geqslant 173$, so long as $Z_1 + Z_2 \geqslant 173$, where Z_1 is the atomic number of the projectile ion and Z_2 the atomic number of the target atom[4]. This approach, however, suffers from a weakness — in the words of Müller[5], the method is "both a curse and a gift"; the rapid time variation of the Coulomb field during the collision leads to ionization of 1s electrons from the collision partners, thereby creating vacancies. This is the "gift", in that spontaneous production of positrons is suppressed by the Pauli exclusion principle if the 1s levels are full. However, the time variation of the Coulomb field is also responsible for induced positron production which can mask the spontaneous process. Calculation of the relative probabilities of the spontaneous and induced processes is difficult; for completely elastic scattering along Rutherford trajectories the two processes cannot be distinguished in principle. There is also a contribution to the total positron production from nuclear mechanisms.

Three experimental groups have used the heavy-ion accelerator at GSI Darmstadt for several years to search for spontaneously produced positrons. In the past two years, these groups have observed a new phenomenon that is difficult to explain[6-8]. For coincident observation of positrons with particles scattered in well-defined angular ranges, and at well-defined ion-beam energies in a narrow interval around the Coulomb barrier, the energy spectra of the positrons produced during a collision can show a distinct 'line' at around 300 keV. Typical spectra are shown in Fig. 1. The width of this line may be used to infer a duration for the collision, simply from Heisenberg's energy-time uncertainty relation. The smallest widths observed so far (about 60 keV) translate into a characteristic time for the positron production process of the order of 10^{-19} seconds. Now it is also possible, from the dynamics of the collision, to estimate the time during which the collision partners are sufficiently close together for the transient 'united atom' Coulomb

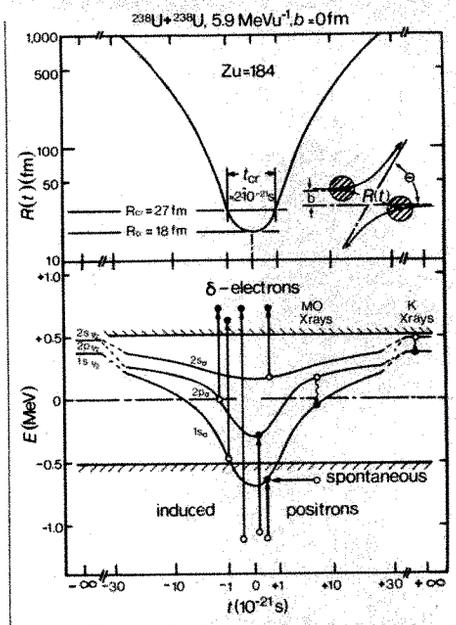


Fig. 2. Electron-binding energies, t, for the three most bound states (top) and the internuclear separation, $R(t)$, for two ^{238}U nuclei as a function of time (bottom) for a collision between a stationary target nucleus and a projectile with energy 1·40 GeV, with impact parameter b = 0. Note that $E(1s)$ is $< -2m_ec^2$ for a time of the order of 2×10^{-21} s for this collision (from ref. 10).

field to be large enough to give rise to spontaneous positron emission. For the typical system ^{238}U + ^{238}U at a beam energy of 1·40 GeV and a head-on collision, this time is ~ 10^{-21} seconds, two orders less than the duration inferred from the width of the positron line (Fig. 2). What is going on?

One possible explanation is the formation, during the collision, of a transient 'giant di-nuclear system' or 'giant nuclear molecule' which lasts for at least 10^{-19} seconds. At present, it is not clear that this explanation is correct or complete, and one problem that is not easily explained is that the most well-defined positron lines are all at an energy of about 300 keV. Nonetheless, the possible creation of an (albeit transient) nuclear system with an atomic mass of about 470 and an atomic number of around 180 is most exciting and has triggered more experimental and theoretical work, in an attempt to understand what is really happening. With the increasing availability of heavy-ion beams of suitable energy it is likely that this phenomenon will soon be better understood. □

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Visual cortex

Stars and stripes present the colours

from Ian Thompson

THE primate prestriate cortex contains several visual areas, each with its own representation of the visual field and a particular set of receptive-field properties. Their major input arises from striate cortex (V1, sometimes termed area 17) either directly, or indirectly via the projection from V1 to V2. How, then, is the observed diversity of function generated by the input from V1 and V2? Does it depend on specific intra-cortical processing within each area or does it arise because each area receives a functionally specific input? Two papers on pages 322[1] and 325[2] of this issue and one in the press[3] provide complementary information on how anatomical and functional inhomogeneities within one visual area (V2) are related to the neuronal projection pattern between V2 and areas in the prestriate cortex concerned with colour (V4) and movement (termed V5 or MT).

Neurones in V4 and in V5 (MT) display marked differences in some of their stimulus specificities. In V5 (MT), they are selective for the speed and direction of a moving object, irrespective of its colour[4-6]. The responses of cells in V4 are much more dependent on the spectral properties of the stimulus[5,6] and may display some form of colour constancy[7]. How do these differences arise?

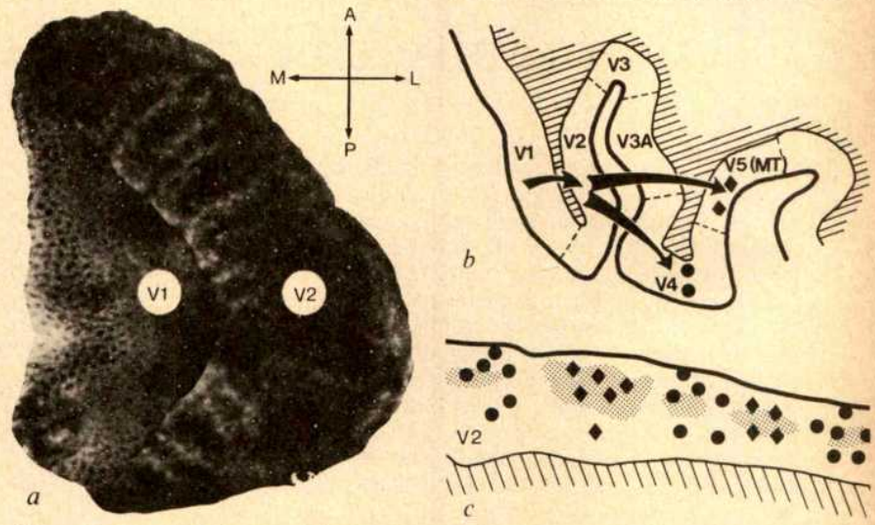
One possibility is that there are separate populations of neurones in V2 projecting to the two areas. Using retrogradely transported tracers injected into V4 or V5 (MT), Shipp and Zeki[1] have demonstrated bands of labelled neurones in V2 that lie orthogonally to the V1/V2 border. Stripes with this orientation in V2 are also seen with another neuroanatomical method, staining for the distribution of the enzyme cytochrome oxidase (part *a* of figure), but Shipp and Zeki note that the spacing of the bands of labelled neurones is twice that of the cytochrome oxidase stripes. In favourable sections, especially from infragranular layers of macaque cortex or from squirrel monkeys, the dark cytochrome oxidase-positive stripes appear alternately thick and thin, separated by light (cytochrome oxidase-negative) interstripe zones. Where identification of thick and thin stripes was possible, Shipp and Zeki report that neurones labelled after a V5 injection coincide with thick cytochrome oxidase stripes, whereas cells labelled from V4 are associated with thin stripes or, occasionally, with the interstripe region (figure *b*,*c*). They conclude that the output neurones from V2 to V4 or to V5 (MT) are clustered into alternating bands. This conclusion is elegantly confirmed by DeYoe and Van Essen[3] who use two tracers that fluoresce at different wavelengths, one injected into V4 and the other into MT (V5). In V2, adjacent bands are labelled with different tracers and no cells are obviously labelled with both.

Given the anatomical heterogenity in V2, is there any corresponding grouping in the physiological properties of its neurones, as suggested by an earlier 2-deoxyglucose study[8]? Using electrophysiological techniques, all three groups[1-3] describe clustering of receptive field characteristics such as colour, orientation and directionality. Unfortunately, the reports differ in the details of the relation between the clusters and the stripes. One contributory problem seems to be reliable identification of thick and thin stripes. DeYoe and Van Essen[3] minimize the problem by combining quantitative electrophysiology, fluorescent tracers and cytochrome oxidase staining. They describe an association of colour selective units with the distribution of cells projecting to V4, which they find can arise from both the thin stripes and interstripes.

The suggested association of colour cells with thin cytochrome oxidase stripes in V2 is interesting because these stripes are thought to be the termination site of the projection from the cytochrome oxidase blobs in V1[9]. Having found previously that V1 blobs were associated with non-oriented double-opponent colour cells[9], Hubel and Livingstone[2] now describe a receptive-field type, associated with thin stripes in V2, which they suggest represents an hierarchical elaboration of the V1 double-opponent cell. The new type is also non-oriented and double-opponent but shows spatial summation over a region smaller than that of its receptive field. An optimally sized spot is thus equally effective in several positions in the receptive field, a characteristic of complex cells. Hubel and Livingstone postulate that this type of receptive field represents a comparable transformation with that occurring between oriented simple and complex cells. The 'non-complex' double-opponent cells are first seen in V1, possibly reflecting a convergence of afferents from the lateral geniculate nucleus in the thalamus, analogous to that generating oriented simple cells[9].

The existence of distinct anatomical pathways within V2 projecting to V4 or to MT (and possibly also to other areas[3]) raises several interesting questions. How is the visual image represented in each pathway? Does each contain a separate map or is the scatter in the topography of V2 sufficient to ensure adequate representation in each projection? Magnification-factor data would be welcome. The anatomical sub-regions are large with the repeat distance from thin stripe to thin stripe between 2.5 and 5 mm. Thus, finer-scale functional representations are possible in the sub-regions, indications of which can be seen in the 2-deoxyglucose results[8]. In fact, it would be surprising if all neurones within a sub-region were functionally identical. Quantitative investigation of distributions of response properties should generate a clearer picture of visual analyses performed in the different visual regions.

Finally, what developmental mechanisms generate the anatomical and functional diversity? The pattern of retrogradely labelled cells in V2[1,3] is reminiscent of the banding of afferent terminals seen when two projections map onto the same neural sheet, for example, ocular dominance columns in striate cortex or in the tectum of



Schematic summary of connectivity patterns within pre-striate cortex taken from refs 1-3. Photomicrograph *a* shows the distribution of cytochrome oxidase staining in a tangential section through V1 (area 17) and V2 (area 18) of squirrel-monkey cortex. A characteristic neuronal connectivity pattern exists between the spots in V1 and the stripes in V2. *b*, V2 projects both to V4 and to V5 (MT) with the cells of origin of both projections clearly segregated into alternating bands in V2 (*c*), depicted by the circle and diamond symbols. An association between the cells of origin and the thick and thin cytochrome oxidase stripes (stippling) is also described in *c*.

three-eyed frogs[10]. Are the two patterns analogous, providing a solution to the problem of accommodating two maps in one structure? If separate maps do occur in one area, then there is a problem with the idea of a one-to-one relationship of function to cortical area. Pursuing the ocular-dominance column analogy, do the projection bands develop from an initially uniform distribution? Recent results from the cat are suggestive — Price and Blakemore have found that cells projecting from area 17 to area 18 initially have a continuous distribution that becomes patchy during development[11]. □

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Protein crystallography

A reaction centre elucidated

from Jim Barber

PHOTOSYNTHETIC organisms contain specialized proteins — reaction centres — that function to convert the energy of an absorbed photon into the chemical potential necessary to synthesize organic molecules. In all classes of photosynthetic organisms the reaction centre is embedded in a membrane matrix with closely associated pigmented proteins acting as light collection systems. Photons absorbed by these light-collecting proteins cause excitations in the pigments, which are efficiently transferred to the reaction centre. On arrival at the centre the excitation causes a rapid charge separation, the primary step in the conversion of light energy to chemical potential.

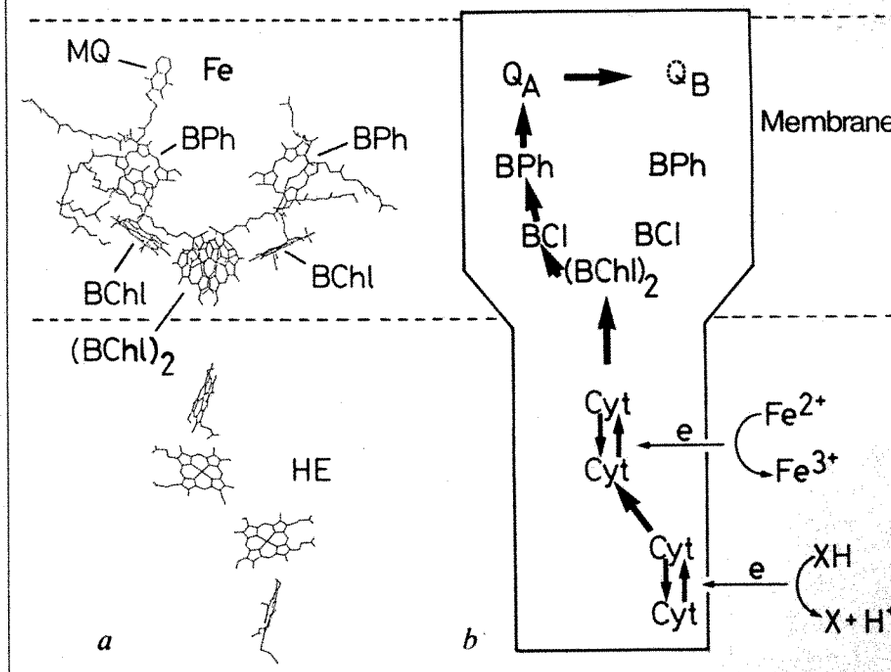
This charge-separation process has some remarkable features which have not yet been reproduced in artificial photochemical systems. The most striking features are the speed of the process, which occurs within a few picoseconds, and its very high efficiency. Although some elegant experimental and theoretical studies have attempted to elucidate the mechanisms by which reaction centres bring about energy conversion, progress had been hampered by the unkown molecular organization of components of the protein. Such restrictions have now been lifted because of the success of a group working in Munich, who have crystallized the isolated reaction centre of *Rhodopseudomonas viridis*.

R. viridis is a gram-negative non-sulphur photosynthetic bacterium first identified only twenty years ago. It contains bacteriochlorophyll-*b* (BChl-*b*) as well as several different carotenoids. The bulk of these pigments make up the light-collecting system but a small percentage of the BChl-*b* and carotenoids is found in the reaction centre protein. The precise composition of the *R. viridis* reaction centre was not known until it was isolated as a complete functional entity by J. Thornber *et al.* (*Biochim. biophys. Acta* **172**, 351; 1969). It was soon established that this reaction centre contained four BChl-*b* molecules, two of which form a 'special pair'. These are able to undergo photo-oxidation, detected as an absorption decrease at 960 nm (P960). The isolated protein also contains two molecules of bacteriopheophytin-*b* (BPhe-*b*), two quinone molecules (menaquinone and ubiquinone), a non-haem iron atom and four cytochrome *c* molecules, two with absorption peaks at 553 nm (C553) and two at 558 nm (C558). Gel electrophoresis indicated that the isolated protein is a complex consisting of four different polypeptides of relative molecular mass (M_r) 38,000, 33,000, 28,000 and 24,000. The last three polypeptides are analagous to those in the reaction centres of other purple bacteria and have been designated H (heavy), M (medium) and L (light) subunits, respectively. The M_r 38,000 component is only found in the *R. viridis* reaction centre and is the polypeptide that binds C553 and C558.

Since the study of Thornber *et al.*, the composition and spectroscopic properties of the *R. viridis* reaction centre have been established using a range of techniques including electron spin resonance, nuclear magnetic resonance, picosecond absorption spectroscopy, fluorescence transients and linear and circular dichroism. As a consquence of these investigations new theories of electron tunnelling and charge stabilization have emerged and structural models have been postulated.

Three years ago, H.Michel successfully obtained crystals of the *R. viridis* reaction centre suitable for high resolution X-ray diffraction analysis which maintained photochemical activity (*J. molec. Biol.* **158**, 567; 1982). In collaboration with J. Deisenhofer, D. Epp, K. Miki and R. Huber, he has now been able to determine the structure of these crystals to a 3 Å level of resolution (*J. molec. Biol.* **180**, 385; 1984). The structural details confirm predictions from spectroscopic measurements as well as revealing some unusual features, still of unknown meaning.

According to X-ray analysis, the protein



Models for the reaction centre of *Rhodopseudomonas viridis*. *a*, Relative positioning and orientation of the prosthetic groups of the reaction centre showing the special pair $(BChl)_2$, two monomeric bacteriochlorophyll-*b* (BChl), two monomeric bacteriopheophytin-*b* (BPh), one non-haem iron (Fe), one menaquinone (MQ) and the four cytochrome haems (HE). The central local symmetry axis runs vertically in the plane of the picture. Broken lines suggest the position of the membrane whose plane is assumed to be orientated perpendicular to the central local symmetry axis. (Modified from Deisenhofer *et al. J. molec. Biol.* **180**, 385; 1984.) *b*, Schematic drawing of the reaction-centre complex showing electron-transport pathways. Q_A, menaquinon; Q_B, ubiquinon, which is lost during cystallization. The special pair $(BChl)_2$, BChl, BPh and Q_A are located in a cylindrical part of the reaction centre. Bound cytochromes (C553 and C558) occur as high potential haems, assumed to be reduced to soluble cytochrome c_2, ($Fe^{2+} \rightarrow Fe^{3+}$) and in closest proximity to the special pair, as low potential haems, which can presumably be reduced by organic acids (XH).

complex has an elongated shape with three clearly distinguishable regions. The central portion consists of the L and M polypeptides and is a 50-Å long cylinder with an elliptical cross-section of diameters between 50 and 70 Å. To each flat face of the cylinder, a globular subunit is attached so that the longest dimension of the entire reaction-centre complex is about 140 Å. One of the globular subunits contains the four haem groups of cytochromes C553 and C558, and the central cylinder is the site for the four BChl-*b*, two BPh-*b* and a high electron-density peak from a non-haem iron atom. The other globular structure is presumably the H-subunit, known not to be directly involved in the photoactivity of the reaction centre.

Although the Munich group has not fully elucidated the complete polypeptide structure, it has obtained details of the spatial relationships between various components involved in charge stabilization (see figure). A prominent feature is that the BChl-*b* and BPh-*b* molecules are arranged with an approximate 2-fold rotational symmetry. The rotation axis runs through (or nearly through) the non-haem iron atom and through two closely associated BChl-*b* molecules, whose pyrrole rings are stacked on each other. These two BChl-*b* molecules are the 'special pair' corresponding to P960; the X-ray diffraction data indicate that a linkage exists between the magnesium atom of one tetrapyrrole head and the acetyl group of ring 1 of the other, a conclusion which invalidates an earlier suggestion that water was involved in bridging. The average distance between the atoms of the tetrapyrrole heads is 3 Å with a Mg-Mg distance of 7 Å, and the angle between the ring planes is about 15°. Interestingly, the other two BChl-*b* molecules are symmetrically spaced from the special pair at a Mg-Mg distance of about 13 Å and an angle of 70° between the planes of the tetrapyrrole head groups. Both the monomeric BChl-*b* molecules contact BPh-*b*, with a spacing between ring centres of approximately 11 Å and a 64° angle between ring planes. Although all the tetrapyrrole rings are on the same 2-fold rotational axis, their phytyl side chains are not.

The non-haem iron atom is positioned on the 2-fold rotation axis and is linked to at least three histidine residues, one of which has an electron density close to it that is indicative of menaquinone (Q_A). The iron-quinone distance is approximately 7 Å and, significantly, in the symmetry-equivalent position to the quinone ring related by the 2-fold axis, is an empty 'pocket' in the protein. Michel and collaborators suggest that this empty pocket is the ubiquinone binding site termed Q_B. Although the haem groups in the cytochrome subunits do have a symmetrical arrangement, they are not positioned on the 2-fold rotational axis relating the BChl-*b* and BPh-*b* molecules. Only the haem closest to the special pair lies on this symmetry axis with an Fe-Mg distance of about 21 Å. Thus, it may be this cytochrome which directly reduces the photo-oxidized special pair ($P960^+$).

From the diffraction analysis it is clear that the L and M subunits form the central cylinder, which consists of five transmembrane helices, each being related by the 2-fold rotational axis. Because of this, both subunits are equally involved in forming the special pair and the non-haem iron-quinone complex. Both the cytochrome and H-subunits also contain helices.

In many ways the structure determined by X-ray crystallography has supported several concepts derived from spectroscopy. Clearly the organization of the components confirms that primary charge separation involves the following electron transfer sequence:

$$(\text{BChl-}b)_2 \rightarrow \text{BChl-}b \rightarrow \text{BPh} \rightarrow \text{menaquinone } (Q_A)$$

with $(\text{BChl-}b)^+_2$ being reduced by cytochrome *c* while menaquinone passes its electron to a ubiquinone (Q_B) molecule normally bound in the vacant pocket. The structural model also supports the chromaphore orientation data obtained from linear and circular dichroism. The puzzling, as yet unaccountable, feature is the symmetrical arrangement of the two monomeric forms of BChl-*b* and BPh-*b*, which suggests two possible electron transfer pathways. However, evidence suggests that only one pathway is preferred so that an electron is always passed to Q_A, the bound menaquinone, before going to ubiquinone (Q_B). It seems, therefore, that one BChl-*b* and one BPh-*b* molecule are not normally involved in the process of charge separation.

It could be argued that the reaction centre of *R. viridis* has evolved from a more primitive reaction centre consisting of one subunit only, and that the dimerization giving rise to the L and M polypeptides facilitated the formation of the special pair and the opportunity for improving charge stabilization by allowing the Q_A to Q_B electron transfer to occur. The precise factors which have given rise to the preferred pathways will become clear as the detailed structure of the polypeptide chains are determined from the electron-density map. Moreover, the role of the H-subunit should become more obvious as Deisenhofer and colleagues continue to elucidate the total structure of the protein complex.

In any event, it is probable that the molecular details of the *R. viridis* reaction centre will not only allow the theoreticians to understand how the high efficiency of energy conversion is attained in this specific protein, but will also act as a model for the reaction centres of other organisms. In fact the *R. viridis* reaction centre is structurally and functionally similar to the reaction centres of several purple non-sulphur bacteria. Even more exciting is that the knowledge gained from these studies will form an important basis for elucidating the molecular basis of the mechanism of water splitting by green plants and algae, because these reactions occur at a reaction centre (photosystem two) having features similar to those of the purple bacteria. We can also look forward to a future where X-ray crystallography will unravel the details of a wide range of membrane processes at the resolution of atomic distances. □

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Palaeoclimatology

Records of past continental climates in deep-sea sediments

from Joseph M. Prospero

EVERYONE talks about palaeoclimate, but no one seems to know much about it. This is especially true for continental climates where the often subtle evidence of past weather conditions is easily obliterated by subsequent events. Even when evidence is found, in many cases it is not possible to date the events. Ocean scientists are more fortunate because a good chronological record of past oceanic conditions (such as water temperature) can often be read in the neatly ordered layers of deep-sea sediments that span many millions of years. Recently, Rea *et al.* (*Science* **227**, 721; 1985) have shown that these sediments also contain information about past continental climates. Their conclusions about atmospheric circulation over the past 70 million years not only challenge conventional views on palaeoclimates, but also provide a test for computer climate models.

Rea *et al.* analysed the windborne (aeolian) mineral dust components in four North Pacific sediment core sections spanning the past 70 Myr and found major changes in particle size, concentration and the mass accumulation rate. They suggest that the accumulation rate data are indicative of the availability of material in the source area and should be related in some way to climate, especially to aridity. The mineralogy of the aeolian grains also provides clues to the climate of the source region and, most importantly, the size distribution can be used to infer the speed of the transporting winds.

On the basis of their assumptions, the authors conclude that the atmospheric circulation was quite vigorous during the late Cretaceous and Palaeocene, but moderated

sharply in the early Eocene (50 Myr ago) before commencing a steady increase that culminated in a maximum associated with the Pliocene-Pleistocene glaciations of 4-2 Myr ago. The most surprising outcome of this work is the implication that circulation during the late Cretaceous was as vigorous as that in the Palaeocene. This is contrary to the long-held belief of geologists and palaeoclimatologists that the Cretaceous was a period of moderate climate and relatively weak circulation, but is supported by recent computer models.

Data such as that of Rea *et al.* are important as relatively rigorous tests of long-term climate models, which otherwise would be little more than interesting intellectual exercises. It is therefore necessary to scrutinize the underlying assumptions of such studies. The most critical assumption is that which relates aeolian particle sizes to the speed of the transporting wind. Rea *et al.* assume that, for any one wind speed, there is a characteristic particle-size spectrum of dust grains which are in 'equilibrium' with the wind, where the "lifting effect of the inherent turbulence must balance the tendency of the grains to settle". On this basis, the ratio of the transporting wind speeds for two different events will be equal to the ratio of the squares of the 'equilibrium' particle diameters.

The concept of a quasi-stationary size distribution related to wind speed is based on work by Gillette *et al.* (*J. geophys. Res.* **85**, 5621; 1980) who studied soil deflation as a function of soil properties and boundary layer micrometeorology. It is, however, debatable whether boundary layer turbulence processes can be extrapolated to the free troposphere where vertical transport and mixing is largely dependent on processes occurring in large-scale weather systems and during convection. Although Rea *et al.* cite some experimental evidence that they believe tends to support their assumption, there are no studies that directly address the relationship of particle size to the speed of the transporting winds.

Even the relationship between soil deflation and aridity is complex. Goudie (*Prog. phys. Geog.* **7**, 502; 1983) has shown that the correlation between dust storms and rainfall is very weak; indeed, the frequency of dust storms is at a maximum in regions with 100-200 mm annual rainfall. This is consistent with growing evidence that water is essential for the production of abundant quantities of fine sedimentary particles (Pye, K. and Sperling, C.H.B., *Sedimentology* **30**, 49; 1983) and for creating surficial environments where these weathering products can be deflated readily (Gillette *et al.*, *op. cit.*). In contrast, many hyperarid areas are relatively blown out; in such regions, the principal features of the surface are rocky plains and dune fields which are primarily comprised of coarse materials that are not readily deflated or transported great distances by winds. These factors suggest that the most abundant sources of soil dust are areas that are changing from moist to arid. This is supported by Prospero and Nees (*Science* **196**, 1196; 1977 and *EOS* **65**, 837; 1984) who demonstrated a threefold increase in the transport of dust out of North Africa and across the Atlantic during both the current drought and that of the early 1970s.

Another aspect that must be considered is the relationship between the meteorology of dust storms and weather. Studies of dust storms in Africa, North America and Asia suggest that if climate were to change, weather would also change and, with it, the sources of the dust, the mechanism by which it is generated and its transport paths. Sediments in the North Pacific would be particularly sensitive to changes in transport paths. Recent atmospheric chemistry studies in this region (Uematsu *et al. J. geophys. Res.* **88**, 5343; 1983) show that Asia is the primary source of the large quantities of dust found in North Pacific winds; it is carried out of Asia on westerly winds and, eventually, some fraction is transported via complex trajectories into the easterlies. As a consequence, dust concentrations decrease markedly in the latitudes south of about 30° N. Therefore, it is conceivable that dust deposition rates in some ocean regions could have changed substantially solely as a consequence of changes in circulation patterns.

Finally, the fact that large quantities of dust could have been produced by climatic factors not directly related to aridity must be considered. Most significant is the considerable evidence (Pye, K. *Prog. phys. Geog.* **8**, 176; 1984) that extensive areas of loessal soils found in North America, Europe and Asia have been formed through the combined action of winds and glaciation. The dearth of the fine particles in these deposits suggests that large quantities of fine soil dust were blown away, although the source region was not necessarily arid.

It is clear that deep-sea sediments contain a strong and varied aeolian signal that must be linked in some way with climatic processes on the continents. Rea *et al.* have taken the first step towards interpreting this signal. Whether their interpretation is correct will be established only by further studies of the areal and temporal variation of this signal in the sediments together with studies of the present-day relationship between weather and processes of dust mobilization and transport. □

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Parasitology

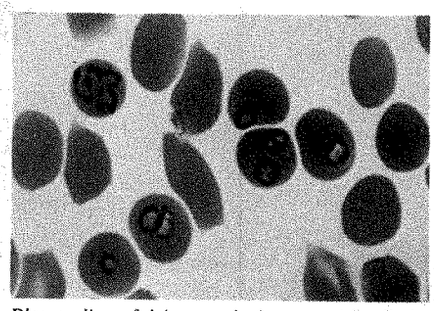
Chromosomes of malaria parasites and trypanosomes

from F.E.G. Cox

OUR understanding of the genetics of the protozoan parasites that cause major diseases in man has been hampered by the fact that we do not even know how many chromosomes they possess. On page 347 of this issue, Kemp and coworkers report the use of a new technique, pulsed-field gradient (PFG) gel electrophoresis, to separate and characterize seven chromosomes from the human malaria parasite *Plasmodium falciparum*[1] (see figure). This technique has enabled several groups not only to enumerate such elusive chromosomes but also to separate and identify them and to elucidate some of their functions. It has already been used to separate 11 of the 17 chromosomes of the yeast *Saccharomyces*[2].

PFG gel electrophoresis involves the separation of large molecules of DNA in an agarose gel[3], and the DNA obtained from the lysed organisms is subjected to alternate electrical pulses at right angles. Because these molecules are larger than the pore size of the gel, they have to orientate themselves end on to pass through the gel; their ability to reorientate at right angles and the time taken to do this provides a basis for separating the molecules. The DNA thus separated can be visualized by staining and blotting on nitrocellulose and the genes located by hybridization techniques. In this way, it is possible to locate and identify DNA molecules in the size range of 30-3,000 kilobases (kb), which happens to include most of the DNA constituting the chromosomes of malaria parasites and trypanosomes.

All that was previously known about the chromosomes of *P. falciparum* was that there are few of them and that they seem to be haploid in the blood stages[4]. Kemp *et al.*[1] studied three strains of *P. falciparum*, one from Papua New Guinea, which was used as a baseline, and others from Ghana and Thailand. Using lysed cultured blood-stage parasites, they found that their DNA separated out into seven discrete zones and, by using yeast chromosomes as markers and complementary hybridization data, they established that these represent complete chromosomes. The four smallest chromosomes were the easiest to identify and, in the Papua New Guinea strain, consist of 0.8, 1.2, 1.6 and 1.8 megabases (Mb). Chromosomes 5-6 were 2.2 and 2.6 Mb and chromosome 7, which overlapped with 6, was 3.0 Mb. The other strains of *P. falciparum* also had seven chromosomes but exhibited considerable polymorphism with respect to the Papua New Guinea strain. For example chromo-



Plasmodium falciparum in human red cells in culture. *P. falciparum* grows easily in culture, so is readily available for laboratory study. In this stained preparation, the nucleus appears as an amorphous dark mass with no detailed structure. Chromosomes can only be identified after separation by pulsed-field gradient gel electrophoresis. (Photograph A R Dluzewski.)

some 1, the smallest, varied between 0.8 and 1.0 Mb and others differed by as much as 20 per cent. There was also variation between the positions of the chromosomes in the agarose gel, but overall a common sequence of seven equivalent chromosomes of increasing size could be identified in each strain. There may also be other chromosomes, probably clustered towards the larger end of the size range where separation presents particular difficulties.

Having separated the chromosomes, hybridization techniques can be used to assign functions to them. The most striking gene products to be identified so far in malaria parasites are the short repeats of amino acids that constitute important antigens. Overall, it seems that the genes for these repetitive antigens are distributed between several chromosomes.

The antigen located on the surface of infected erythrocytes[5] is encoded by chromosome 1 and also, to a lesser extent, by chromosome 7; the secreted (S) antigen[6] is also associated with chromosome 7. It is of particular interest that five other repetitive antigens are associated with both chromosomes 5 and 6. Analysis of the results of hybridizations suggests that chromosome 5 is an exact but incomplete copy of chromosome 6 and that this chromosome, 5-6, is diploid, whereas chromosomes 1-4 are haploid.

The chromosomes of trypanosomes have not revealed their secrets so easily. The DNA of trypanosomes is divided between the nucleus and a unique organelle, the kinetoplast, which considerably complicates the study of the genetics of these organisms. Nevertheless, PFG gel electrophoresis is beginning to produce clues about the genes encoding the variant surface glycoproteins (VSG) that coat the trypanosome and are the basis of its ability to evade the immune response by antigenic variation.

Conventional agarose separation of the DNA of *Trypanosoma brucei* produces two fractions, a minichromosome band and the remainder of the DNA that stays at the starting position. PFG gel electrophoresis separates *T. brucei* DNA into four components, a minichromosomal fraction containing about 100 DNA molecules in the 50–150 kb range, about 6 chromosomes of 200–700 kb, 3 or more larger chromosomes of 2 Mb and a large heterogeneous fraction that hardly migrates at all from the starting point[7]. The genes encoding the VSG seem to be distributed among several chromosomes and are seen most readily in the minichromosomes. Recombination involves the transposition of large numbers of base pairs from one chromosome to another, resulting in major changes in the size distribution of the minichromosomes. It is possible to relate these size changes to changes in the expression of particular VSG genes, which cannot be done using more conventional techniques[8]. This leads to the question of whether minichromosomes are essential for antigenic variation. To answer this, van der Ploeg and colleagues have made a comparative study of several species of trypanosomatid flagellates[9]. PFG gel electrophoresis reveals tremendous differences between the chromosomes of different species. In contrast to *T. brucei*, *T. equiperdum* has few minichromosomes and *T. vivax* has no DNA smaller than 2,000 kb (ref. 9); as both can undergo antigenic variation it seems that minichromosomes are not essential for this process.

For a long time, parasitological problems have been emerging faster than the techniques available for their solution, which has resulted in frustrating gaps in our knowledge. PFG gel electrophoresis will quickly resolve some of these questions; the applicaton of this relatively simple technique represents a milestone in modern parasitology. It should soon possible to distinguish between genes encoding antigens important in protection and those used by parasites to evade the host immune response. It might also be possible to distinguish between virulent and avirulent strains of parasites and to pinpoint the basis of drug resistance. □

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Geomagnetism

On the trail of Earth's tail

from Stanley W.H. Cowley

NEARLY all of the planetary bodies in our Solar System are believed to possess long magnetic tails extending for many tens to hundreds of planetary radii in the direction away from the Sun, but none has been so thoroughly investigated as that of the Earth. That has been particularly so since 1983 when the ISEE-3 spacecraft was sent on a series of passes extending about 230 Earth radii along the geomagnetic tail. Altogether, ISEE-3 spent around 8 months beyond the lunar distance, and provided unique and detailed data on the distant tail and its environs. Twenty papers arising from initial analyses of these data have recently been published in an issue of *Geophysical Research Letters*. These papers deal principally with the overall structure of the tail and the properties of plasmas and fields both in the magnetic tail lobes and in the central current sheet that separates the lobes.

Magnetic tails are formed by an interaction with the solar wind, the tenuous hydrogen plasma which blows continuously outwards from the Sun at speeds of around 500 km s^{-1}. In the case of a magnetized planet, such as Mercury, Earth, Jupiter and Saturn, the tails are thought to result from magnetic reconnection on the dayside of the planet, a process which produces 'open' magnetic field lines mapping directly from the planet's polar regions into the solar wind. The flow of the wind then carries the field lines downstream from the planet and stretches them out into a nightside tail consisting of two bundles of oppositely directed magnetic flux (mapping to either pole) squashed together into a long cylinder. The field lines in the two tail lobes eventually reconnect across the internal interface between them, returning 'closed' field lines to the planet and 'disconnected' field lines to the solar wind.

Non-magnetized planets such as Venus and probably Mars also possess magnetic tails, but in their case the solar wind plasma becomes slowed on the dayside of the planet by its interaction with the planet's ionosphere. Field lines embedded in this slowed plasma become draped around the planet and stretched into an 'induced' tail as their distant 'ends' continue to move with the wind away from the Sun. A similar process is thought to be responsible for the formation of Type 1 comet tails. The phenomenon of magnetotail formation is therefore very common in our Solar System.

Although these basic notions have been commonly accepted for a number of years, detailed spacecraft observations of magnetotail plasmas and fields had been very limited before ISEE-3. Even in the case of the Earth's magnetotail, most observations had been confined to the region within the lunar orbit (60 Earth radii), a distance representing only about one-twentieth of the tail's theoretically estimated length. The papers in *Geophysical Research Letters* show how drastically the situation has been altered by the travels of ISEE-3.

Two of the papers demonstrate in very

different ways that the field lines in the tail lobes are indeed magnetically 'open', that is they eventually map across the outer surface of the tail into the surrounding solar wind. Changes in plasma bulk parameters, such as density, temperature and velocity, during ISEE-3 traversals of this boundary are reported by Gosling *et al.* [1], who find that well-defined transitions in the magnetic field (sharp changes in field magnitude and direction) are often accompanied by gradual variations in plasma parameters. These observations indicate the presence of direct magnetic flux linkage across the boundary, allowing solar wind plasma a relatively easy access. Abrupt plasma changes also occur, indicating a locally magnetically closed surface.

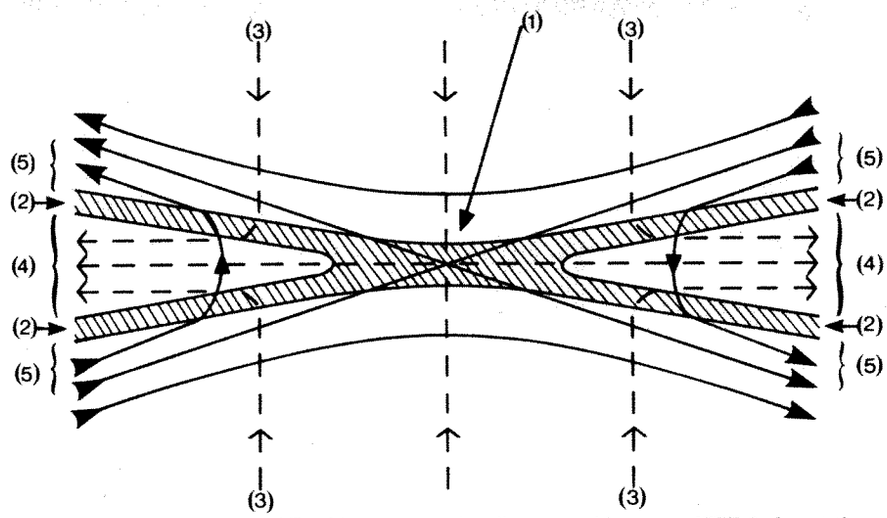
The second paper deals with the direction of the field in the tail and its dependence on the solar wind field direction, measured simultaneously upstream from the Earth by the IMP-8 spacecraft. Tsurutani *et al.* [2] find that the values of that cross-tail field component that lies approximately parallel to the central current sheet are positively correlated with the corresponding component in the solar wind. In effect, the lobe field (and that in the central current sheet) does not point directly along the tail axis towards or away from the Earth, but is somewhat skewed towards the direction of the solar wind field. This is precisely the result expected for an 'open' tail structure[3], arising from the effects of the magnetic stresses exerted on the plasma by the open field lines.

Equally interesting discoveries have been made about the hot plasma sheet particle population that carries the cross-tail current between the two magnetic lobes of the tail. A previous article in these columns[4] described the initial finding that beyond about 100 Earth radii, the flow of this plasma is directed almost exclusively away from the Earth, indicating that the tail reconnection region usually lies less far from Earth. The appearance of closed field loop 'plasmoids' in the distant tail about 30 min after the onset of magnetically disturbed periods has provided evidence of rapid tail reconnection near the Earth. Confirmation is provided by several papers describing energetic-particle, plasma and magnetic field observations and their relationship[5-7].

100 Years Ago

THE verdict of the jury who considered the case of the Usworth Colliery explosion is probably the first expression of opinion from a public body of this class that coal-dust and a small percentage of fire-damp can play the part that has hitherto been usually ascribed to fire-damp alone. They found that the explosion was caused by a shot, the fire of which acted upon "*the coal-dust and a small percentage of gas.*". The convenient and time-worn "outburst of gas" theory, which consigned the helpless miner to the vicissitudes of chance and exonerated colliery owners and their agents from all responsibility, seems on the point of giving way before the coal-dust theory.

From *Nature* **32** 61, 21 May 1885



The reconnection geometry of Earth's geomagnetic tail suggested by recent ISEE-3 observations. Broken lines are plasma streamlines, solid lines are magnetic field lines, and hatched areas represent regions of high electrical current density. A single current layer or diffusion region (1) at the site of reconnection bifurcates into slow mode shocks (2) in the downstream flow. Plasma flowing from the tail lobes (3) is accelerated across the shocks to form a hot plasma sheet (4) that flows away from the reconnection site on either side. Suprathermal particles, escaping upstream of the shocks, form boundary foreshock regions of streaming particles (5) located on reconnected tail-lobe field lines just outside the plasma sheet. (No attempt has been made to represent the asymmetries that would result from the observed flow of lobe plasma away from the Earth.)

An important subsequent discovery is that the interface between the tail lobe and the plasma sheet often has the character of a slow-mode shock wave[8]. The formation of such shocks resulting from magnetic reconnection was suggested by Petschek[9] over 20 years ago, but ISEE-3 has provided the first evidence for them. The field and plasma structure implied by the observations are shown in the figure. The single current layer separating the tail lobes at the site of reconnection (the 'diffusion region', where the field diffuses through the plasma) bifurcates on either side into two slow shocks standing in the plasma flow. Tail-lobe plasma flowing across the shocks is accelerated away from the reconnection region by the magnetic stresses to form a hot accelerated plasma (the plasma sheet) which is mainly confined beteween the shock waves[10]. However, high-speed ions and electrons are observed to escape upstream of the shocks to form 'boundary layer' or 'foreshock' regions of streaming supra-thermal particles, located on tail-lobe field lines between the surface of reconnected field lines mapping to the diffusion region and the shock waves (see figure)[11,12].

Scarf *et al.*[13] describe a well-defined plasma wave morphology associated with these structures. Electron plasma oscillations of a few kHz are observed in the foreshock region in association with a 'heat flux' formed by field-aligned streaming electrons that have energies above a few hundred eV. These waves therefore probably result form the Landau 'bump-on-tail' instability. A similar association seems to occur in this region between streaming energetic ions (of tens of keV) and the magnetosonic waves reported by Tsurutani and Smith[14]. Within the shock current sheet itself, a spectral peak in electric field noise at frequencies of a few hundred Hz, possibly identifiable as ion acoustic waves, is found[13]. An estimate of particle scattering by these waves suggests that they do not lead to appreciable resistivity; instead the lower hybrid drift instability (at too low a frequency to be measured by ISEE-3) may play an important role[13]. In my view, another important factor determining the structure of the current sheet is likely to be finite ion inertia.

An important question raised by these findings is whether the boundary of the plasma sheet on the earthward side of the tail reconnection region also has the nature of a slow shock. It would be surprising if this were not so in the more distant tail, but the near-Earth regime will be complicated by two factors. First, field line convergence at the Earth mirrors accelerated particles back in to the tail. Second, it is possible that the earthward-streaming plasma observed at the outer surface of the near-Earth plasma sheet predominantly results not so much from local acceleration as from the denser tail-lobe plasma found at somewhat larger distances. A deeper understanding of magnetotail physics will undoubtedly ensue from these and other considerations of the consequences of the ISEE-3 observations. □

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The quest for pure electron lattices

SIR — The very interesting work on observing three-dimensional electron lattices, reported in News and Views[1], is even more widely relevant than discussed in the brief space available. We note here other laboratory experiments and also, perhaps surprisingly, some astrophysical theory. An essential point of departure stems from the (correct) observation in the article that it is "taken for granted that an electron lattice can form only if embedded in a neutralizing sea of positive electric charge". An electron lattice can be formed *without* such a neutralizing background.

It has been known for years that a magnetized Penning trap can stably store single charged particles for extremely long periods. Such a trap consists simply of an axially symmetric electric quadrupole (two negative electrodes with a positive ring electrode in between, preferably with hyperbolic cross-sections of the appropriate curvature) and a magnetic field along the symmetry axis. The magnetic field prevents a charged particle from escaping across the magnetic field and the quadrupole electric field confines the particle (if negatively charged for the above charging of the electrodes) along the axis. Until fairly recently, it was not understood what happens if one fills such a trap with a large number of particles. Older literature vaguely discussed 'clouds' of trapped particles. It is now known that the charged particles condense effectively to form what appears very much to be a liquid. That is to say that the particles are confined to a spheroidal volume of constant density with a sharp interface to the surrounding vacuum. These sharp discontinuous surfaces were predicted theroretically for both the laboratory and astrophysical cases[2,3]. The surface thickness separating the plasma from the vacuum is shown to be only of the order of the Debye length, which is small compared with the system scale in the neutron star case even for million-degree plasmas, and in the laboratory case for the relatively cool plasmas that can be produced there (room temperature or less). Moreover, such systems ('non-neutral plasma' is the usual term in laboratory circles) have been created in the laboratory with both electrons[4] and ions[5]. Indeed, in the case of the ions, the particles have been cooled to a temperature (0.1 degrees kelvin) where the system should not only appear liquid-like (confinement to a variable shape volume of constant density) but actually be a liquid in the sense of large particle–particle correlations. Future experiments aspire to produce a trapped solid of ions (D.J. Wineland, personal communication). The workers at the University of California at San Diego have trapped the electrons in a similar configuration, using ring electrodes at the ends of a long solenoid that parallels the magnetic field axis. It may be feasible to cool such trapped electrons to temperatures where they are liquidized or solidify[6]. In either case, the formation of an ideal 'theoretician's' lattice of totally non-neutral plasma free of interfering background neutralizing charges seems within reach.

In the case of astrophysics, solidified states of matter have been expected for neutron star interiors (for example, refs 7,8). However, it now seems increasingly possible that the exteriors of magnetized neutron stars may, in many cases, be surrounded by such quasi-liquid non-neutral plasmas pulled from their surfaces. The essential ideas were forwarded by a number of people such as Holloway[9] who pointed out the likelihood of vacuum–plasma interfaces, Rylov[10] who first speculated on how such plasmas might be distributed about pulsars, and Jackson[11] who also argued for condensation of such plasmas about neutron stars. The laboratory effort itself goes back at least to Trivelpiece[12], and an entire book has been written on the properties of nonneutral plasmas[13].

Not only do these remarkable plasma experiments open up the possibility of creating pure liquid and solid plasmas, but they also underscore an interesting convergence in research on solid-state physics[14], with laboratory experiments on non-neutral plasmas and, surprisingly, some astrophysical theory as well.

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Selfish DNA and the origin of introns

SIR — The selfish DNA or transposon theory of the origin of introns[1-9] is dismissed far too readily by Cornish-Bowden[10]. As introns are very often not at domain boundaries[11,12], the hypothesis of evolutionary exon shuffling to recombine protein domains is hardly the "most plausible suggestion" for their "function". It does not adequately explain either the origin or the evolutionary maintenance of RNA splicing. Although fusion of two genes by recombination between existing introns probably has been evolutionarily important, it does not explain the origin of the introns "in the first place". *De novo* intron formation by fusing two genes lacking introns is far more complex than fusion to produce an unsplit gene; it could hardly have occurred in a single step in an organism previously lacking RNA splicing[7]. Split genes and RNA splicing could not have evolved merely because they might millions of years later help a new protein evolve: evolution lacks such foresight[2].

The transposon hypothesis can explain the origin of introns and also of RNA splicing itself[3-7]. The presence of introns in mitochondria and chloroplasts, in particular, is best explained by transposition from the nucleus[5-7]. The different positions of the introns of the chloroplast *psbA* genes of *Chlamydomonas*[13] and *Euglena*[14], coupled with their absence from the ancestral cyanobacteria[13], supports insertional origin for chloroplast introns, rather than one from primordial gene fusions that created the *psbA* gene. The coding of proteins involved in splicing by certain introns in fungal mitochondria[4], and in a chloroplast gene[15], supports the thesis that such introns originated as defective transposons in which the RNA-splicing enzymes evolved from pre-existing DNA splicing enzymes specific for their termini[3-5].

This also can explain the origin of introns in general [3,4,7]. Suppose a transposon capable of precise excision inserted into a cellular gene, thus inactivating its transcript[4]. This first 'intron' would be exactly pliced out of the RNA if the DNA excisionase of the transposon mutated so as to be able to cut RNA instead of DNA, but retained its specificity for the sequence at either end of the transposon; this would simultaneously prevent its self-transposition and rescue the cell from its harmful insertion mutation.

This mutation would protect the cell from insertion mutations by any transposons generating similar terminal sequences upon insertion, and therefore have allowed the rapid intragenomic spread of kindred non-defective transposons; moreover these would have provided DNA excisionase activity allowing the rapid intragenomic spread of the defective RNA-splicing transposon itself[7]. So many genes would quickly acquire introns that RNA splicing could never be lost[1,2]. All descendants of this cell would be permanently infected with useless introns. RNA splicing could later acquire real functions for at least some genes, such as differential splicing to make different proteins from the same transcriptional unit[16]. This rescue hypothesis[4] provides an immediate and powerful mode of spreading for introns and RNA splicing. At one and the same time it involves intragenomic kin selection favouring the spread of selfish DNA, and conventional selection at the cellular level for protection from the harmful effects of certain selfish transposons. Alternatively, RNA splicing might have originated in a virus[7] before being taken over by the cell.

The transposon theory of intron origins best fits the nuclear mRNA and rRNA splicing mechanisms, both of which depend on specific sequences within, as well as at the end of, introns[17]. I suggest that they originated in an early eukaryote and were never present in prokaryotes. The small nuclear RNAs involved in nuclear pre-mRNA splicing might initially have been synthesized from transposon promoters lying on the DNA strand opposite the transcribed strand of the cellular gene into which it had inserted. But nuclear tRNA splicing does depend on specific intron sequences and may have originated independently; the presence of similar tRNA introns in the archaebacterium *Sulpholobus*[18] suggests that it originated before archaebacteria and eukaryotes separated. Chloroplast tRNA introns seem unrelated to nuclear and archaebacterial tRNA introns; they resemble mitochondrial and nuclear rRNA introns and have features suggestive of defective transposons[6,15,19].

The improbability of losing every single intron by accidental deletion[2,4], and the extra complexity that splicing poses for the origin of protein coding[4], make it improbable that RNA splicing was the general rule in the primordial cell, and favour the view that archaebacteria and eukaryotes both evolved from a eubacterium lacking split genes[4]. Once introns evolved, their number and total length per genome would increase or decrease in response to generalized selection for larger or smaller total genome size[1] and transcript size, as well as to selection for specific functions[16] for at least some introns.

The relative uniformity of exon length in protein-coding genes is not, as claimed by Lonberg and Gilbert[12], good evidence against an insertional origin. The periodic structure of chromatin may prevent totally random insertions. The correspondence between the three major peaks in exon size distribution and the DNA lengths in nucleosome core particles, linkers and whole nucleosome[20], suggests that the junctions between linkers and core particles have been 'hotspots' for intron insertion and therefore that introns in protein genes originated after the origin of eukaryotic chromatin. By contrast, exon length in mitochondria [20] and chloroplasts[13,14,21,22], which lack histones, is much less uniform and more random than in nuclei.

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Chemical warfare evidence unconvincing

SIR — Rosen *et al.*[1] fail to substantiate their contention that environmental samples from south-east Asia provide evidence of chemical warfare. They base their argument on reports of trichothecene mycotoxins in six samples from alleged chemical attack sites[2,3]. In arguing that the toxins they reported were not of natural origin, they astonishingly say that the US Army Chemical Systems Laboratory found no trichothecenes in samples from south-east Asia.

This incorrectly implies that the Army's samples were controls. In fact, at least 60 of the Army's samples, like those of Mirocha and Rosen, were from alleged chemical attack sites. As far as we know, the Army laboratory has not found trichothecenes in any sample from such sites. Mirocha and Rosen have known of this since at least October 1983, when one of us (M.M.) wrote to them asking "Why does the Army find no positives out of 60 samples while Mirocha plus Rosen find six out of six positive?" The evidence for trichothecenes at sites of alleged chemical warfare is inconclusive at best: 6 positives out of 6 samples compared with 60 negatives out of 60 samples.

Rosen *et al.* ignore the fact that it is pollen, and not trichothecenes, which is the consistent and confirmed finding in samples of yellow rain, the alleged chemical warfare agent. Since 1979, many samples have been turned in by Hmong refugees from Laos, and then examined by US and other investigators. In November 1982, the head of the US Army laboratory referred to above stated "most of the samples that are of yellow rain are fairly dry and they have a high level of pollen grains in them"[4].

To our knowledge, *all* samples of yellow spots and powders from sites of alleged chemical attack that have been examined under the microscope, including those analysed by Mirocha and Rosen, consist largely of pollen. This is also true of honeybee faeces. Moreover, our analysis of the pollen types in samples of the alleged agent collected by Hmong in April 1981 and in March 1982, and comparison with pollens gathered by local honeybees, strongly supports the identification of yellow rain as the faeces of south-east Asian honeybees[5].

Rosen *et al.* also ignore descriptions of the alleged agent by Hmong refugees. Summarizing interviews with Hmong for the period 1978-82, the official reports of Secretaries of State Alexander Haig and George Shultz state that the alleged agent is yellow and falls like rain[6,7]. This resembles mass defaecation flights of the giant Asian honeybee, *Apis dorsata*, which two of us (M.M. and T.D.S.) observed in Thailand in March 1984[8]. Although now realized to be common, such flights were previously unknown, perhaps because they occur at so great a height, even though hundreds of thousands of bees may be involved. Further, nearly all the Hmong to whom we showed bee faeces on leaves failed to identify them. Some said they were *kemi*, a Hmong term for the alleged chemical warfare agent.

Our conclusion that yellow rain is probably the faeces of honeybees and not a chemical warfare agent is thus supported by Hmong accounts of its appearance by pollen analysis, and by observations of the behaviour of honeybees in south-east Asia.

Studies of the yellow rain phenomenon in several countries, particularly in government laboratories, have still to be made public. It is to be hoped that the responsible officials will make every reasonable effort to move further investigation of this problem into the normal channels of scientific communication.

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Somatotopic map of the flying fox

SIR — In the interesting article by Calford *et al.* on the unusual somatotopic cortical map in the grey-headed flying fox, *Pteropus poliocephalus*, and also in the comment by Jones[2] on the article, one point escaped emphasis: the caudal shift of the somatotopic representation of the distal forelimb has taken place by reflection from rostral to caudal (that is the first digit or thumb remains lateral and adjacent to the face), instead of by rotation from rostral to caudal (in which event the thumb would lie medial and the fifth digit would be located adjacent to the face). In contrast to the reflection of the representation of the forelimb, the representation of the hindlimb appears to have rotated through about 90 degrees, so that it points medially (instead of rostrally as in most mammals). Whether this difference in the shift of the fore- and hindlimbs is related to their different functions (flying and feeding in the case of the forelimb, landing from flight in the case of the hindlimb) remains an open question.

Jones[2] has addressed a related issue: what is the advantage of representing the body surface on flat cortical maps instead of as a bolus of cells? Rostral/caudal and medial/lateral appear to be the spatial relationships in the periphery that need to be maintained in the cerebral cortex; dorsal/ventral seems a less important direction, being coded sometimes as caudal/rostral (for example in the axial/distal parts of a limb in quadrupes) and sometimes as lateral/medial (for example in the position of the head relative to the limbs and trunk). If, then, one direction in the periphery does not have to be maintained in the cortex, a flat map can achieve all that a bolus would achieve, with the advantage of much less complex connections between thalamus and cortical surface.

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Spatial relationship and extrafoveal vision

SIR — A recent letter by Rentschler and Treutwein[1] describes a qualitative difference in human visual acuity between central foveal vision and a region 2° from the fovea when the task is to discriminate between stripe patterns that differ in appearance but are mathematically similar in everything but phase relationship between first and third spatial harmonics. For the pairs that are easily discriminated both foveally and at 2°, the appearance of the stripes differs in the contrast of a narrow dark band on a lighter grey stripe. In the patterns that can be discriminated foveally and at 2°, the darker bands in each discrimination task are identical in quality and differ only in that their position with respect to the lighter bands is mirror-image reversed in the two images.

Rentschler and Treutwein conclude that (1) there is a qualitative difference between foveal and nonfoveal vision for spatial localization tasks but not for contrast detection tasks, and that (2) because of this difference between foveal and nonfoveal vision for one type of task but not the other, there must be two types of visual processing involved. We suggest that there might not be a qualitative difference between foveal and nonfoveal vision, but rather a quantitative difference — spatial localization might simply decline faster than contrast detection as a function of eccentricity — and that this difference can be explained at least partly in terms of receptive field size and stimulus resolution in complex cells at various levels in the visual pathway, as a function of eccentricity.

Both the letter itself and the accompanying News and Views article by Watt[2] are couched in terms of the Fourier-analysis theory of cortical function; curiously no mention is made of known receptive field properties of cortical cells. As an alternative to their interpretation, which assumes that the early stages of vision can be regarded as a number of parallel spatial filters (a view that our receptive field studies do not seem to support), we would propose an interpretation (or perhaps a more physiological translation of their interpretation) in terms of the known properties of *complex* cortical cells. At a given eccentricity, the territory of visual field over which a complex cell can be activated is usually much larger than that of a simple cell, but the optimum stimulus size is approximately the same. A complex cell responds optimally to lines or slits in a specific orientation, but for an optimal response the line width is also critical. The optimal line evokes a response wherever it is shone over a wide area, even though a line thick enough to cover this entire area evokes no response at all. The optimal line width is roughly the same as the width of the region from which a simple cell can be activated, or the diameter of a retinal ganglion cell receptive field centre (for the same eccentricity), implying a preservation of acuity but loss of the spatial localization conveyed by simple cells.

This relative loss of what may correspond to spatial localization without loss of acuity is seen not only in complex cells in area 17, but even more strikingly in prestriate visual areas. Rovamo and Virsu[3] have shown that human visual acuity at different eccentricities varies as a linear function of cortical magnification in area 17, and Rentschler and Treutwein therefore use magnification to scale the size of their two test patterns for eccentricity: they compare foveal and nonfoveal discrimination using only this one stimulus size ratio. This may be justified if one assumes that resolution (that is, acuity) as a function of eccentricity does not change as one goes further along in the visual pathway; physiological comparisons of areas 17 and 18 (see our paper, this issue[4]) support this assumption. But we think that magnification in area 17 might not be an appropriate scaling factor for tasks involving spatial localization. Spatial localization could well depend on the size of complex receptive fields, and that clearly does increase beyond 17, for example, in 18, V4 and MT, and also increases more rapidly for nonfoveal than for foveal vision.

The difficulty in discriminating mirror-image patterns is more likely to be related to spatial localization, and consequently tailoring stimulus size to cortical magnification in area 17 may be inappropriate. The mirror-image (spatial localization) discrimination is already poorer than the contrast discrimination in the fovea (Fig. 2 of ref. 1) and falls off more rapidly with eccentricity. This parallels the behaviour of complex cells at more central levels of the visual system than area 17. Thus the finding would seem to suggest not a qualitative difference in handling of form foveally and peripherally, but a difference in rates of deterioration with eccentricity between acuity as commonly defined, and something else, possibly spatial localization.

We further suggest that these results do not necessarily implicate two separate visual processes for contrast discrimination and spatial localization. The fact that the relative loss of spatial information seen physiologically in single cells is paralleled in these psychophysical observations suggests to us that spatial localization and contrast information are probably encoded in the same cells and not by separate processes. Only if psychophysical spatial localization were better than predicted by the physiology of complex cells would one need to invoke a separate process for it.

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1 Rentschler, I. & Treutwein, B *Nature* 313, 308-310 (1985).
2 Watt, R J *Nature* 31, 266-267 (1985)
3 Rovamo, J & Virsu, V *Expl Brain Res* 37, 495-510 (1979)
4 Hubel, D H & Livingstone, M S., *Nature* 315, 325-327 (1985)

Lysosomes and prohormone activation — correction

IN the letter from C. N. Hales under this title in *Nature* of 7 March (Vol. 314, 20) an editorial change altered the sense of the final paragraph which should read:

> The sequence information which has emerged to date is consistent with the lysosomal hypothesis, suggesting that it may provide a useful framework for making and interpreting structural comparisons in this area.

The hypothesis referred to is outlined in the second paragraph of the letter and in more detail in the ref 3 and 4 in the list cited.

How many orbiting lasers for boost-phase intercept?

from Richard L. Garwin

A constellation of laser battle stations in low Earth orbit could intercept intercontinental ballistic missiles (ICBMs) in their boost phase. If such lasers are available decades hence, they will face fast-burn ICBMs and find their task more difficult. How difficult is shown by an elementary analysis taking into account laser retarget time.

PRESIDENT Reagan's Strategic Defense Initiative (SDI) for countering Soviet nuclear-armed intercontinental-range ballistic missiles (ICBMs) and submarine-launched ballistic missiles (SLBMs) puts great stress on interception of the missiles in boost phase. Although the feasibility of a star wars defence will stand or fall by the ease of countering the system, it is of some interest to know how many satellites of given characteristics would be required to destroy boosters in the numbers and with the characteristics that may be available by the time such a defence could be mounted. (See ref. 1 for a technical introduction to boost-phase intercept.)

The emphasis given in this discussion to the need to destroy most ICBMs while the rocket booster is still burning is consistent with the general supposition by SDI supporters that this would be essential even to partial defence, because the missile is then more visible and more vulnerable than later, when the reentry vehicles and any decoys may have been deployed; moreover, in the boost phase there are fewer targets to be destroyed.

The Soviet silos lie deep within the Soviet Union, and two basing modes have been suggested for boost-phase interception. The first entails large numbers of low-orbit satellites carrying powerful lasers or homing rockets, so that there will always be enough within range of any silo to destroy the ICBM launched therefrom. The second involves pop-up interceptors based on submarines near the Soviet Union, that would be launched on information that a massive ICBM launch had taken place and which would destroy missiles in boost phase with X-ray lasers powered by nuclear explosions. If the X-ray laser is assumed feasible and effective, the need to bring it above the curve of the Earth during the duration of boost of the ICBM requires an interceptor mass far larger than the payload mass itself. Thus[2] an interceptor based 7,400 km from the silo, required to climb in 120 seconds to an altitude giving a line of sight with 185-km Earth-limb clearance to a booster which has climbed to 370 km altitude, would have an initial mass of 660 tons to lift an X-ray laser payload of 1 ton. This assumes no structural mass needed for the rocket, a specific impulse of 300 seconds (exhaust velocity of 3 km s^{-1}) and instantaneous burn of the interceptor rocket propellant. To reach the same altitude in 60 seconds would require twice the speed and an initial mass of 430,000 tons. These are some of the reasons why what follows concentrates on the characteristics of the first basing mode for SDI, a satellite constellation.

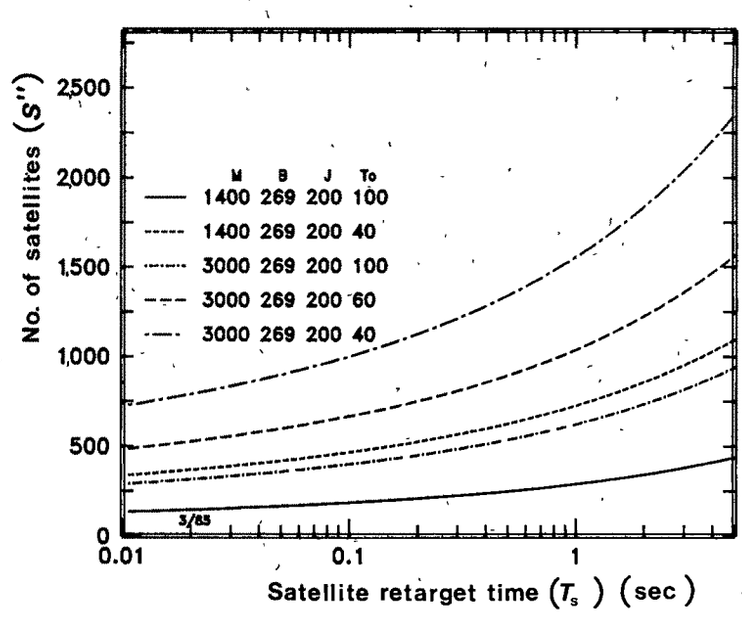


Fig. 1 The number S'' of our standard laser satellites is plotted against assumed laser retarget time for two assumed booster numbers and 3 assumed engagement times during boost phase. Against the clustered launch of 3,000 boosters of 40-s engagement time, 1,344 satellites of 0.5-s retarget time in optimum orbit are required, working perfectly reliably, to dispatch the boosters.

Counter measures

In what follows, I consider the likelihood that there will emerge counters to the assumed defences, for example, by the evolution of the Soviet ICBM force to small single-warhead missiles equipped with fast-burn boosters. Such missiles, perhaps burning out in 50 seconds at an altitude of 80 km, would negate the pop-up interceptor in two ways: first, it could not climb to line-of-sight within 50 seconds, while the soft X-rays could not penetrate to 80-km altitude within the atmosphere. Studies made for the US government in July 1983, by McDonnell-Douglas and Martin-Marietta, indicate that such fast-burn boosters with MIRVs and penetration aids could be built at a cost of only some 10–15 per cent more than optimized (normal-burn) boosters. Indeed, given that the cost of the missile guidance systems may be much reduced, a silo-based single-warhead ICBM could even be cheaper per warhead than a new MIRVed large missile because of economies of scale in production, and the much cheaper development and test programme.

Missile defence based on satellites in low Earth orbit would provide continuous visibility from some satellite to each booster, but fast-burn missiles would again negate the capability of both X-ray lasers and neutral particle beams (hydrogen-atom beams of 100-MeV energy) because of the screening of the atmosphere. Homing interceptors would have little time to reach the boosters and the atmosphere at 80-km altitude would

interfere with their infrared homing. Satellites carrying powerful continuous-wave or repetitively pulsed infrared lasers can, at some wavelengths, see down to the clouds and might have significant capability against individual missiles, even fast-burn boosters. We assume that a 50-second-burn booster could be engaged for 40 seconds by such a satellite fleet, but note that such large satellites would need to be protected against Soviet antisatellite weapons.

There are few published analyses of required numbers of laser battle stations, and these are examples of methodology rather than conclusions, since they neglect the relationship between a satellite's vulnerability and its altitude, assume zero retarget time, and make other simplifying assumptions. Carter[3] chooses to deploy his satellites on 1,000-km altitude orbits and, for booster hardness half that assumed here, estimates 160 satellites required to handle the present 1,400 Soviet boosters as they are now distributed. For the same boosters deployed "in one region", Carter estimates that 500 satellites are required.

Other estimates[4] point to a requirement of 300 lasers, or 800 lasers (the latter estimate involving satellites at 1,000-km altitude deployed in orbits of optimum inclination and assumed to destroy a booster out to 3,000-km range in the 7 seconds required at 3,000 km). The assumption that satellites are deployed for uniform coverage of the entire globe[5] leads to a requirement for some 2,400 satellites[6].

In unpublished Los Alamos documents of 4 May and 6 August 1984, G. H. Canavan[7] assumes satellites distributed uniformly over the entire globe and asserts that for boosters uniformly distributed over a very large region, satellite numbers required grow only as the square root of booster numbers (or hardness) because satellites uniformly deployed will be closer together and will destroy boosters at shorter average range. These results are cited in ref. 8. For deployment in a small region, Canavan also obtains satellite numbers proportional to the number of boosters.

C. T. Cunningham[9] assumes 300-km orbit altitude, with 60-deg. orbits; using a dynamic simulation, he finds that only 60 satellites are required—noting however that his simulation requires "that the switching time be much less than the dwell time of 42 milliseconds at the minimum target distance of 100 km."

The present analysis explicitly considers retarget time for both distributed and regional basing of ICBMs. We consider a constellation of satellites assumed to provide, over the region of ICBM silos, an areal density s (number of satellites per unit area) adequate to destroy all ICBMs, assumed launched simultaneously and burning out in time T_0.

The derivation of the overall number of satellites S from the required local areal density s entails the calculation of s as a function of orbit parameters. Satellites in a mixture of orbits giving uniform density everywhere will occupy $4\pi E^2 = 509(\mathrm{Mm})^2$ of area, so that $S = 509s$. Launch sites clustered at the North Pole could be attacked most effectively with polar-orbit satellites, which have a much larger latitude-band-averaged density at $\lambda = \pm 90°$ than elsewhere. Silos limited to the Equator could be efficiently addressed by equatorial low-Earth-orbiting satellites. In general, there will be an optimum orbit to attack boosters launched from silos spread uniformly from latitudes λ_1 to λ_2. If we assume satellites uniformly phased in orbits of optimum inclination i, the band-averaged satellite density

$$\langle n \rangle_{\lambda_1,\lambda_2} \equiv \frac{\int_{\lambda_1}^{\lambda_2} n(\lambda) \cos\lambda \, d\lambda}{\int_{\lambda_1}^{\lambda_2} \cos\lambda \, d\lambda} \qquad (1)$$

where $n(\lambda)$ is the satellite density averaged by Earth rotation for latitude λ. This is an optimistic measure. For satellites of very short range, the constellation capability is limited by $\min(n(\lambda))$ over $\lambda_1 < \lambda < \lambda_2$. For long-range satellites, those outside the latitude range will have to be considered, which will reduce the average density below that calculated here, so that the concentration factor y derived here is an over-estimate, especially because the granular satellite density is assumed uniform.

For N satellites on any orbits of inclination i, the longitude-averaged

$$n(\lambda) = (N/2\pi^2)/(\sin^2 i - \sin^2\lambda)^{1/2} \qquad (2)$$

for $\lambda < i$, and $n(\lambda) = 0$ for $\lambda > i$. Averaging over a band from λ_1 to λ_2, we have for such orbits

$$\langle n \rangle(\lambda_1, \lambda_2) = (N/2\pi^2) \times \frac{[\sin^{-1}(\sin\lambda_2/\sin i) - \sin^{-1}(\sin\lambda_1/\sin i)]}{(\sin\lambda_2 - \sin\lambda_1)^{-1}} \qquad (3)$$

(for $\lambda_1 < \lambda_2 < i$, and with obvious modifications for $\lambda_2 > i$). For silos spread uniformly between 50° and 60°, the maximum concentration factor y is reached with orbital inclination $i = 60°$, and is $y = 3.1$, which we have used in constructing Table 1 and Fig. 1. Thus we multiply required satellite densities by 164 $(\mathrm{Mm})^2$, confident that the resulting number of satellites will always be an underestimate.

We assume a steady or rapidly pulsed laser on each satellite, provided with an optically perfect diffraction-limited mirror of diameter D which is refocused at the range of that booster which is the target of the moment. (The Hubble space telescope will have $D = 2.4$ m; demonstration of even megawatt diffraction-limited chemical lasers is several years away in the SDI programme.) The laser is assumed to have a steady output power P and wavelength λ. For a uniformly illuminated output aperture of area $A = \pi D^2/4$, the maximum possible power density at any point in the focal spot at range r is obtained from the brightness B of the laser (power per steradian of solid angle) as $p \equiv B/r^2$. In turn, B is given precisely by $B = PA/\lambda^2$, which can be shown by integrating over the radiating aperture to obtain the electric field a short distance away, and comparing with the electric field when the entire aperture is phased to provide a focus at distance r.

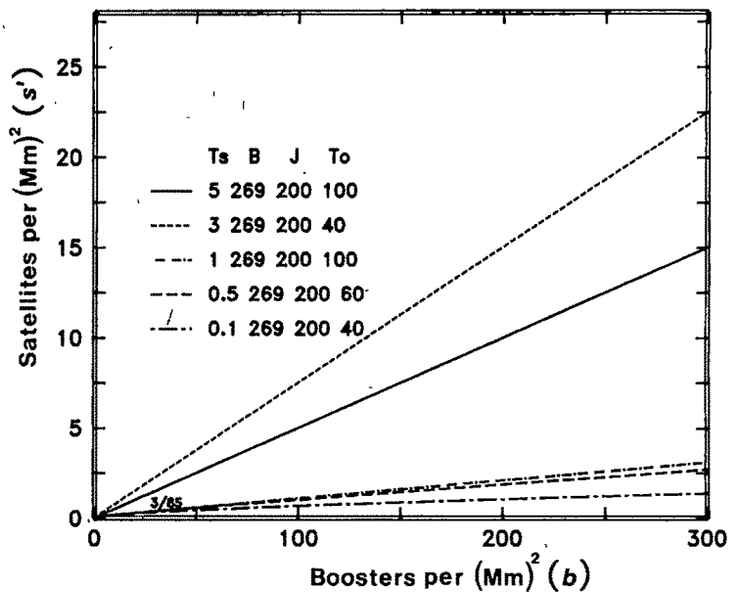


Fig. 2 Perfect satellites as in Fig. 1 are uniformly distributed at optimum (near-zero) altitude above a distributed booster density of b and are constrained to shoot within their tiling hexagons. Soviet missiles are said[3,4] to number 1,400 and be distributed over an area of 10 $(\mathrm{Mm})^2$; thus $b = 140$. With a 5-sec laser retarget time and 100-sec engagement time, some 5.8 satellites per $(\mathrm{Mm})^2$ are needed—or 950 in optimum orbit world-wide.

In the numerical examples we consider $P = 25$ MW, $D = 10$ m, $\lambda = 2.7$ micrometre (hydrogen fluoride). We thus take $B = 2.69 \times 10^{20}$ watts per steradian. At $r =$ 3,000 km or 3 Mm, the power density is thus 30 MW m^{-2}. We assume a booster hardness $J = 200$ MJ m^{-2}, which is sufficient to evaporate a layer of carbon 2 mm thick. Then the time $t(r) = Jr^2/B$ required to deliver the assumed lethal heat is 6.7 seconds. Such calculations assume that the satellites can see the boosters sufficiently clearly and can point sufficiently accurately and stably that the power density at the peak within the 1-m diameter spot (at 3-Mm range) is not diminished by spot wander exceeding a few tens of cm during the 40 km or so travel of the booster during the 7-second engagement.

As one considers countering missiles with boost times ranging from the 300 seconds or so of the Soviet SS-18 to the 180 seconds of the US MX missile and to the 40 seconds of a fast-burn ICBM, the need arises either for more satellites or for satellites at closer range. Furthermore, particularly for powerful satellites or for close-range engagements, the time T_s required for the laser satellite to retarget, to slew and settle the beam to the necessary accuracy of 0.1 microradian or better, plays an important role in determining the size of the constellation.

Our programme is first to calculate, for a flat Earth and a planar satellite constellation at altitude h, the number of missiles launched from a region that could be killed by a constellation of satellites with particular density and laser characteristics. We then extend that calculation to the more realistic case of a spherical Earth, obtaining a reasonably accurate closed-form expression (Fig. 1). Next we calculate the missile-killing potential of an assumed satellite constellation density above a uniform areal distribution of boosters, for which each satellite is assigned the boosters within its circle of responsibility (Fig. 2). This would be a reasonable assignment strategy for boosters covering the entire Earth at uniform density, but it has also been used to justify "square-root scaling" of satellite numbers against booster numbers for boosters deployed over 10 (Mm)^2, taken to be the deployment area of the 1,400 current Soviet ICBMs[7,8]. The results are tabulated in Table 1 and discussed for some cases of interest.

Although there seem to be no published results on laser constellation size for finite retarget time, our calculations show the great importance of the time required to move the beam from one booster to the next.

Clustered launch

We now turn to the problem of relating local satellite density to the number of boosters to be destroyed. For M_0 boosters all launched simultaneously from a point, and if a satellite constellation of local density s and brightness B, deployed at altitude h, is assigned to kill them, we assume a continuous distribution of satellites and that the beam is always normal to the booster surface. Since the time to kill a booster at distance r is Jr^2/B, a satellite at distance r can kill $m(r) = (BT_0/Jr^2)$ boosters in the available boost time T_0.

The missile-killing potential of the constellation against clustered launch is then obtained by integrating $m(r)$ over all satellites from those directly above the launch site ($r = h$) to some maximum r. We cut off the integral at $r = U$, defined as the distance beyond which a satellite can no longer kill even one booster. Then U is given by $m(U) = 1$, or $U^2 = BT_0/J$. This calculation optimistically takes credit for fractional kills above unity. Each satellite closer than U is assigned a different booster and heats it for the required time before being assigned and moving instantly to heat another. The number of boosters that can be killed, M, is then

$$M = \int sm(r)2\pi\rho \, d\rho = (BT_0/J) \int_{\rho=0}^{\rho=\max} \frac{2\pi s\rho \, d\rho}{(h^2+\rho^2)} \quad (4)$$

where the coordinate system is fixed at the booster launch point, the integration runs over the various satellites and ρ is the radius from the origin to a sub-satellite point. With $a \equiv r^2 = h^2 + \rho^2$,

$$M = \pi s(BT_0/J) \int_{a=h^2}^{a=U^2} da/a = \pi s(BT_0/J) \ln(U^2/h^2) \quad (5)$$

$$= \pi s(BT_0/J) \ln(BT_0/Jh^2) \quad (6)$$

For the 25-Mw 10-m diameter HF laser described earlier, we have $B =$ 269 Mw(Mm)^2 m^{-2}, $J = 200$ MJ m^{-2} and, for $T_0 = 100$ s, $U = 11.6$ Mm. For $h =$ 500 km (0.5 Mm), the logarithm is about 6 and varies only slowly with h.

A planar constellation of one satellite per square megametre and zero retarget time, consisting of some 164 satellites in optimum orbit covering only Soviet ICBM sites, could thus destroy 2,657 missiles of the assumed characteristics. But, as will be seen, the recognition of satellite retarget time of 0.1, 0.5, or 3.0 seconds reduces this capability to 2,475 2,107 and 1,468 boosters respectively. The imposition of horizon limitations for the case of a curved Earth will then further reduce the capability of the constellation.

Table 1 Required satellite densities s, and numbers S.

M boosters	J (MJ m^{-2})	T_0 (s)	T_s (s)	s' (per (Mm)^2)	S'	s'' (per (Mm)^2)	S''
1,400	200	100	3	4.24	697	2.32	381
1,400	200	40	3	10.54	1,732	5.80	953
3,000	200	40	3	22.5	3,699	12.43	2,044
1,400	200	100	0.5	0.89	146	1.53	251
1,400	200	40	0.5	1.96	322	3.82	628
3,000	200	40	0.5	3.97	653	8.18	1,344
3,000	200	100	0.5	1.71	281	3 27	537
1,400	200	100	0.1	0 48	79	1.14	187
1,400	200	40	0.1	0.84	138	2.85	468
3,000	200	100	0.1	0.76	126	2.44	401
3,000	200	40	0.1	1.39	228	6.10	1,003
Boosters distributed over 10 (Mm)^2 (solution of Eqn. 18): (Lasers assigned local areas of responsibility) (Flat Earth, satellite numbers will be greater than shown)							
Concentrated boosters with horizon cutoff of satellite effectiveness: (Round Earth, solution of Eqn. 13, optimum altitude $h^2 = (BT_s/J)$) For $T_s = 3$, 0.5, 0.1 seconds, h is 2,010, 820, or 370 km, respectively. The point-launch approximation holds for $G \leq h$, where G is the diameter of the launch field							

Orbiting satellites carrying 25-MW lasers of 2.7 μm wavelength and with 10-m diameter perfect mirrors (brightness $B = 2.69 \times 10^{20}$ W per steradian) are placed in near-optimum 60° inclination orbits for boost-phase intercept of ICBMs near 55° latitude. The last two columns present the local satellite density s'' to destroy the indicated number of missiles of boost time (engagement time) T_0, with satellites of retarget time T_s, assuming the missiles concentrated in a region not much bigger than the altitude of the satellites. Total number S'' of satellites needed is obtained by multiplying s'' by the surface area of the Earth—509 (Mm)^2—divided by a concentration factor of 3.1. For this concentrated launch, optimum orbit altitude (Eq. 12) is 2,010, 820, or 370 km respectively for retarget times of 3, 0.5, or 0.1 s. Columns s' and S' are an improved analysis of an assumed distribution of boosters clearly inapplicable to the parameters discussed here—as if boosters were deployed at constant density everywhere, with satellites constrained not to shoot at a booster if there is a satellite closer to that booster. This model would clearly require more satellites than if an optimum assignment were used, as in calculating the density s'' against clustered launch. Equally clearly and primitively, distributed boosters should be an easier task for the defence—a correct intuition contradicted by the comparison of 697 satellites and 381 satellites in the first row of the table. The discrepancy arises because the assumed model[7,8] is inappropriate.

Flat Earth and non-zero retarget time. A 10-m diameter mirror cannot move instantly from one target to another. The Hubble space telescope has a settling time of 3 seconds for the smallest motion within a beam-width (about 0.2 μradian), and will take several minutes to move to a new target. Because of the large laser power at the mirrors, it is not possible to make use of tricks often used in optical systems for compensating imperfect figure or orientation of large elements by the deformation or motion of smaller elements. For the same reason, and because the beam must be moved typically 6° or so between targets (some 4×10^5 beamwidths) electro-optical phase-shifting coatings cannot do the job. It might be possible to retarget a 10-m mirror to within 0.1 microradian or less in, say, $T_s=3$ seconds. We shall consider $T_s=3$, 0.5, and 0.1 s. Kill time Jr^2/B then becomes T_s+Jr^2/B, which is equivalent to replacing r^2 by (r^2+BT_s/J) in the calculation of M. We then have

$$M=\pi s(BT_0/J)\ln\left[\frac{(U^2+BT_s/J)}{(h^2+BT_s/J)}\right] \quad (7)$$

Round Earth. The horizon (tangent plane to the Earth at the booster launch location) intersects the satellite constellation sphere at a distance

$$H=[(E+h)^2-E^2]^{1/2} \quad (8)$$

Satellites beyond that distance are below the horizon and shielded from the booster at low altitude. For example, $H=1.94$ Mm for $h=300$ km, far less than the upper limit of the integral for calculating M in the flat-Earth case, for which U exceeds 11 Mm. Apart from small effects relating to the somewhat reduced range to a satellite on the basing sphere at ρ, the spherical distribution of satellites can be taken into account. With

$$U'\equiv\min[(U),(H)] \quad (9)$$

Equation (7) becomes

$$M=(\pi sBT_0/J)\ln\left[\frac{(U'^2+BTs/J)}{(h^2+BT_s/J)}\right] \quad (10)$$

or, for the normal horizon-limited case,

$$M=(\pi sBT_0/J)\times\ln[1+2hE/(h^2+BT_s/J)] \quad (11)$$

In order to use the satellites to best advantage, we maximize M with respect to h. The logarithm is maximum when the argument is maximum, which for the horizon-limited case, yields the criterion

$$h^2=BT_s/J, \quad (12)$$

For this optimum altitude, we then have

$$M=(\pi sBT_0/J)\times\ln[1+E(BT_s/J)^{-1/2}] \quad (13)$$

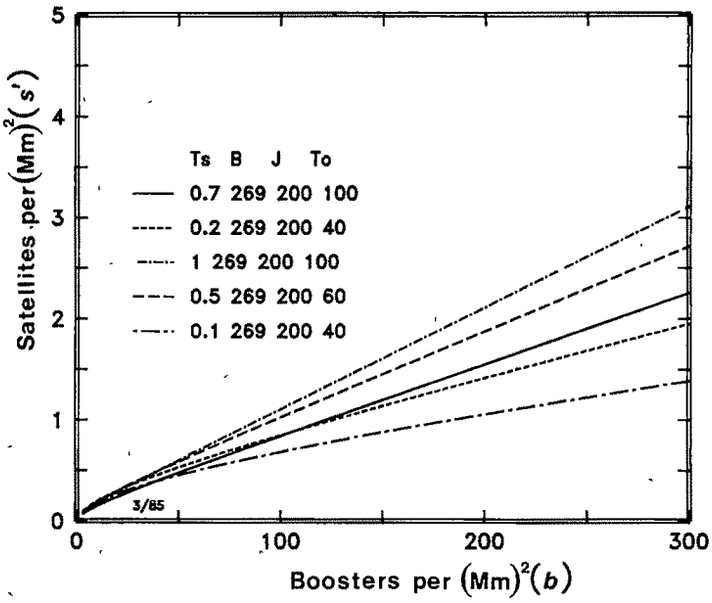


Fig. 3 Shorter retarget times T_s than in Fig. 2 are modelled here. Note that the "square-root scaling"[3,4] is confined to low booster density and is replaced by a linear relationship at high booster density. Satellite density s' exceeds the greater of bT_s/T_0 and $(Jb/2\pi BT_0)^{1/2}$. Thus a model which is inapplicable because of geographic distribution also fails to give favourable square-root scaling at large threats when finite retarget time is considered

valid so long as $H^2<U^2$, or so long as $2E(BT_s/J)^{1/2}+(BT_s/J)<U^2$. For $B=269$, $J=200$, this condition is satisfied up to $T_s=9.8$ seconds for $T_0=40$ and $T_s=17.5$ s for $T_0=100$. The results for the numbers of boosters killed are plotted in Fig. 1.

Distributed boosters

We now turn to the question of the validity of the claimed "square-root scaling" of satellite density with increasing booster density. To calculate the constellation densities to destroy boosters distributed uniformly (with density b) over a plane, we assume satellites on a regular triangular (hexagonal close-packed) lattice at altitude h, with nearest-neighbour satellite spacing $2R$. To each satellite is assigned the task of destroying every booster in its own circle of responsibility of radius R (which strictly is a hexagon whose area is 10.3 per cent greater than that of the intended circle). The capability of each satellite is exhausted when the sum of the times taken to kill each booster (including retarget time) exceeds the available engagement time T_0. For a given booster density b, we calculate the time T required for a satellite to kill its "own" boosters, which is a function of the parameters b, J, h, R, B and T_s. If the laser parameters B and T_s are fixed, we maximize constellation capability with respect to h for a given satellite density $s\equiv1/\pi R^2$ and thus determine b, which gives a point on the b–s curve of booster density against satellite density (or number). If $t(r)$ is the time to kill a booster at distance r, and ρ is now the radial coordinate on the ground, with origin at the sub-satellite point, the time required to kill all boosters is

$$T\equiv\int_{\rho=0}^{\rho=R}t(r)b2\pi\rho\,\mathrm{d}\rho \quad (14)$$

The integral extends over all the boosters within radius R of an individual satellite. With $t(r)=T_s+Jr^2/B$ and $a\equiv r^2+(BT_s/J)$, the time T becomes $\pi Jb/B\int a\,\mathrm{d}a$ between limits which are the extreme values of a (differing by R^2), which leads to the result

$$T_{\min(h)}=(2\pi Jb/B)(R^4/4)\times[1+(2/R^2)(BT_s/J+h^2)] \quad (15)$$

This time is a minimum at $h=0$, whence

$$T_{\min(h)}=(2\pi Jb/B)(R^4/4)\times(1+2BT_s/JR^2) \quad (16)$$

and

$$T_0=(Jb/B)(\pi R^2)^2(2/4\pi)\times(1+2BT_s/JR^2) \quad (17)$$

$$s^2=(Jb/BT_0)(1/2\pi)\times[1+(2\pi T_sB/J)s] \quad (18)$$

Satellites cannot be in orbit at zero altitude, so that s defined by this quadratic equation will again be an underestimate. The equation determines the satellite density s required to cope with an infinitely extended booster distribution of density b.

A more flexible assignment of targets would require fewer lasers, but we take our restrictive assignment scheme as a prerequisite (but not a sufficient condition) for a claimed square-root scaling, in order

to explore the actual scaling law. The solution of the equation is

$$s = (T_s/T_0)(b/2) \times\{1+[1+(1/b)(2/\pi)(T_0J/BT_s^2)]^{1/2}\} \quad (19)$$

If $b \gg b^* \equiv (2/\pi)(T_0/T_s)(J/BT_s)$, then $s \simeq bT_s/T_0$ and is linear in booster density. For the parameters assumed, our $b^* = 5.3$ per $(\mathrm{Mm})^2$ is far below the existing 140 per $(\mathrm{Mm})^2$ density of Soviet ICBMs, and the number of satellites required is linear in the number of boosters deployed. In terms of satellite density, $(2\pi T_sB/J)$ is 25.36 for satellite retarget time $T_s = 3$ seconds. Thus the second term in equation (18) dominates the first for $s > 0.04$ satellites per square megametre. Much weaker satellites, or shorter retarget time, or tougher boosters will be required to obtain "square-root scaling," even for infinite distributions of boosters and satellites[7,8].

Equations (18) and (19) teach us that "square-root scaling" can enter only at low satellite density or low booster density, respectively. But a stronger inference can be drawn from Equation (19) regarding the scaling of numbers of satellites of given characteristics versus numbers of boosters of infinite extent. Clearly, the asymptotic behaviour for large numbers of boosters is $s = bT_s/T_0$—each local satellite destroying a booster each retarget time, for the entire burn or engagement time. But Equation (19) tells us that satellite numbers are always greater than that given by the asymptotic formula, and in the linear range this residue of square-root scaling requires an additional satellite density $s_+ = J/2\pi BT_s$. Thus we know that even on the assumption of widely deployed boosters, the required local density of satellites is at least the local density of boosters multiplied by T_s/T_0, and the ratio of the cost of the satellite constellation to the cost of the booster constellation exceeds

$$(C_s/C_b) \times (T_s/T_0) \times (4\pi E^2/yA) \quad (20)$$

where C_s is the cost of a satellite, C_b the cost of a booster, and A the area over which the boosters are deployed. Adopting the proposed[7,8] assignment strategy that satellites should be restricted to shoot within their circles of responsibility (as required for square-root scaling), and assuming the deployment area to be 10 square megametres and a satellite concentration factor $y = 3.1$ from optimum orbits, satellite numbers are $16T_s/T_0$ times the number of boosters. With 3-second retarget time and 100-second engagement time, one satellite must be deployed for every two boosters, and the defense would be overcome by a cheaper offensive deployment if a booster cost less than half the price of a laser battle station.

Since the laser must have been launched into orbit by a rocket, and weighs far more than the ICBM payload, and because lasers must be deployed over an area very much larger than the booster deployment area (to which active lasers are limited by the assignment strategy needed for square-root scaling), boost-phase intercept can hardly be achieved cost-effectively by satellites armed with powerful lasers. Furthermore, for non-zero retarget time and for reasonable satellite and booster characteristics, satellite numbers are proportional to booster density even for infinitely extended booster deployment. Because satellites are assumed to shoot only at local boosters in the distributed launch case, there appears to be little difference between the planar constellation and round-Earth results for distributed launch, although the smaller values of S' in Table 1 would be increased by recognition of horizon limitations which impose $h > 0$.

With the simplistic assignment of satellites to kill widely distributed boosters in their immediate vicinity, Equation (18) shows a number of satellites linear in booster density except for near-zero retarget time, and then only for satellite altitude zero or scaled with satellite separation, and also only for low booster densities. According to Equation (19), large booster density, short burn time, reduced booster hardness, enforce linear scaling; while poor laser brightness and short retarget time are conducive to square-root scaling. Figure 2 and the columns in Table 1 labelled s'' and S'' demonstrate near-linear scaling for many cases of interest with distributed boosters.

This rather primitive assignment of satellite resources is in fact far from optimum in some of the cases considered here, and since a defensive system will be two decades or more in evolution, it is realistic to consider the satellite numbers required to overcome a more responsive threat—the clustered launch of smaller missiles.

The last two columns in Table 1 present the satellite density and numbers required to handle the indicated booster threat for launch of boosters from an area some hundreds of km diameter. We assume 25 MW of 2.7 micrometre laser light; 10-m diameter perfect optics; retarget time T_s of 0.1, 0.5, or 3.0 seconds as indicated; engagement duration T_0. For concentrated launch, the required number of satellites is proportional to the number of boosters.

Figure 3 demonstrates that only for retarget time of 0.1 second is there such a thing as "square-root scaling" at existing booster numbers. Furthermore, Table 1 and Fig. 1 illustrate that the Soviets have a far more effective response than to build additional silos distributed over their vast territory—namely to cluster them in a region 1,000-km diameter, or so, and to pay the modest additional cost to shorten burn time to 40–50 seconds. This would also negate boost-phase intercept by satellites carrying X-ray lasers, particle beams, and by any pop-up weapon. Such a response would ensure that boost-phase intercept could not meet the requirement stated for the US Government on 20 February by P. H. Nitze that it "be cheap enough to add additional defensive capability so that the other side has no incentive to add additional offensive capability to overcome the defence."

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Nuclear collisions at intermediate energies: achievements and prospects of GANIL

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At energies of tens of mega-electronvolts per atomic mass unit, nuclear collisions exhibit phenomena more complex than the essentially collective behaviour at lower energies or the more individualistic nucleon interactions observed at much higher energies. This intermediate regime is being uniquely explored at the French national heavy-ion accelerator GANIL.

THE French facility for heavy-ion physics, GANIL (Grand Accélérateur National d'Ions Lourds), has been operating since 13 January 1983. The first beam from this accelerator, which uses a series of three cyclotrons to bring the velocity of ionized atoms to about one third of the velocity of light, had been obtained two months earlier, in November 1982. From the start, GANIL was thought of as a national facility. This had two consequences: first, the formation of a very able team of engineers and technicians from all French laboratories; second, the choice of an accelerator that would represent a significant improvement on anything previously built in France, but which was within reach.

The nature of the beams to be accelerated and the type of the accelerator were decided in 1972–74. At that time, heavy-ion research had just established itself in the study of atomic nuclei. For some years, many physicists had considered it to be a backwards step from the precise and successful analysis of discreet nuclear states that the accelerators of light ions, such as protons and α-particles, had provided. Massive projectiles, such as the ions of carbon, calcium and heavier elements, with poor energy resolution, were described by some as awkward tools for the study of the subtle quantum structure of nuclei—not much better, perhaps, than the use of boxing gloves to put thread through the eye of a needle. Yet, in about 1970, it was clear that nucleus–nucleus collisions, as made possible by heavy-ion beams, were unique means of studying the collective coherent behaviour of nuclei. New phenomena were discovered and the lexicon of nuclear physicists had to be expanded to include new phrases such as dissipative phenomena, temperature, time evolution and friction, which undoubtedly reflected increasing knowledge about nuclei. French groups had been particularly active in this field, with a major part being played by the ALICE accelerator in Orsay.

What was the next step? One problem arose from the mismatch between the turnover time for an experiment, typically 6 months to 2 years, and the time needed to design and build a heavy-ion accelerator, typically 5–10 years (constructing an accelerator for particle physics takes about the same time but experiments last several years. At the other extreme, in most fields of physics, the turnover time of experiments is measured in months, as in nuclear physics, but the equipment is usually lighter and can be transformed at a similar rate). Hence, at the beginning of the 1970s, French nuclear physicists had to design an accelerator for the 1980s and 90s while hoping that the next experiments woud help to elucidate the mechanisms of dissipation of excited nuclear systems within the next year. But choices had to be made, and three orientations were agreed upon: (1) The new accelerator had to accelerate ions up to velocities of 100 MeV AMU^{-1} with no provision for extending that range further. (2) The structure of the accelerator would consist of a small compact injector cyclotron followed by two large separated-sector cyclotrons operating in series (Fig. 1). Other possibilities were discarded. A linear accelerator would have opened up a regime field not very different from that well explored by GSI Darmstadt. Cryogenic cyclotrons would have proved difficult to design and operate safely, a risk that a relatively small community could not afford. The coupling of an ion source of high ionization ability with a synchrotron would, it was felt, limit the intensity of the beams and rely too heavily on the overcoming of technical problems not then solved. The choice made seemed to represent the best compromise between innovation and security. (3) The new laboratory had to be a national facility, therefore all agencies and laboratories had to contribute in manpower and financially to ensure the successful completion of the GANIL programme.

Some 30 articles dealing with experimental work done at GANIL have been published so far. They are systematically compiled and discussed in *Nouvelles du GANIL*, published bimonthly and sent on request to all interested scientific institutions.

Among the general papers (unpublished) dealing with GANIL nuclear physics are:

M. Lefort *Lectures to the Cargese Summer School* 'Heavy-Ion Collisions from Nuclear Collective Motion to Quarks' (September 1984; preprint IPN-Orsay IPNO-DRE-84-33).

B. Tamain *Lecture to the 16th International School on Nuclear Physics* (Mikolajki 1984; preprint LPC Caen 84-03).

C. Detraz *Mt Fuji Symposium on Heavy-Ion Physics* 'First results from GANIL' (Tokyo, August 1984).

Intermediate energy physics

Next, I discuss the reasons that determined the choice of the beam energies available at GANIL. The idea was to study nuclear collisions in an energy range where a transition between two regimes occurs. Figure 2 shows the domain available with GANIL in comparison with other existing or projected accelerators. At energies of several tens of MeV AMU^{-1} the processes at work when two nuclei collide should change markedly. I will address four such arguments.

First, the wavelength λ associated with a nucleon of a projectile with an energy of say 5–10 MeV AMU^{-1}, available with major cyclotrons and tandems, is much larger than the mean distance d between two nucleons of the target nucleus. Thus, the incoming nucleon will 'see' (that is, interact with) several target nucleons. The collision process will then seem to be essentially collective. In the 1960s and 70s one could thus study the fusion process in which the two colliding nuclei fuse into a single excited nuclear system, and the so-called deep inelastic collisions where a temporary binuclear system is formed before the two partners separate again. The latter, an unexpected process, offered significant new information on the collective properties of nuclear matter and even, at a more elementary level, on the mechanisms that allow the propagation and dissipation of energy and angular momentum.

At the other extreme, say at 1,000 MeV AMU^{-1} energy, the situation is clearly the opposite, λ becoming smaller than d. The reaction mechanism results from the superposition of nucleon–nucleon interactions. The degree of overlap of the two colliding nuclei determines the dominant behaviour. In central collisions complete explosion is observed, whereas in peripheral ones a

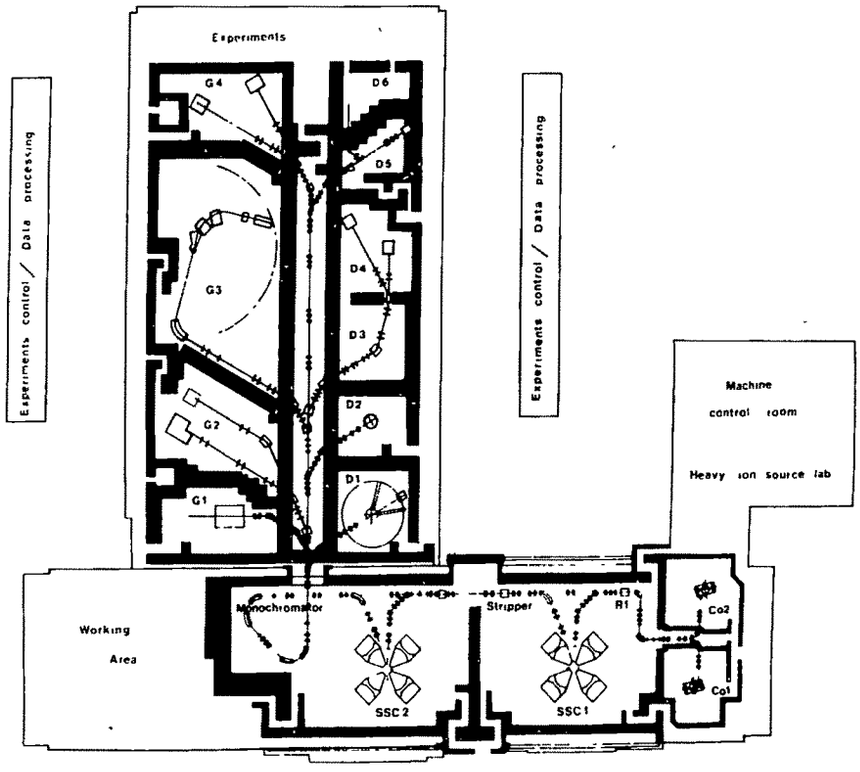


Fig. 1 Layout of the GANIL accelerating system, consisting of one compact cyclotron injector and two large separated-sector cyclotrons (SSC). A thin carbon-foil stripper increases the charge stage of the accelerated ion before entering the second SSC. An α-shaped magnetic analyser selects the momentum of the accelerated particles before the beam is transported to the various experimental areas.

participant zone, highly excited and emitting many fast nucleons, can be distinguished from the spectator zones corresponding to the non-overlapping parts of the nuclei. Despite their very low beam intensity, those high-energy machines that could accommodate heavy ions, such as the Bevalac in Berkeley and the synchrophasotron in Dubna, confirmed this overall picture.

Second, the quantum nature of the two colliding nuclei means that the interaction of two of their nucleons, which are fermions, is only possible if they can be scattered into unoccupied discrete states. Because all the quantum states below the Fermi level are occupied in both nuclei, this requires that the energy available in the nucleon–nucleon collisions be large enough to promote these nucleons into highly excited states. In terms of velocity instead of energy, individual nucleon–nucleon interactions play a significant part in the nuclear collision only if the relative velocity of the nucleons is larger than the Fermi velocity, which is equivalent to ~35 MeV AMU^{-1}.

Third, the velocity of sound in nuclei can be calculated from what is already known about the elementary modes of excitation of nuclear matter. An elementary local perturbation of the wave function of nucleons propagates with a velocity of ~30 MeV AMU^{-1}. When the relative velocity of colliding nucleons is far below this value, the perturbation induced can be distributed all over the nuclear system during collision time. In other words, thermalization can be achieved but above this value it is impossible and thus the nucleons ejected remain very energetic.

Fourth, it has long been known that two nuclei can fuse only if the total energy transformed into excitation energy of the compound nucleus is not too high. Above an average value of 8 MeV per nucleon, the nucleons become unbound and the nucleus is then beyond its boiling point. Such a situation is reached in a collision of nuclei with similar masses when the bombarding energy in the laboratory reference frame is ~30 or 35 MeV AMU^{-1} but never in the few experiments performed above 100 MeV AMU^{-1}.

First results from GANIL

The long-term goals of the research programme at GANIL proceed from the above arguments. The energy available allows us to explore the transition region where the nuclei evolve from a coherent state to a collection of individual nucleons. One can formulate the regime in terms of a thermodynamic phase transition. The nuclear system formed in low-energy collisions can be described by collective variables; it exhibits a high degree of coherence and is not unlike a liquid. The concepts of viscosity, temperature, friction and the shape parameters seem to be useful. In high-energy collisions, the system behaves like an assembly of loosely interacting individual nucleons, rather similar to a gas.

The GANIL energies encompass the transition region. How does such a phase transition proceed and what phenomena occur when the nucleus looses its coherence? To observe and analyse these features is the primary experimental objective of the accelerator. More fundamentally, perhaps, these observations should allow physicists to address the following question: out of the very large number of degrees of freedom of the system formed by the excited assembly of nucleons, can the few relevant parameters that describe its structure and evolution be identified? This question defines a basic problem in statistical physics. The excited piece of nuclear matter formed in the collision has very distinctive features: it consists of a finite number of fermions, carries a high internal energy and is governed by the laws of quantum mechanics. Present-day statistical mechanics have not answered to this problem. It cannot provide parameters to derive the relevant variables nor laws to describe the evolution of such a system. These answers must come from nuclear physics itself.

Yet the experimental information obtained from these nuclear collisions seems hopelessly complex. A large variety of nuclear fragments with a wide energy spectrum are emitted at all angles and an accurate description of the actual reaction mechanism must be obtained from this mass of information. In heavy-ion physics, the additional wide variety of target, projectile and energy combinations adds both to the amount of information and to the difficulties of its interpretation.

The search for a thorough description of nuclear matter at the limits of its coherence, which, as stated above, is the goal of GANIL physics, could then start from hundreds of arbitrarily chosen points of view. One could say: let us investigate whether fast oxygen fragments are emitted forwards in coincidence with isotopically emitted α-particles, when argon and uranium nuclei collide. One would certainly observe such events with a probabil-

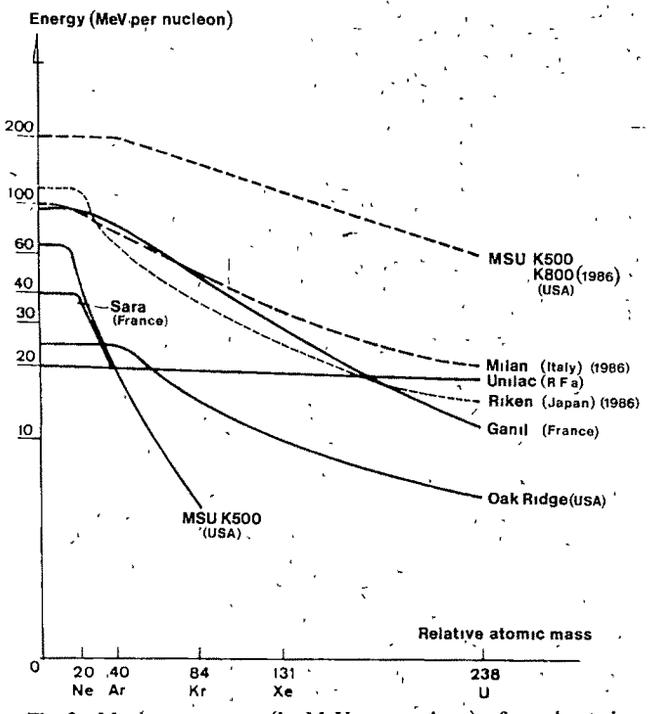


Fig. 2 Maximum energy (in MeV per nucleon) of accelerated ions from several accelerators, as a function of the atomic mass of the ion. Solid lines refer to operating facilities and dashed lines to projects.

ity that could be measured, then move to another event. However, little insight would emerge from such a random approach.

At GANIL, physicists started from known facts. As stated above, they had reached a detailed understanding of the dissipitative phenomena that occur in lower energy collisions. Similarly, the well-documented fragmentation process observed at high energy could be used as a reference. These initial points of reference have been used extensively since the first experiments of January 1983.

In dissipative interactions, the two colliding nuclei are linked for a time, rotating around their centre of mass and progressively equilibrating the various parameters that describe their respective structure, such as the ratio of neutrons to protons (N/Z), the energy and the angular momentum. Of these parameters, the one that equilibrates fastest is the N/Z ratio. This means, for instance, that a ^{28}Si projectile (14 p, 14 n) interacting with a heavy target such as ^{208}Pb, which has a large neutron excess (82 p, 126 n), will emerge as a neutron-enriched fragment. Such an equilibration occurs in $\sim 2 \times 10^{-22}$ s. It seemed of interest to see whether such a fast equilibration process would still occur at higher incident energies, that is, for shorter interaction times.

An unambiguous answer was achieved with incident beams of ~40 MeV per nucleon. Although the interaction times are long enough to accommodate the fast N/Z equilibration process, the average value of the N/Z parameter of the emitted fragment was found not to be influenced by the target. Rather, it closely reflects the value of the projectile, hence pointing to a fragmentation process. The forward-emitted nucleus results from the breaking of the projectile in the field of the target. No binuclear state is formed that would allow for some equilibration of the parameters. It took only two weeks to obtain the expected, although reassuring, information that the GANIL energies were high enough to observe the transition from low energy to high energy processes; it took a few months to confirm it with other projectile–target systems. Since then, however, a large amount of beam time has been necessary to qualify the observation that, at energies as low as 40 MeV per nucleon, collisions result in fragmentation.

Several well-established properties of the forward-emitted fragments are incompatible with a pure projectile-fragmentation mechanism. First, it was observed that the isotope distribution of the fragment (that is, the variation of the yield of isotopes of a given element Z with their mass A), depends somewhat on the nature of the target. More precisely, the more neutron rich the target is, the more probable is the emission of a neutron-rich fragment. Hence, the so-called fragmentation of the projectile includes some exchanges between the two colliding nuclei, a property obviously at variance with a pure fragmentation process. Second, some of the forward-emitted fragments actually gained one or two neutrons or protons as compared with the incident projectile, which again is incompatible with pure fragmentation. Finally, the energy characteristics of these fragments differ from those observed at higher energy. Instead of being centred around the velocity of the projectile, as expected and observed for fragmentation products, the velocity spectrum extends to lower values, giving evidence for the importance of relaxation processes during the interaction.

This accumulation of facts for and against a model of a reaction discussed here must be seen as an example of the approach pursued at GANIL during the first one or two years. Similar studies have been performed on the fate of central collisions. The ability of the two nuclei to fuse and the possibility that the resulting nucleus absorbs all the momentum carried by the projectile have been studied for different systems and for various energies.

This first stage of research is basically complete, at least for projectiles no heavier than krypton. The main facts are collected and published, but, at this stage, the conclusions remain ambiguous and difficult to formulate. This should not be surprising. Again, the results have been interpreted within the framework of known reaction mechanisms. Physicists have been looking for footprints they could identify and so they ascribe the results to the contributions of several mechanisms. This situation will not last: at the limits of nuclear coherence, when the internal motions inside nuclei and the relative motion of the two colliding nuclei carry similar velocities, new processes, as yet unknown, will provide a simpler description. So far we approximate them through conceptually understood mechanisms described for other situations. But the procedure that helps us describe the apparently complex properties of the system should not be mistaken for the as yet undefined new set of original concepts, effective variables and simplifying models.

Future research

We have now entered a new phase of development along the lines discussed above. Both experimental and theoretical physicists (perhaps not as readily distinguishable in heavy-ion physics as in other fields of nuclear physics) have concentrated on examining systematically the relevance of the variables commonly used in the light of the first results. For example, can we define a temperature and find unambiguous ways to measure it in the systems under study? This new phase should bring us closer to selecting the relevant parameters.

Simultaneously, the need to move from inclusive measurements to more exclusive ones results from the gross features of reactions already observed. For instance, when one observes a so-called fragment of an argon projectile after collision with, say, a nickel nucleus, which other fragments or particles emitted during that process have gone unobserved; at which angles were they emitted; and how much of the initial energy did they carry?

These questions call for large multidetectors with an ability to detect and measure fragments in all directions. For each single collision, several hundreds of parameters must be read to collect all the information gathered by all the detectors. It would have been unrealistic to begin with such capabilities: how could intelligent use of 700-dimensional information be made? Now that the basic features of the reactions have been observed, such second-generation studies become not only legitimate but necessary. These new experiments have already started at GANIL, where >200 parameters can be read. For example, two-proton interferometry is used to study the size of the emitting hot zone of the so-called participant nucleons described earlier.

This method is very similar to the two-photon interferometry used to measure the size of stars.

We are ready to develop detectors and data-acquisition that can measure up to 700 parameters. I do not believe that this implies a blind jump into experimental megalomania. This programme is extrapolated from systems already operational and derives naturally from results obtained by physicists already involved in understanding the dynamics of the collisions.

Here, I have not given a full review of all the results obtained and all the ideas advanced to understand them, but have attempted, by the use of examples, to outline the means by which original properties of nuclear matter in the transition energy region opened by GANIL are being investigated. Indeed, this is the major challenge, yet the potential of high-intensity medium-energy heavy-ion beams for nuclear physics is, in my view, already well established by some of the results obtained. The use of projectile fragmentation to observe new neutron-rich isotopes through a triple-focusing magnetic analysis system allows us to extend the limits of known nuclei by one or two isotopes: ^{23}N, ^{29}Ne and ^{30}Ne were seen in the first attempt. The high excitation energy that can be transferred to nuclei has allowed the observation of high-lying excited states of simple configuration, probably multi-phonon excitation, of up to ~70 MeV. The cooperative creation of pions below the nucleon–nucleon threshold has been observed at the lowest energy ever.

Those are only some examples of the results obtained. In the coming years, several new machines at Catania, East Lansing (Michigan), Tokyo, Lanzhou and elsewhere will achieve the performance of GANIL. This interest reflects the novelty and scientific importance of a wide field now opened. One can anticipate that major developments in the understanding of the properties of nuclear matter will result from such a large-scale effort. The challenge for the GANIL physicists will be to play a leading role in the emergence of the new ideas.

ARTICLES

A quantum potential approach to the Wheeler delayed-choice experiment

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We apply the quantum potential approach to a 'delayed-choice' experiment considered by Wheeler and show that there is no need to conclude that the past has had no existence except insofar as it is recorded in the present.

WHEELER[1] has given a detailed description of several possible types of 'delayed-choice' experiment designed to show that, according to the quantum theory, the choice to measure one or another complementary variable at a particular time can apparently affect the course of events for considerable periods of time before such a decision is made. Wheeler interprets this using Bohr's principle of complementarity and his emphasis on the phenomena and experimental arrangement as forming an indivisible whole. He concludes that "no phenomenon is a phenomenon until it is an observed phenomenon", so that the "Universe does not 'exist out there' independently of all acts of observation. It is in some strange sense a participatory Universe. The present choice of the mode of observation ... should influence what we say about the past ..., the past is undefined and undefinable without the observation."

We discuss here the same question raised by Wheeler using a different approach that is completely consistent with quantum mechanics, namely the quantum potential approach[2,3]. (This question has also been treated by Bell[4] who came to a similar conclusion emphasizing different aspects of the problem.) Our approach agrees with Wheeler in concluding that this is a participatory Universe but differs in that the whole process, past and present, is treated as uniquely determined in itself, and not merely by what we are led to say about it through our observations. That is to say, there is a definable and defined overall process that includes both the observer-participator and the rest of the Universe in one undivided whole. Thus, we concur that the behaviour of the Universe is not independent of acts of observation, but also add that the past has had an existence independent of these acts.

Delayed-choice experiment

To bring out the difference between Wheeler's approach and our own through the quantum potential model, we will first re-examine one specific experiment described by Wheeler (the generalization to other more complex experiments is straightforward).

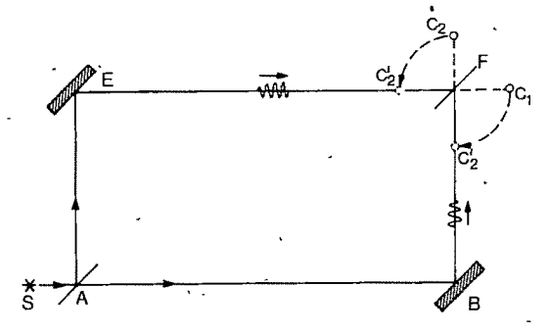


Fig. 1 Schematic view of the delayed-choice experiment for photons passing through a half-silvered mirror at A. Counters C_1 and C_2 can be moved into positions C_1' and C_2'

Two photodetectors are mounted so that they can be moved easily and quickly either to positions C_1 and C_2 or to the positions C_1' and C_2' (Fig. 1). Suppose a single quantum of electromagnetic energy passes through a half-silvered mirror at A Then, with the detectors in positions C_1 and C_2, the relative counting rates will determine the phase difference between the two routes. With the counters in positions C_1' and C_2', one can infer which route the photon takes. Thus, we can choose whether to perform either a two- or a single-beam interference experiment by the choice in the positions of the two detectors. Furthermore we can make this choice some time after the original photon has entered the apparatus but before it reaches F, thus giving rise to a delayed-choice effect. It is as if we could determine by our choice whether the photon has travelled along both routes or only one route.

It should be emphasized that Bohr has already pointed out that, in his view, the notion of the route that the photon or

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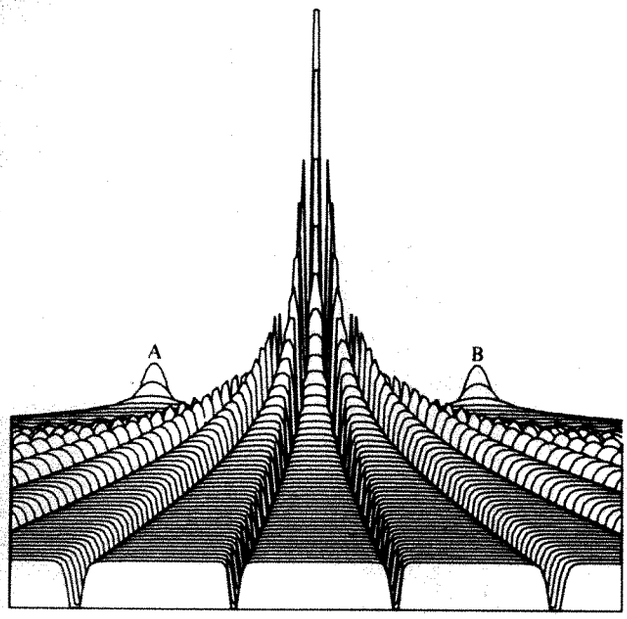


Fig. 2 Quantum potential for the two-slit experiment. The two slits are at A and B while the interference fringes appear in the foreground.

particle travels along has no meaning. Indeed, he[5] cites this very experiment, and in commenting on it, states, "it is just arguments of this kind which recall the impossibility of subdividing quantum phenomena and reveal the ambiguity in ascribing customary physical attributes to atomic objects". He further states that "all unambiguous use of space-time concepts in the description of atomic phenomena is confined to the recording of observations which refer to marks on a photographic plate or to similar practically irreversible amplification effects like the building of a water drop around an ion in a cloud-chamber".

The very idea of a 'micro-reality' (such as photons, electrons) that exists in some sense independently of the experimental context is thus denied. To be consistent with Bohr's point of view, the discussion should be solely in terms of what is observable and no attempt should be made to discuss paths of particles that would provide an explanation or account of how the experimental results obtained at successive times are connected.

Heisenberg[6] has a view similar to that of Bohr in many respects but different in that he argues that it is meaningful to talk about an orbit after an observation has taken place. In fact he writes "... the 'orbit' comes into being only when we observe it". For example, if an electron is observed at A and then later at B, one could say that the orbit went from A to B but the subsequent motion of the electron beyond B would be unpredictable. A further measurement at C might show that it actually was, but then the movement beyond C would be unpredictable. Thus, the concept of an orbit has a meaning only in the past of the act of observation.

Note, however, that there is no way to verify that the inferred trajectories were the actual orbits by additional experiments. Thus, it would be quite possible that the particle did not follow a straight line between A and B or, indeed, that there was no particle at all in the intermediary space and time. It would be equally consistent to assume that the particle appears only in the act in which it is made manifest. Thus, this discussion of the paths of particles has only the function of giving an explanation of how the phenomena are connected and is not capable of being verified experimentally.

Wheeler, of course, agrees basically with Bohr's point of view, but he has used Heisenberg's way of discussing this situation to show that the assumption that the past did exist independently of present observation leads to absurdities. This he regards as a support for his notion "that no phenomenon is a phenomenon until it is an observed phenomenon". In doing this, Wheeler does not use specific particle trajectories, but instead uses wave-packets to connect the present to the past. When the particle does not show interference effects, this packet corresponds approximately to a somewhat indistinctly determined trajectory, but when the experimental arrangement allows interference, the connection between the present and the past is made by the two packets acting together.

The approach of Bohr and Wheeler is possible in the sense that it is consistent. We feel, however, that the quantum potential approach, which we will outline below, treats the whole question in a more intelligible way. Here it is consistent to say that the past existed in a unique way that does not depend on what happens in the present. Nevertheless, some of the key points raised by Wheeler are retained but are comprehended differently.

Quantum potential approach

A consistent treatment of the electromagnetic field using the quantum potential approach is possible, and the outline of such a treatment has indeed already been elucidated[2,7]. However, the details are rather complicated and would perhaps obscure the main point. Therefore, we confine our attention in this paper to the interference of particles such as electrons and neutrons, for which we can apply the quantum potential approach in a straightforward way[8]. Indeed, delayed-choice experiments of the type suggested by Wheeler, but using neutrons, have actually been proposed by several authors[9,10].

We begin by first writing the wavefunction in standard polar form

$$\psi(\boldsymbol{r}, t) = R(\boldsymbol{r}, t) \exp\left[\frac{iS(\boldsymbol{r}, t)}{\hbar}\right] \tag{1}$$

From Schrödinger's equation it follows, writing $P = R^2$, that

$$\frac{\partial P}{\partial t} + \nabla \cdot \left(\frac{P \nabla S}{m}\right) = 0$$

and

$$\frac{\partial S}{\partial t} + \frac{(\nabla S)^2}{m} + V + Q = 0 \tag{2}$$

where Q is the quantum potential given by $Q = -(\hbar^2/2m)(\nabla^2 R/R)$ and the velocity is assumed to be given by $V = \nabla S/m$.

To interpret these two equations, we regard, say, a neutron as consisting both of a particle, which has a definite position and a definite momentum (and therefore a definite trajectory), and a field which is inseparable from the particle. The field is just the wavefunction as expressed through the two real fields R and S. It satisfies the Schrödinger equation which determines the time behaviour from the initial conditions.

Before proceeding further, we stress again that the particle and the field are indissolubly linked and we make no assumptions as to why or how this arises. Here we are simply attributing to the field those properties that are necessary to account for the quantum phenomena. To explain the origins of this field will require us to go deeper and make new physical assumptions of the type used by Bohm and Vigier[11] or by Cufaro Petroni and Vigier[12] or, indeed, some other appropriate ideas. It is not necessary to address such questions here as they are not relevant to the principles we wish to discuss. As a result, we use concepts such as 'wave-packet' that are applicable in the one-body case, but cannot be generalized in such a simple way to the many-body system which would require a field in the multi-dimensional configuration space. Therefore, such concepts are simply a convenient way of dealing with the phenomena and should not be taken as a definitive description of the nature of reality at this level.

Returning now to the main discussion, it is evident that equation (2) is similar to the Hamilton-Jacobi equation but it differs in that it has the additional quantum potential term Q, which leads to the suggestion that the particle follows an orbit different from the classical one. This difference is the result of

the quantum potential and is one of the major factors accounting for the quantum properties of matter.

To account for the rest of the quantum properties of matter, we must first consider the single valuedness of the wavefunction that leads to a kind of modified Bohr-Sommerfield quantum rule

$$\oint \nabla S \cdot \mathrm{d}x = nh \tag{3}$$

(The difference from the Bohr-Sommerfield quantum rule is that, in the former, S is obtained from the classical Hamilton-Jacobi equation, whereas here it is obtained from the quantum Hamilton-Jacobi equation (2).)

Finally, we have to interpret equation (1), which is evidently a conservation equation. We can do this consistently by assuming a statistical ensemble of similar systems all having the same wavefunction ψ. We further postulate that P is the probability distribution in this ensemble. This postulate is consistent in the sense that if it holds at the time $t = 0$ the conservation equation will guarantee that it will hold at all times.

Wigner[13] has objected to this approach by arguing that Q depends on R and that R refers only to the ensemble of systems. He concludes that it is unreasonable to have a theory in which the individual depends on the ensemble. Our answer to this criticism is that we do not primarily regard R as referring to the ensemble but rather as part of a field that belongs to the individual system as much as the particle does. The major effect of the field is to produce the force derivable from the quantum potential. But we have shown that the field can also describe the probability in an ensemble of systems that have the same field; that is, that have the same wavefunction except for a constant factor. This requires, of course, a further special assumption on the statistical ensembles. But the consistency of this assumption depends on the properties of the individual systems that make up the ensemble and, therefore, the individual does not depend on the ensemble but the ensemble depends on the individual in the way that is customary, for example, in classical statistical mechanics. The only new feature is that both quantum potential and probability are obtained from the same field R.

As an example, we give here the two-slit interference experiment[14]. We assume as an initial condition an ensemble of particles each having a plane wave that passes through a pair of slits. After passing through the slits, the two waves interfere and produce a very complex quantum potential shown in Fig. 2. At some distance from the slits, the form of this potential is that of a set of plateaus separated by a set of deep valleys. Figure 3 shows the paths of the particles in the ensemble. Clearly the net effect of the potential is to bunch the trajectories so as to produce the pattern of the well-known interference fringes in the statistical ensemble.

To understand this result, it is crucial to note that the quantum potential is not changed when the wavefunction is multiplied by an arbitrary constant, that is, that the quantum potential is determined only by the form of the wavefunction and not by its magnitude. This means that the effects of environmental features such as the slits can be felt by the particles at long distances. This is not in itself a form of non-locality because the effect is propagated locally via the wavefunction to which Schrödinger's equation applies. However, it represents the possibility that the behaviour of the particle cannot be separated from the whole context of the environment which is involved, for example, through a determination of the experimental conditions. We also add here that through such a dependence on the environment, the particle does not necessarily travel in a straight line at constant speed in what appears to be empty space. Rather, as shown in Fig. 3, it may have a complicated and irregular motion.

Let us now proceed to use this approach to account for Wheeler's[1] experiment. A detailed treatment using the quantum potential would be more complex than that required for the two-slit experiment described above, but the basic principles involved are the same. Therefore, we can use the approach to account in a general way for the experiment shown in Fig. 1.

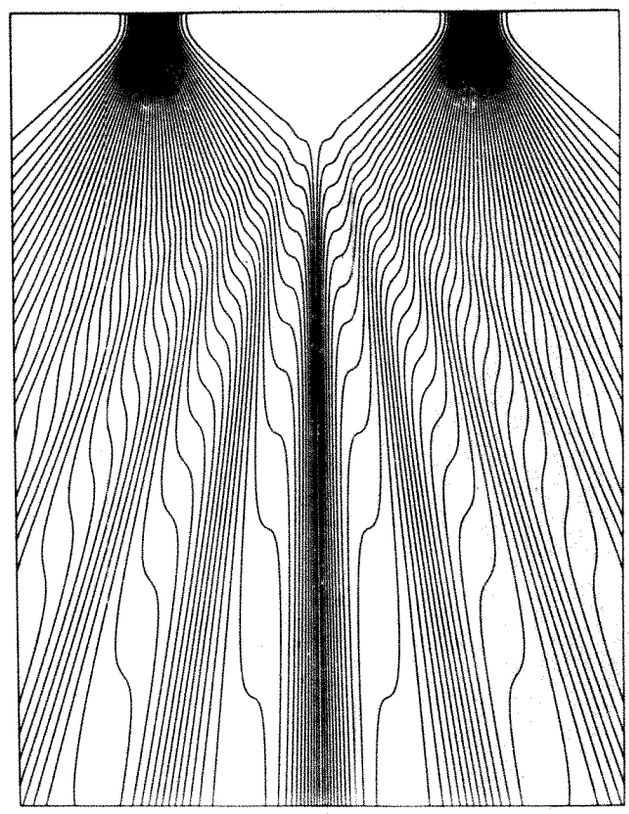


Fig. 3 Particle trajectories for particle passing through the two slits at the top and forming interference fringes at the bottom.

Each individual member of the ensemble goes one way or the other. That is, one of the wave-packets contains the particle and the other does not. As explained in our discussion of the measurement[2], if there is a particle detector for each packet, then the one actually containing the particle will be the one that registers. If, however, we allow interference to occur before we detect the particles, there will be a region of complex fluctuating quantum potential where the wave-packets overlap. When the particles enter this region, the trajectories will no longer be straight lines but rather will bunch into interference fringes in a way similar to what happens in the two-slit experiment. If the particles are detected, then the ensemble will show the interference pattern[15].

In no case is the behaviour of the system in the past modified. Each individual system will develop two wave-packets after passing through the half-reflecting mirror A (see Fig. 1). In each case the particle will go one way or the other, while the wave-packet is both transmitted and reflected. Indeed, which wave-packet the particle enters at A is determined by its (unknown) initial position in the incoming wave-packet (as can be shown by considering the scattering of a wave-packet from a square potential barrier[16]). No matter what is done later on in the experiment, for example, moving the pair of detectors from one position to another, this is not altered.

Until the two wave-packets travelling the two routes to F overlap, each particle follows a trajectory determined by a quantum potential independent of the existence of the alternative route. Detection at this stage by C_1' and C_2' simply gives information concerning which route the particle took. When the wave-packets overlap, the resulting quantum potential is determined by the physical conditions along both routes. In this region the trajectory of the particle is modified in such a way that the ensemble of trajectories produces an 'interference pattern' even though the particle has only travelled along one path. However, what we do with the detectors can affect the quantum potential that each particle experiences between the mirror and

the detector and in this way we obtain either a simple distribution of particles between alternative wave-packets or an interference pattern.

Conclusion

Here, we have shown that, using the quantum potential approach, it is possible to give a simple and intelligible account of a typical delayed-choice experiment. It is not necessary to suppose that the behaviour that we ascribe to a system in the past will depend in any way at all on what is done to it later. As we stated earlier, we feel this is more satisfactory than the approaches either of Heisenberg, who says that the orbit comes into being only when we observe it, or of Wheeler, who implies that the past has had no existence except in as far as it is recorded in the present.

Of course, to achieve this result we had to assume the particle trajectories that are not observable in detail, at least in any domain in which quantum mechanics is valid[11]. In this respect, our approach is similar to that of Heisenberg, who makes statements about the past that are also unverifiable by observation to give an account of how certain phenomena at different times may be related. However, by bringing in the quantum potential, we are able to understand the characteristic results of the quantum theory; for example, interference[14], barrier penetration[16], the Einstein-Podolsky-Rosen situation[17], the Schrödinger cat paradox and the measurement problem[2].

In this regard, our trajectories have essentially the same purpose as the trajectories of Heisenberg (although we think that our trajectories achieve much more). We do, however, find it difficult to understand why many authors reject the quantum potential approach on the grounds that it provides no new experimental consequences beyond those of the usual theory, while they are ready to engage in discussions of what may happen between measurements, discussions that equally have no further experimental consequences. Rather we agree with Wheeler who says "We search here not for new experiments or new predictions but for new insights". In this connection, we emphasize also that we are not proposing the quantum potential as a definitive explanation of the nature of reality. We do appreciate that the quantum potential is an arbitrary assumption, but think that the use of this assumption helps to understand the meaning of the quantum theory in an intuitively clear way. Many questions that are unclear in the usual interpretation (for example, the Einstein-Podolsky-Rosen experiment, the experiment discussed in this paper) became obvious in meaning when put in terms of the quantum potential.

Moreover, this interpretation gives results entirely compatible with Bohr's views on wholeness and complementarity. We regard our approach as complementing Bohr's instead of opposing it. To support this, we point out that our approach is generally more consistent with the spirit of the interpretations of Bohr and Wheeler than it is with the one adopted by Heisenberg. In other words, if one does want to use some sort of trajectory to explain the meaning of quantum mechanics, then the quantum potential is necessary if this is to be consistent and fully compatible with the experimental meaning of Bohr's interpretation.

Finally, as stated in the introduction, we agree with Wheeler that this is a participatory Universe. However, we suggest that this comes about because the observer-participator in, for example, determining the environmental context needed to observe a particle, creates a situation in which the fundamental mode of behaviour of the particle is strongly affected by this context (for example, the slit system can bring about a very irregular trajectory even when the particle is far away from it). Thus, the Universe cannot be analysed into elements that are essentially independent when they are far apart. In this way, the participation of the observer in the rest of the Universe (and of the rest of the Universe in the observer) has a far more profound significance than it has in classical physics. Through the quantum potential a certain insight into this significance has been made possible.

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Role of lithospheric strength heterogeneities in the tectonics of Tibet and neighbouring regions

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Unlike rigid oceanic plates, continents show diffuse deformation, for example the Tibetan plateau that has accommodated much of the convergence of India with Asia. We interpret the Tertiary deformation of Asia as the three-dimensional strain of a non-linear, viscous continental lithosphere. Lateral strength contrasts modify this deformation; stress concentration around the relatively strong Tarim basin gives rise to local crustal thickening in the Tien Shan to the north and to strike slip in the Altyn Tagh along its southern margin.

THE region accommodating the convergence between India and the Asian landmass is one of the clearest examples of non-plate-like behaviour of the continental lithosphere[1]. This deformation has been explained in several different ways. One interpretation involves the thrusting of India under the entire Tibetan plateau[2-4] but, although there is some present-day underthrusting of Indian crust underneath Tibet, recent seismic reflection[5,6] and palaeomagnetic[7,8] studies suggest that most of



Fig. 1 Locations of principal features referred to in this study. The 2,000- and 4,000-m contours are shown, with land above 4,000 m stippled; the position of the Altyn Tagh fault is taken from the map of Tapponnier and Molnar[9], to which reference should be made for positions for other major faults in the region.

the post-Eocene convergence between India and Asia has been taken up by shortening in Asia.

Continuum models for this intraplate deformation fall into two groups. The first emphasizes the role of strike-slip faulting in the present-day tectonics of Asia and interprets the deformation in terms of the predictions of plane-strain slip-line calculations[9], or by analogy with unscaled plane-strain plasticine models[10]. The second group represents the continental lithosphere by a thin non-linear viscous shell that can be analysed quantitatively as to its predictions about crustal thickness, strain and strain-rate fields and other parameters of finite deformation[11,12]. This approach recognizes the three-dimensional nature of the Tertiary deformation in Asia and accounts for the relatively greater importance of lateral motions at the present day in terms of the buoyancy forces generated by the elevation contrasts produced during the thickening of the Tibetan crust[9,12,13]. What these calculations have not contained so far is an analysis of the effect of a large-scale heterogeneity that is represented, for example, by the relatively aseismic and apparently undeforming Tarim basin[14] (Figs 1, 6). Here we pursue the implications of the thin sheet approach to continental tectonics[11-13,15,16] by assessing the influence of heterogeneities in lithospheric strength on the tectonics of Asia.

Mathematical model

The thin-sheet model has been described in detail elsewhere[12]; the lithosphere is represented by a thin layer of non-newtonian viscous fluid; for mathematical tractability we assume that horizontal gradients of crustal thickness and topography are small and use a depth-averaged constitutive relation of the form

$$\bar{\tau}_{ij} = 2\eta\dot{\varepsilon}_{ij} \tag{1}$$

where $\bar{\tau}_{ij}$ is the depth-averaged deviatoric stress component, $\dot{\varepsilon}_{ij}$ is the corresponding strain rate and η is the depth-averaged effective viscosity

$$\eta = \frac{B}{2}\dot{E}^{(1/n-1)} \tag{2}$$

where $\dot{E}$ is the second invariant of the strain-rate tensor and B is a depth-averaged strength coefficient. In the experiments described here, $n > 1$, so the effective viscosity is a function of position in the solution region.

The horizontal velocity components are assumed to be indepdent of depth in the layer and the solution is constrained by boundary conditions on the stress or velocity as shown in Fig. 2. The force balance equations for creeping flow are solved by finite element techniques.

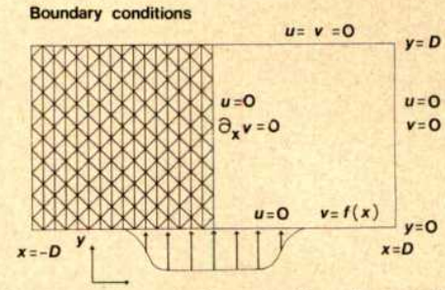


Fig. 2 Initial geometry, coordinates and boundary conditions for the numerical experiments. The x-y plane is horizontal, the line $x = 0$ is an axis of symmetry and the region is divided into triangluar elements as shown in the left. Velocities are zero on the boundary, except on the part of it that initially concides with the x-axis, where the region $-D/2 < x < D/2$ moves in the y-direction, representing the edge of an indenter. The motion of this boundary deforms the sheet (see Fig. 3). The velocity function that describes the indenter is: $v(x) = 0$ for $D/2 < |x| < D$, $v(x) = u_0$ for $0 < |x| < D/4$ and $v(x) = u_0 \cos^2(2\pi(x/D - 1/4))$ for $D/4 < |x| < D/2$. For convenience in the text, the positive y-direction is referred to as north, the positive x-direction as east, etc.

The rate of thickening or thinning of the crust is determined locally by the horizontal divergence of the calculated velocity field

$$\frac{1}{S}\frac{\partial S}{\partial t} = -\nabla \cdot \boldsymbol{u} \tag{3}$$

where S is the thickness of the crust and $\boldsymbol{u}$ is the velocity. Under the influence of gravity the thickened crust tends to spread horizontally, introducing a force proportional to the gradient of the square of crustal thickness into the dimensionless stress balance. The constant of proportionality is called the Argand number (Ar) by England and McKenzie[11] and is a measure of the magnitude of this gravitational spreading force relative to the viscous resisting forces.

The constant B(equation (2)) contains the depth averaged temperature dependence of the effective viscosity (see appendix of ref. 11) and hence is dependent on the geotherm, the depth to the Moho and lithological variations. Previous calculations[11-13] have assumed for simplicity that B is a constant. However, it has been suggested[14,16,17] that regions such as the Tarim basin are stronger and more resistant to deformation than the surrounding continental lithosphere. In the experiments described here, we investigate the consequences of including a small region of anomalously high strength in an otherwise

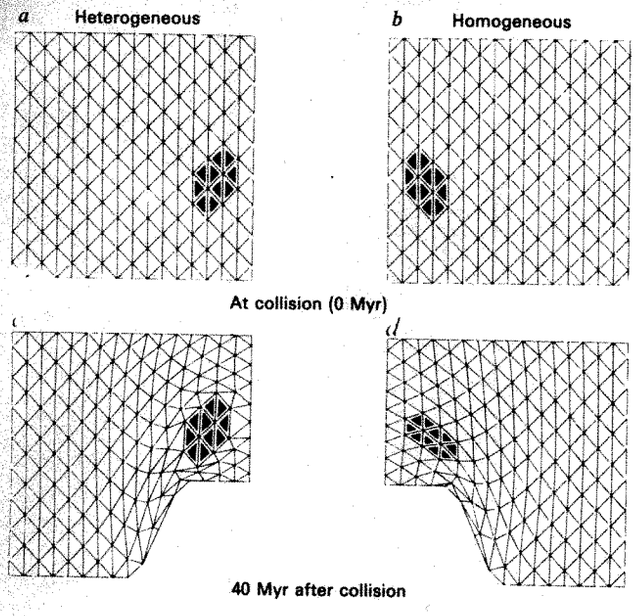


Fig. 3 *a*, *b*, Initial and *c*, *d*, final ($t = 40$ Myr) mesh configurations for the left and right halves, respectively, of the two symmetrical solutions for: *a* and *c*, the case where the effective viscosity coefficient (*B*, equation (2)) is increased by a factor of 10 in the shaded elements and *b* and *d*, the laterally homogeneous case, with the corresponding elements shaded for comparison with *a* and *c*. For both experiments $n = 3$ and $\mathrm{Ar} = 3$.

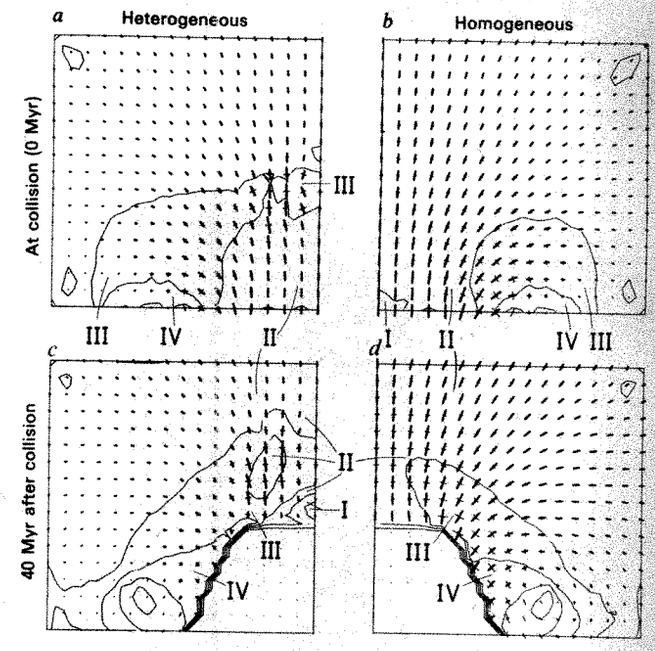


Fig. 4 Principal deviatoric stress axis orientation and magnitude for *a*, *b*, $t = 0$ and *c*, *d*, $t = 40$ Myr. The length of each arrow is proportional to the magnitude of the stress and the longest arrow represents a stress of *a*, 63.9 MPa, *b*, 43.5 MPa, *c*, 95.1 MPa and *d*, 44.2 MPa. Superimposed are contours of the parameter $\tan^{-1}(\dot{\varepsilon}_2/\dot{\varepsilon}_1)$, where $\dot{\varepsilon}_1$ and $\dot{\varepsilon}_2$ are principal horizontal strain rates. These contours divide the box into regions that show a consistent style of faulting (see text for II and III; I is a region of pure thrust faulting and IV is a region of normal plus strike-slip faulting).

laterally homogeneous layer; the constant B is increased by a factor of 10 in the shaded region of Fig. 3*a*. As a rough estimate of the effect of this modification, it follows from equations (1) and (2) that if the stress field is constant and the strain-rate field is uniaxial, then

$$\dot{E} \propto B^{-n} \tag{4}$$

For $n = 3$ the strain rates in the anomalously strong region would be only 10^{-3} of those in the surrounding regions. In fact, because of changes to the stress field (Fig. 4) and the triaxial nature of the strain-rate field, the contrast is in the range $0.5\text{–}1.0 \times 10^{-2}$.

Because of computation limitations, calculations were performed with an axis of symmetry along the perpendicular bisector of the indenter (Fig. 2). For the calculation shown in the left halves of Figs 3–6 there are two anomalously strong regions disposed symmetrically about the axis and having approximately the same positions relative to the indenter as do the Tarim and Qaidam basins to the arc of the Himalayan mountains (Fig. 1). We contrast these results with the calculation shown in the right halves of Figs 3–6 for the symmetrical laterally homogeneous case. By presenting in this format half of each solution, we mean to highlight the differences in tectonic style between western Tibet and Xinjiang province on the one hand and eastern Tibet and Gansu province on the other.

Results

We first describe the results of a calulation with $n = 3$ and $\mathrm{Ar} = 3$, as these parameters give reasonable agreement with observation, but also note that effective values of $n \leq 10$ and $\mathrm{Ar} \leq 10$ can give reasonable results (Fig. 6). Figure 3 shows the initial and final configuration ($t = 0$ and 40 Myr) of the finite element mesh for the two calculations. The vertices of each triangle are fixed to and carried along by the deforming medium. The strong region is barely deformed in Fig. 3*c* and has been translated and rotated practically as a rigid body; this behaviour should be compared with that of the corresponding region in the laterally homogeneous case (Fig. 3*b*, *d*).

The deviatoric stress fields in Fig. 4 show that the stresses in the strong region are amplified by the more rapid creep rates in the weaker material next to it (compare with ref. 18). At $t = 0$ the maximum stress in Fig. 4*a* is 47% greater than that of the corresponding laterally homogeneous case (Fig. 4*b*) and is found in the strong region rather than on the corner of the indenter. After 40 Myr (Fig. 4*c*), the maximum stress in the basin has increased by a further 49%. There is also a slight rotation of the principal stress axes near the viscosity heterogeneity.

Superimposed on the stresses of Fig. 4 are contours separating regions with a different style of surface faulting, assuming that the upper brittle crust deforms with the same strain rates as the underlying ductile layer. In region II, both strike-slip and thrust faulting must occur, although there is a greater rate of moment release from the thrust faulting component. In region III, the same two fault styles would be observed, but the rate of moment release from the strike-slip component is greater in this region.

For both calculations (with the strong region, Fig. 4*a*, *c*, and the homogeneous case, Fig. 4*b*, *d*) region III increases in lateral extent as the indenter moves further north, because as the crust is thickened the gravitational forces tend to resist further crustal thickening (thrust faulting) and an increasing percentage of the deformation is accommodated by strike-slip faulting[11,13]. When the strong region is present, strike-slip deformation is predominant on its east and west margins throughout the deformation (Fig. 4*a*, *c*). At the centroid of the element next to the southern corner of the strength heterogeneity, the rate of moment release for thrust faulting is 3.7 times that for strike-slip faulting at $t = 0$, but at $t = 40$ Myr the strike-slip component is greater than that of the thrusting by 46%. For the same material point in the homogeneous calculation, thrust faulting is dominant throughout, although the ratio of thrust moment to strike-slip moment falls from 3.6 to 1.2 during the 40 Myr. The region next to the south-east margin of the basin, which at 40 Myr shows the maximum strain rates and strongest crustal thickness gradients (Fig. 6*a*), is thus found to be undergoing predominantly strike-slip deformation (Fig. 4*c*) during the last 10 Myr of the calculation. This region is in the same position relative to the basin as is the Altyn Tagh fault system relative to the Tarim basin (Fig. 1).

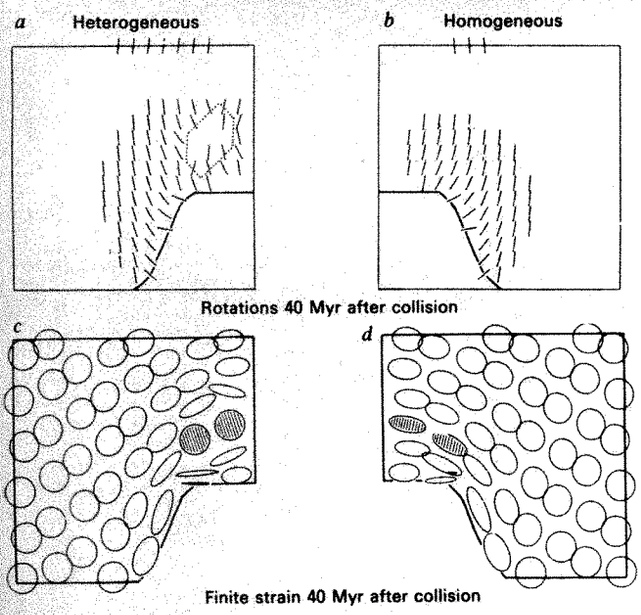


Fig. 5 *a*, *b*, Finite rotations (half of the time-integrated vorticity) at $t = 40$ Myr; *c*, *d*, finite deformation ellipses at $t = 40$ Myr; for *a*, *c*, the case with the strong region and *b*, *d*, the laterally homogeneous case. For *a* and *b* the angle between the small bar and the *y*-axis is the magnitude of the rotation; only rotations $>5°$ are shown. For *c* and *d* the ellipses show the finite strain, after 40 Myr, of initially circular horizontal material elements. The shaded ellipses in *c* lie in the basin and the corresponding ellipses are shaded in *d*.

Figure 5 shows the finite rotation and deformation in the two cases. Only rotations $>5°$ are shown so the strong region in Fig. 5*a* is defined clearly by the small magnitude ($\sim 5°$) and opposite sign of its rigid-body rotation, compared with the anticlockwise rotation of 30–40° of the material to its north-west and south-east. The rotations on the northwestern margin of the basin are significantly greater than they would be in the absence of the viscosity heterogeneity (Fig. 5*b*) and significant clockwise rotations ($\sim 20°$) are also found on its north-east and south-west margins; otherwise the distribution and magnitude of rotation is much the same as in the homogeneous case.

Figure 5*c*, *d* is an alternative presentation of the data shown in Fig. 3*c*, *d*. Each ellipse shows the finite deformation undergone by a particular triangular element. The strain ellipses from the strong region (shaded) show ratios of major to minor semiaxes of 1.010 and 1.007 respectively, compared with the ratios for the corresponding ellipses in the homogeneous case of 2.39 and 2.35.

Figure 6*a*, *b* illustrates the elevations calculated for this model at 40 Myr after collision, compared with those observed in Asia at the present day. Because of the assumption of Airy isostasy in these calculations, contrasts in elevation are proportional to contrasts in crustal thickness. The contours of Fig. 6*a*, *b* show the most obvious effect of the viscosity anomaly; crustal thickening is inhibited within the strong region, whereas it is enhanced both to the north and south of the anomaly. Assuming isostatic balance, the topography would show a basin in the area corresponding to the anomalously strong region; after 40 Myr (Fig. 6*a*) the deepest part of the basin still has a 35-km-thick crust. To the south of the basin there is a steep topographical gradient where the crustal thickness increases by >20 km as the indenter is approached and near the corner of the indenter the crustal thickness is more than doubled to 75 km. Immediately north of the indenter on the axis the crustal thickness is ~64 km, some 6 km thicker than for the laterally homogeneous calculation (Fig. 6*b*). To the north of the basin there is another local maximum of 55–60-km-thick crust. This local maximum bears the same geographical relation to the basin as does the Tien Shan mountain range to the Tarim basin (Fig. 1). In comparison,

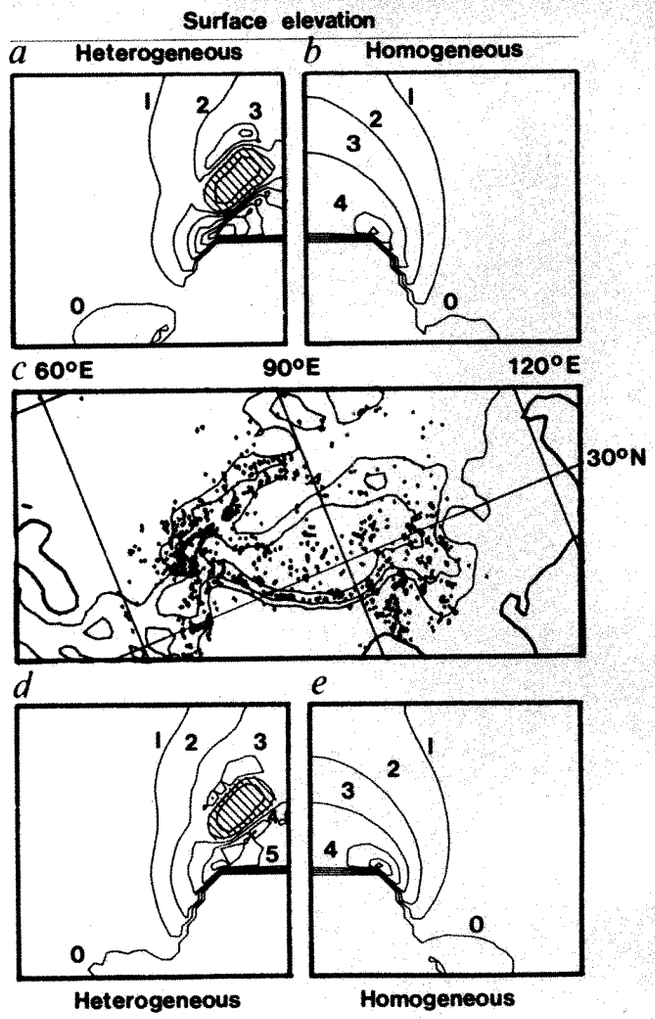


Fig. 6 Contours of elevation 40 Myr after collision for *a*, *d*, heterogeneous and *b*, *e*, homogeneous thin sheets, compared with *c*, contours of elevation in Asia. We assume an initial crustal thickness of 35 km, and changes in elevation $(e - e_0)$ are related to changes in crustal thickness $(S - S_0)$ by $(e - e_0) = (S - S_0)(1 - \rho_c/\rho_m)$. For $\rho_c = 2{,}800\ \mathrm{kg\ m^{-3}}$ and $\rho_m = 3{,}330\ \mathrm{kg\ m^{-3}}$ (ref. 22) difference in elevation of 1 km is equivalent to a difference in crustal thickness of 6.3 km in the model. In *a* and *b*, $n = 3$ and $\mathrm{Ar} = 3$; the shaded region in *a* has a contrast in *B* of a factor of 10 relative to its surroundings. In *d* and *e*, $n = 10$ and $\mathrm{Ar} = 10$ and the shaded region in *d* has *B* greater by a factor of 2 than its surroundings. Contours of topography in *c* are taken from Lee's[23] $1° \times 1°$ averages, smoothed with a gaussian filter of width 2°; contouring intervals are 1 km. Dots indicate earthquakes in the interval 1964–1980, shallower than 100 km and reported to the International Seismological Centre by more than 25 stations. Note the relative quiescence in the seismicity of the Tarim basin.

the laterally homogeneous case (Fig. 6*b*) shows a monotonic decrease in crustal thickness northwards of the indenter.

A second important result of these calculations is illustrated in Fig. 6*d*, *e*, which shows the results of a calculation similar to that illustrated in Fig. 6*a*, *b* except that $n = 10$, $\mathrm{Ar} = 10$ and the contrast in *B* for the strong region in the heterogeneous calculation is 2 (giving approximately the same contrast for uniaxial strain rates (equation (4)) as does a contrast of 10 when $n = 3$). With the minor difference that the peak crustal thickness near the corner of the indenter is decreased, the distribution of the topography in Fig. 6*c* resembles closely that of the topography in Fig. 6*a*. The resemblance emphasizes that the role of

heterogeneous inclusions in determining the scale lengths of continental deformation may be as important as that of the deformation mechanisms.

It would be difficult to distinguish, on the basis of the calculations shown in Fig. 6, between a model with a power-law exponent of 3, appropriate to a lithosphere governed by the deformation of olivine, and one with an effective power law exponent of 10, perhaps reflecting a significant contribution to the vertically averaged rheology from friction on faults.

Discussion and conclusions

The magnitude of the strength contrast required to produce the features described here is surprisingly small; for $n=3$ a factor of 10 is more than adequate, and for larger values of n a smaller strength contrast would be sufficient. Assuming that the strongest part of the lithosphere is the uppermost mantle, we may use the power-law rheology given for olivine by Goetze[19], and the expression of England[20] for the vertically averaged strength of a lithosphere governed by such a rheology, to estimate the drop in temperature at the Moho that is implied by increasing B (equation(2)) by a factor of 10. For $n=3$ and Moho temperatures in the range 600–800 °C, the implied temperature decrease is ~100 °C, which is consistent with the suggestion of Molnar and Tapponnier[14] that strength contrasts in the Asian lithosphere may be related to differences in geothermal regime in the region. However, we must caution against too literal interpretation of these calculations in terms of a specific rheological model, in view of the many uncertainties involved in applying the results of laboratory measurements to the deformation of the continental lithosphere.

We have shown that the strain-rate fields and distribution of deformation in a thin viscous layer acted on by a rigid indenter are modified strongly by the presence of a strength heterogeneity in the layer. The most important features predicted by the calculations here are the preservation of a relatively low-lying region that is surrounded by thicker crust and higher topography, relatively small strain rates and total strain within this basin and a narrow zone of steep crustal thickness gradients and predominantly strike-slip deformation separating the basin from the plateau of much thicker crust to its south. It seems probable that if the symmetry requirement were dropped and the calculation were performed with a single heterogeneity corresponding to the Tarim basin, there would be a net eastward mass flux across the line that bisects the indenter and enhanced strike-slip motion at the southern edge of the high strength inclusion. The above features are all consistent with the interpretation of the Tarim basin as a relatively strong lithospheric block emplaced in an otherwise laterally homogeneous thin viscous layer; in this context the model explains the formation of the Tien Shan range and the Altyn Tagh strike-slip fault system. It seems likely that lithospheric strength heterogeneities (for example, the stable region of central Iran[21]) have similar roles in other collision orogenies.

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Abrogation of metastatic properties of tumour cells by *de novo* expression of H–2K antigens following *H–2* gene transfection

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H-2 gene transfection was used to restore expression of H-2K antigens in metastatic and non-metastatic subclones of a murine fibrosarcoma that lack their major histocompatibility complex-encoded H-2K antigens. De novo *expression of H-2K reduced tumorigenicity and abolished the formation of metastasis in syngeneic mice. Expression of H-2K may lead to effective recognition of the disseminating tumour cells by the host immune system.*

THE major histocompatibility complex (MHC) of the mouse (*H-2*) encodes a group of cell-surface antigens that are involved in various pathways of the immune response. Thus, cytotoxic T lymphocytes (CTL) can recognize cells infected with virus or altered by neoplastic transformation only in association with an H-2 antigen (reviewed in ref. 1). It depends on the antigen as to which of the three known H-2 class I antigens, K, D or L, serves as a restriction element for CTL[1]. Hence, it is conceivable that the control of tumour growth by the immune system would depend not only on the existence of tumour-associated cell-surface antigens, but also on the expression of MHC-encoded antigens on the tumour cells. Therefore, modulation of H-2 antigens of tumour cells may alter their growth properties because of alterations in the immune response of the host to the neoplastic cells.

Recent studies have demonstrated that the metastatic properties of certain mouse tumours are correlated with the relative expression of class I MHC antigens[2–9]. A notable example is the methylcholanthrene-induced T10 sarcoma of ($H\text{-}2^b \times H\text{-}2^k$) F_1 origin. Two distinct sets of T10 clones were isolated, differing not only in their metastatic potential but also in their expression of class I MHC antigens[4,5]. According to their genotype, the tumour cells should express four different H-2 molecules, namely K^b, D^b, K^k and D^k. However, both sets of clones were

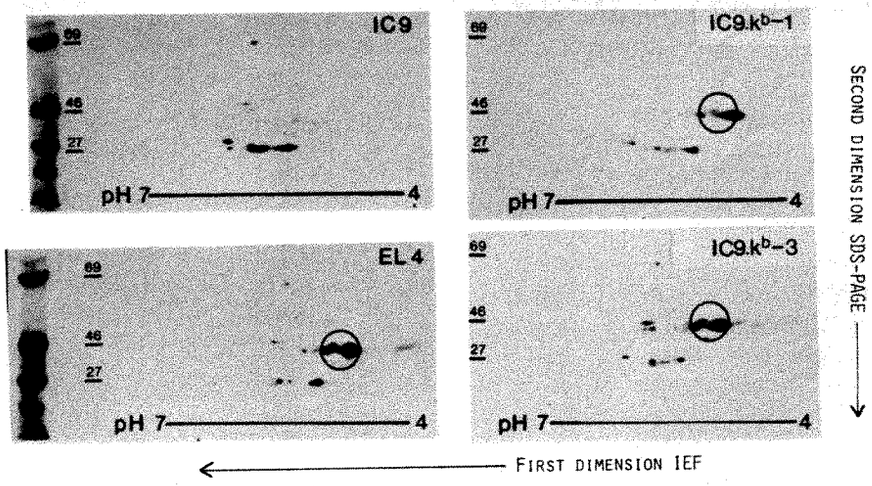


Fig. 1 Two-dimensional isoelectric focusing (IEF) gel of H-2K region associated antigens. $1-3\times10^6$ tumour cells (IC9, K^b-transfected IC9 and C57BL/6-derived EL4 lymphoma) were pulse labelled for 6 min at 37 °C in the presence of 200 μCi ^{35}S-methionine and lysed in ice-cold Tris buffer containing 1% Triton X-100. Cell lysates were preabsorbed with Protein A-Sepharose 4B for 2 h. H-2 proteins were immunoprecipitated using a mixture of monoclonal anti-H-2K^b antibodies K10-56, K9-136, K7-309, K9-178 (ref. 13) (20× concentrated culture supernatant) followed by Protein A-Sepharose for 5 h. Two-dimensional IEF gels were performed according to O'Farrell[30]. Second-dimension gels were 10% acrylamide. Gels were processed with 2,5-diphenyloxazole dried, and exposed for 2 weeks using Kodak XAR-5 film. β_2-Microglobulin, relative molecular mass 12,000, runs through the second-dimension gel and is therefore not visible on autoradiograms. Circles indicate positions of H-2K^b proteins. EL4 yields the same K^b spots as spleen cells from the C57BL/6 mice used for breeding of the C3H×C57BL/6 mice used in this study (not shown).

found to lack the K end-controlled K^b and K^k antigens. The non-metastatic clones express only the D^b molecule whereas the metastatic clones express both the D^b and D^K molecules (refs 6, 7; see also Table 1). Further studies suggested that the D^k molecule contributes to the metastatic potential of these clones[8].

Here, we seek to determine whether expression of H-2 molecules encoded by the K end of the H-2 complex would influence metastatic spread and tumorigenicity of the T10 fibrosarcoma clones. To elucidate this problem we transfected clones of the T10 sarcoma with cloned *H-2K* genes and tested the malignant properties of the transfected tumour cells in syngeneic mice.

Restoration of H-2 expression

For transfection with *H-2* genes a representative non-metastatic tumour clone (designated IC9) and a metastatic clone (IE7) were selected from the two sets of T10 fibrosarcoma clones. Cells were co-transfected with *H-2K^b* (ref. 10) or *H-2K^k* plasmid DNA[11] and plasmid pAG60 containing the neomycin resistant gene (*neo^r*) (ref. 12) and subsequently selected in medium containing Geneticin, which selects for cells that have productively integrated the *neo^r* gene. We identified colonies expressing transfected *H-2* genes using specific monoclonal H-2 antibodies at a frequency of ~10-30%. After cloning of positive colonies,

Table 1 H-2 phenotype of transfected IE7 and IC9 tumour clones

Cell line	Transfected genes	H-2 antibody (c.p.m. obtained) Anti-D^b	Anti-K^b	Anti-D^k	Anti-K^k	H-2 phenotype
IE transfected lines						
IE7	—	27,849	580	6,438	697	D^b, D^k
IE7.neo^r-1	*neo^r*	27,069	270	6,072	294	D^b, D^k
IE7.neo^r-2	*neo^r*	20,051	768	7,188	524	D^b, D^k
IE7.K^k-1	*neo^r*, *K^k*	16,461	949	4,964	5,785	D^b, D^k, K^k
IE7.K^k-2	*neo^r*, *K^k*	21,176	683	5,382	7,413	D^b, D^k, K^k
IE7.K^{k}K^b-1	*neo^r*, *K^b*, *K^k*	16,160	13,664	5,539	6,992	D^b, D^k, K^b, K^k
IE7.K^b-1	*neo^r*, *K^b*	10,447	9,350	3,433	851	D^b, D^k, K^b
IE7.K^b-2	*neo^r*, *K^b*	27,780	14,877	6,505	419	D^b, D^k, K^b
IE7.K^b-3	*neo^r*, *K^b*	17,350	12,631	4,817	913	D^b, D^k, K^b
IC9 transfected lines						
IC9	—	28,259	453	397	507	D^b
IC9.neo^r-1	*neo^r*	16,758	252	187	254	D^b
IC9.K^b-1	*neo^r*, *K^b*	10,629	15,944	848	653	D^b, K^b
IC9.K^b-2	*neo^r*, *K^b*	18,118	14,496	414	478	D^b, K^b
IC9.K^b-3	*neo^r*, *K^b*	21,806	28,420	719	718	D^b, K^b
IC9.K^k-1	*neo^r*, *K^k*	17,023	514	745	4.282	D^b, K^k
IC9.K^k-2	*neo^r*, *K^k*	19,316	471	516	10.305	D^b, K^k

Transfection was performed according to Wigler *et al.*[26] with some modifications. pBR322 plasmids containing the 10.5-kilobase (kb) *Eco*RI fragment of the *H-2K^b* and *H-2K^k* genes, respectively, were generously provided by Drs R. Flavell[10] and B. Arnold[11]. The *H-2K^k* gene (cos10.7) originally came from Dr M. Steinmetz. The plasmid pAG60 (ref. 12) containing *neo^r* was obtained from Dr B. Dobberstein. IC9 and IE7 cells were seeded at a density of ~10^6 cells per 10-cm Petri dish. Various mixtures of *H-2* DNA (4 μg), *neo^r* (0.4 μg) and salmon-sperm DNA (20 μg), precipitated with calcium phosphate, were added. After 5 h at 37 °C the medium was replaced by 5 ml 15% glycerol or a 41% solution of polyethylene glycol 4000 for 2 min. After overnight incubation with medium, the cells were collected with EDTA buffer and distributed into two COSTAR 3524 multiwell plates in selection medium containing 0.7 mg ml^{-1} Geniticin (G418, Gibco). Growing colonies (1-10 per 10^6 input cells) were expanded, tested for H-2 expression by cytofluorography or radioimmunoassay and cloned by limiting dilution. (Without glycerol or polyethylene glycol almost no transfectants were obtained.) The table shows a representative cellular radioimmunoassay which was performed as described previously[27]. Briefly, 10^6 cells were incubated in microtitre plates with specific anti-H-2 monoclonal antibody (anti-D^b, hybridoma B22-249, ref. 28; anti-K^b, K10-56, ref. 13; anti-D^k, 15-5-5S and anti-K^k, 16-3-22, ref. 13). After washing, ^{125}I-labelled Protein A was added and the amount of bound radioactivity determined. Values represent the mean of triplicate wells. The standard deviation ranged between 5 and 10% (not shown). Background with irrelevant antibodies was always <1,000 c.p.m. (not shown). The c.p.m. obtained with different H-2 antibodies cannot be compared because the affinity and amount of specific antibody in the hybridoma supernatant are probably different.

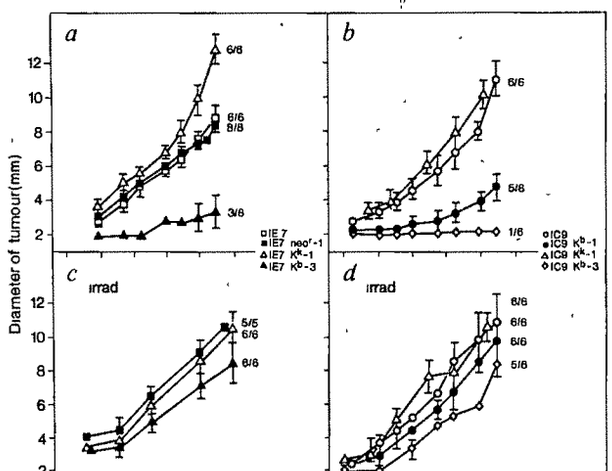


Fig. 2 Growth of parental and *H-2K*-transfected IE7 and IC9 cells. 10^4 *neo*^{r} and *H-2K*-transfected IE7 (*a*, *b*) or IC9 (*c*, *d*) were injected i.f.p. into intact (C3H.eB×C57BL/6)F_1 mice (*a*, *c*) and into sublethally irradiated (550 rad) F_1 mice (*b*, *d*). Tumour size was recorded using a caliper. The growth curves represent the mean tumour size of all mice inoculated in each experimental group ±s.e.m., including the diameter of legs of mice which rejected the inoculated cells. Mean diameter of legs of control non-inoculated mice is 2 mm. The numbers designated above each growth curve represent the proportion of mice that developed a tumour of the entire experimental group. *a*, *b*, □, IE7; ■ IE7.neo^r-1; △, IE7.K^k-1; ▲, IE7.K^b-3. *c*, *d*, ○, IC9; ●, IC9.K^b-1; △ IC9.K^k; ◇ IC9.K^b-3.

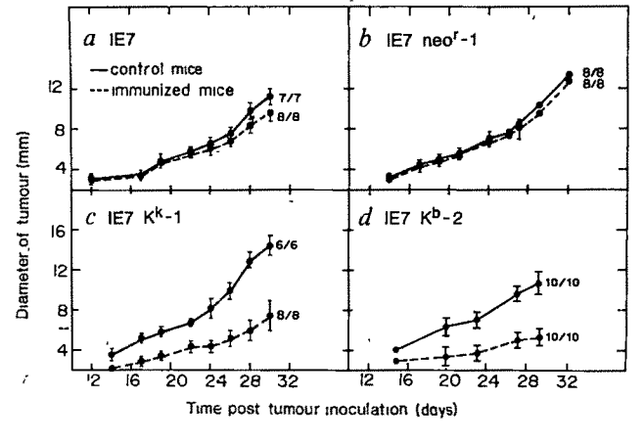


Fig. 3 *In vivo* immunogenic properties of IE7, parental and *H-2K*-transfected cells. (C3H.eB×C57BL/6J)F_1 female mice, aged 2 months, were inoculated with: *a*, 1×10^6 IE7 parental tumour cells; *b*, with IE7 cells transfected with *neo*^{r} gene only; *c*, with *H-2K*^{k}-transfected IE7 cells; *d*, with *H-2K*^{b}-transfected IE7 cells. One week later, the tumour-inoculated leg was amputated as described in Table 2 legend (immunized mice). Mice were then re-inoculated i.f.p. with 10^4 tumour cells of the immunizing origin. (Control mice were amputated as well.) Tumour growth in the immunized and control mice were measured using a caliper. Growth curves represent the mean tumour diameter of all mice inoculated ±s.e.m., including the diameter of legs of mice that did not develop tumours. Mean diameter of legs of control mice is 2 mm. The numbers designated above each growth curve represent the proportion of mice that developed a tumour.

the H-2 phenotype was determined in four different assays: (1) cytofluorography; (2) cellular radioimmunoassay; (3) lysis by alloreactive H-2-specific CTL; and (4) immunoprecipitation with monoclonal H-2 antibodies. All tests yielded identical results, but for the sake of brevity only a typical cellular radioimmunoassay using monoclonal H-2 antibodies and ^{125}I-labelled Protein A and a representative selection of transfected cell lines is presented.

Parental IC9 cells or IC9 transfected with *neo*^{r} alone express only the D^b molecule, whereas IC9 cells transfected with *H-2* genes also synthesize the K^b- or K^k antigens (Table 1). Correspondingly, parental IE7 cells or IE7 transfected with *neo*^{r} alone carry only D^b and D^k, whereas *H-2*-transfected IE7 also express K^b or K^k antigens. K^b- or K^k-positive cells were obtained only after transfection with the appropriate *H-2K* genes and never after subcloning of parental tumour cells or after transfection with *neo*^{r} alone (>500 colonies tested).

Because the intention of the work was to study the influence of *H-2* expression on interaction of the tumour cells with a syngeneic host, it was important to establish that the transfected *K*^{b} and *K*^{k} molecules are not modified in some way, for example by intragenic recombination of the transfected *H-2* gene with the host genome. Neither serological analysis with >20 monoclonal antibodies against distinct epitopes[13] on the H-2 molecules (data not shown) nor two-dimensional gel electrophoresis revealed any abnormalities (Fig. 1). The K^b spots immunoprecipitated from *K*^{b}-transfected IC9 cells (marked with a circle) are identical to those obtained from a K^b-positive cell line of C57BL/6 origin, EL4. In addition, Northern blot analysis of H-2K end-specific messenger RNA, which is found in IC9 and IE7 cells only after transfection with *H-2K* genes, did not show any unusual H-2-specific mRNA (E. Meyer and G.J.H., unpublished). It is not yet clear why the parental IC9 and IE7 cells fail to express their H-2K antigens. That the respective *H-2K* genes are present in these tumours is shown by genomic Southern blot analysis with an H-2K-specific probe (E. Meyer and G.J.H., unpublished).

Two points can be made from these findings. First, transfection with cloned *H-2* genes can be used to restore H-2 expression in cells that have lost expression of their H-2 antigens. Second, there is no evidence for an alteration of the H-2 molecules encoded by the transfected genes.

Metastatic properties

Tumorigenicity and metastatic potential of the H-2K-transfected tumour cells was investigated by inoculation of 10^4–10^6 tumour cells into the hind foot pad of syngeneic (C3H×C57BL/6)F_1 mice. Expression of either K^b or K^k antigens on the surface of IE7 cells resulted in loss of their metastatic potential (Table 2). IE7 cells transfected with the *neo*^{r} gene alone retained the metastatic properties (for example, cell line IE7.neo^r-1). Consistent results were obtained with nine additional IE7 clones transfected with *neo*^{r} alone and with five additional IE7 lines derived from transfection with *K*^{b} or *K*^{k} genes (data not shown). In contrast to lack of metastasis in normal mice, the H-2K-transfected IE7 lines were found to metastasize in irradiated (C3H×C57BL/6)F_1 mice (Table 2). For irradiation, 550 rad were used, a dose that strongly reduces host immune reactivity. The findings suggest that the immune system of the host controls the ability of transfected cells to metastasize. Transfection of the non-metastatic IC9 cells with *H-2K* genes did not change their inability to metastasize in normal mice (Table 2). However, like transfected IE7, these cells were able to metastasize in irradiated mice.

Simultaneously, we tested whether the tumorigenic properties of the H-2K-transfected cells were also affected. We inoculated (into the foot pad) 10^4 transfected IE7 cells into syngeneic (C3H×C57BL/6) F_1 mice and recorded tumour growth (Fig. 2). K^b-positive IE7 cells (IE7.K^b-3) were found to grow significantly slower than the control IE7 cells (Fig. 2*a*). In fact, only three out of six mice developed a tumour. The transfected IE7 cells growing *in vivo* were still K^b-positive when analysed for H-2 expression (not shown) and, therefore, the tumour growth cannot be attributed to loss of K^b. In contrast, IE7 cells transfected with *K*^{k}, (IE7.K^k-1) or with *neo*^{r} alone (IE7.neo^r) grew like parental IE7 in all mice inoculated (Fig. 2*a*). Similar growth patterns were obtained with four other *K*^{b}, two *K*^{k} and nine *neo*^{r}-transfected IE7 cell lines and when different doses of transfected IE7 cells were injected (10^5, 10^6 cells per mouse; data not shown). Thus, expression of K^b antigens on IE7 cells

decreased their tumorigenic properties. In irradiated mice, K^b-transfected IE7 grew like parental cells (Fig. 2*b*), suggesting that the immune system of the host also controls growth of the local tumour. The fact that IE7 cells transfected with K^k or K^b genes are both non-metastatic but manifest different growth rates suggests that expression of H-2K antigens on the tumour cells blocks metastasis irrespective of the growth kinetics of the tumour.

The tumorigenic properties of the non-metastatic IC9 clone were also affected by transfection with *H-2K* genes. K^b-positive IC9 cells were found to grow more slowly in syngeneic F_1 mice than in parental IC9 or IC9 transfected with *neo*^{r} alone (Fig. 2*c*). In fact, cells of the transfected IC9.K^b-3 line were rejected in five out of six mice (Fig. 2*c*). For other K^b-transfected IC9 lines, the percentage of rejection was in general lower and varied between 5 and 20%. The higher rate of rejection of IC9.K^b-3 compared with other K^b-transfected IC9 lines seems to correlate with the amount of K^b expressed on these cells (see Table 1). The K^b-transfected IC9 growing in mice still expressed H-2K^b (not shown). Similar growth curves were recorded when 10^5 and 10^6 cells of IC9 transfected cells were inoculated, but the percentage of mice without a tumour was lower than with 10^4 injected cells (data not shown). All transfected IC9 lines were able to grow like parental IC9 in irradiated mice (Fig. 2*d*). The results presented so far indicate that transfection of IC9 and IE7 cloned cells with *H-2K* genes leads to the abolition of metastatic competence and a decrease in tumorigenicity. Our experiments with irradiated mice suggest that the immune system is involved in reduction of metastasis and tumorigenicity.

Immunogenic properties

We next tested whether the reduction in metastasis in *H-2K*-transfected IE7 cells is associated with increased immunogenicity. F_1 mice were immunized with transfected tumour cells and then challenged with homologous tumour cells (Figs 3, 4). IE7 parental cells (Fig. 3*a*) and IE7 cells transfected with *neo*^{r} gene only (Fig. 3*b*) showed the same growth rate in immunized and control mice and, therefore, do not seem to be immunogenic. In contrast, immunization with IE7.K^k-1 (Fig. 3*c*) or with IE7.K^b-1 cells (Fig. 3*d*) led to retarded growth of the corresponding tumour cells. In agreement with previous observations[10], the non-metastatic parental IC9 cells proved to be slightly immunogenic (Fig. 4*a*). However, expression of K^b on transfected IC9 cells led to a drastic increase in immunogenicity, resulting in complete rejection in respectively immunized mice (Fig. 4*b–d*).

Table 2 Metastatic potential of H-2K-transfected IE7 and IC9 cells in normal and irradiated syngeneic (C3H×C57BL/6)F_1 mice

Tumour	H-2 phenotype* D^k	K^k	D^b	K^b	Mice inoculated	Weight of lungs (mg)	No. of nodules in lungs
IE7 transfected lines							
IE7	+	−	+	−	Normal	397±70	12, 200, 200, 200, 200
					Irradiated	460±20	200, 200, 200, 200, 200
IE7.neo^r	+	−	+	−	Normal	420±15	200, 200, 200, 200, 200
					Irradiated	395±40	200, 2000, 200, 200
IE7.K^k-1	+	+	+	−	Normal	186±8	0, 0, 0, 0, 2, 6, 8
					Irradiated	381±15	200, 200, 200, 200, 200
IE7.K^kK^b-1	+	+	+	+	Normal	170±3	0, 0, 2, 2, 4
					Irradiated	401±20	200, 200, 200, 200, 200
IE7.K^b-1	+	−	+	+	Normal	168±8	0, 0, 0, 0, 0
					Irradiated	326±45	200, 200, 200, 200, 200
IE7.K^b-2	+	−	+	+	Normal	182±10	0, 0, 0, 0, 0, 0
					Irradiated	345±50	200, 200, 200, 200, 200
IE7.K^b-3	+	−	+	+	Normal	180±5	0, 0, 0, 0, 0, 3
IC9 transfected lines							
IC9	−	−	+	−	Normal	172±16	0, 0, 1, 3, 9, 200
					Irradiated	255±52	3, 200, 200, 200
IC9.K^b-1	−	−	+	+	Normal	162±6	0, 0, 0, 0, 0, 5
					Irradiated	270±8	200, 200, 200, 200
IC9.K^b-2	−	−	+	+	Normal	154±4	0, 0, 0, 0, 0
					Irradiated	283±5	200, 200, 200, 200, 200
IC9.K^k-1	−	+	−	+	Normal	171±11	0, 0, 0, 0, 0

A single-cell suspension of 10^5 *neo*^{r}- and *H-2K*-transfected IE7 and IC9 cells were inoculated intra footpad (i.f.p.) into intact and sublethally irradiated (550 rad Co) (C3H×C57BL/6)F_1 mice. When the tumour diameter reached a size of 8–10 mm, the tumour-bearing leg was amputated using a 3/0 silk tie, which was ligated beyond the iliofemoral joint of the hind leg to prevent bleeding. In each experimental group, mice were killed 21 days post-amputation, and their lungs removed and assessed for metastatic load by weight and by number of nodules counted after fixation in Bouin's solution. Without excision of the primary tumour the number of metastatic nodules is much lower[29]. Corresponding results were obtained with both protocols, but only data generated with the excision protocol are presented. Other independent experiments yielded identical results (not shown).

* H-2 phenotype according to results presented in Table 1.

Table 3 Generation of CTL specific for transfected tumours in (C3H×C57BL/6)F_1 mice

Immunization	Target cells (% specific lysis) IC9	IE7	(IC9.K^b-3)	(IE7.K^b-1)	(IC9.K^k-1)	(IE7.K^k-1)	EL4	L929
IC9	0	0	0	0	0	0	0	0
IE7	0	0	0	0	0	0	0	0
(IC9.K^b-3)	0	0	45	28	0	0	0	0
(IE7.K^b-1)	0	0	40	35	0	0	0	0
(IE7.K^k-1)	0	0	0	ND	ND	59	0	5

(C3H×C57BL/6)F_1 mice were immunized three times at weekly intervals with 10^6 tumour cells irradiated with 3,000 rad. After 7–10 days, spleen cells were restimulated *in vitro* with the same tumour cells irradiated with 10,000 rad. Cytolytic activity was determined at different effector-to-target ratios against ^{51}Cr-labelled target cells including EL4 (H-2^b) and L929 (H-2^k) as controls Data show per cent specific lysis obtained with an effector/target ratio of 50:1. ND, not determined.

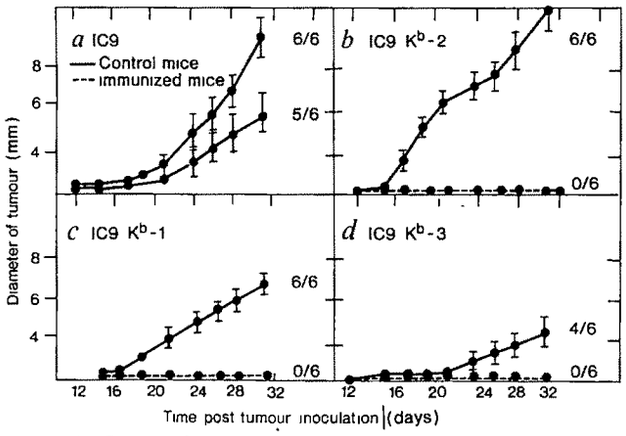


Fig. 4 *In vivo* immunogenic properties of IC9 parental and $H\text{-}2K^b$-transfected cells. Syngeneic F_1 mice were immunized against. *a*, IC9 parental cells and, *b–d*, against $H\text{-}2K^b$-transfected IC9 cells. Immunization and the immunogenic properties of the different tumour cells were followed as described in Fig. 2 legend.

In conclusion, expression of H-2K antigens on IC9 and IE7 cells confers highly immunogenic properties on these cells.

Generation of cytotoxic T lymphocytes

Spleen cells from syngeneic $(C3H \times C57BL/6)F_1$ mice immunized with irradiated tumour cells were restimulated *in vitro* and their cytotoxic activity was determined against a panel of target cells (Table 3). Immunization with parental IC9 or IE7 cells did not lead to generation of CTL. In contrast, specific Thy-1.2$^+$ CTL were obtained, after immunization with K^b-transfected IC9 or IE7 cells. These CTL lysed only K^b-transfected IC9 and IE7 but not parental IC9 or IE7 cells, or EL4, an unrelated K^b-positive tumour. The findings suggest that the CTL can recognize an unknown tumour-associated antigen on IC9 or IE7 only in conjunction with K^b. Likewise, K^k-restricted CTL were found after immunization with K^k-transfected IE7. The fact that CTL induced with K^b-transfected IC9 can also lyse K^b-transfected IE7, and vice versa, indicates that both IC9 and IE7 carry the same tumour-associated antigen.

Discussion

The data presented here suggest that manipulation by gene transfection of H-2K expression on $(H\text{-}2^b \times H\text{-}2^k)F_1$ mouse-derived T10 sarcoma cells leads to changes in the malignant properties of these cells. Thus, the H-2K-negative and highly metastatic IE7 cells lost their capacity to metastasize when made to re-express H-2K antigens by *H-2K* gene transfection. Moreover, K^b-transfected IE7 or IC9 cells manifested a decrease in their tumorigenic properties. The crucial question is whether the modulation of tumour growth does indeed result from the *de novo* expression of H-2K antigens. The most convincing argument is that all IE7 clones expressing only the *neo*^{r} gene still metastasize and grow *in vivo* like the parental IE7 cells. Similarly, all IC9 clones transfected with *neo*^{r} alone grow like the non-metastatic parental IC9.

IE7 cells transfected with either *H-2K* or parental and transfected IC9 metastasized when inoculated into sublethally irradiated mice, suggesting that in the case of these tumour cells the immune system is involved in preventing metastasis formation. Indeed, the efficient immunization with H-2K-positive IE7 and IC9 cells (Figs 3, 4) and the induction of CTL by *H-2K*-transfected cells (Table 3) demonstrates that the *de novo* expression of H-2K converts the tumours into highly immunogenic cells. The resulting CTL lysed only H-2K-positive, but not parental, cells. These findings indicate not only that the presence of H-2K antigens on the tumour cells is required for induction of immunity, but also that H-2K is part of the target structure recognized by effector cells.

The results presented here are consistent with many reports showing that CTL raised against foreign cellular antigens can be preferentially restricted by the H-2K or the H-2D molecules depending on the antigen and *H-2* haplotype involved[1]. Thus, it is conceivable that in the case of the IC9/IE7 tumour system, the D^b and D^k molecules fail to act as H-2 restriction elements for T-cell recognition. In contrast, the putative tumour associated antigen on IE7 cells, the nature of which is unknown, can be efficiently recognized in conjunction with the transfected K^b or K^k, resulting in the elimination of disseminating metastatic cells. As only expression of K^b but not K^k leads to retarded growth of the primary tumour, it seems that K^b renders the cells more immunogenic than K^k. However, the resulting K^k-restricted immune response seems to be strong enough for elimination of metastasizing K^k-transfected IE7 cells, possibly because the disseminating cells are more accessible than the locally growing tumour. In addition to the potential failure of H-2D molecules to function as H-2 restriction elements, accumulating data suggest that H-2D antigens may play an active role in the prevention of immune responses[14–17]. In fact, recent experiments suggest that the D^k molecule on IE7 cells exerts suppressogenic effects on the host response[9]. Other non-immunological effects of H-2 antigens cannot be excluded at this point.

The frequently observed absence of class I MHC antigens on tumours[2,3,18,19] may have important implications for the immune surveillance of neoplastic cells. This notion has been substantiated here and by other recent reports[20–22]. While this manuscript was in preparation, Hui *et al.*[23] reported that restoration of H-2K^k expression by gene transfection led to reduced tumorigenicity of a non-metastatic AKR thymoma, which is in agreement with our observations on the solid T10 sarcoma. The functional importance of class I MHC antigens in the animal systems described above may also have implications for the phenotypic expression of malignancy of human neoplasms. Indeed, there is some recent evidence that the malignancy of certain human tumours seems to be correlated with expression of HLA class I antigens[24,25].

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RS Ophiuchi — first radio detection of a recurrent nova outburst

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Recurrent novae and classical novae belong to the cataclysmic variable class of objects. Of these, only classical novae outbursts have been hitherto observed at radio wavelengths, and then no earlier than 50 days after the outburst. Here we report the first radio detection of an outburst from a recurrent nova (RS Ophiuchi). The observations begin only 18 days after the optical maximum and show a radio 'light' curve which is different from that of classical novae. The brightness temperature of $\sim 10^7$ K, calculated for this event, greatly exceeds the 10^4 K seen in classical novae, and suggests a non-thermal origin for the radio emission from RS Ophiuchi. Although the later emission has a classical $(t-t_0)$ 'light' curve, the radio emission mechanism appears not to turn on until 14 days after the optical nova and takes a further 10 days to reach the $(t-t_0)$ curve. Analogies may be drawn between the radio development of RS Ophiuchi and that of young supernovae.

In both recurrent and classical novae, the outbursts may result from thermonuclear runaway on the surface of a white dwarf in a close binary system, which leads to a rapid increase in luminosity and mass ejection[1]. Based on this model there are two major differences between recurrent and classical novae. First, the secondary component of the binary system in recurrent novae is thought to be a late-type giant rather than a main-sequence dwarf as in classical novae. Second, the inter-outburst timescale is tens of years rather than 10^4–10^5 years as predicted for classical novae; for example, the recurrent nova RS Ophiuchi has previously undergone very similar outbursts in 1898, 1933, 1958 and 1967 (ref. 2).

Following the nova outburst reported by Morrison[3] on 26 January 1985, observations were made with the new Jodrell Bank Mk IA, Mk II 400-MHz bandwidth interferometer system operating at 5 GHz (S.P. and R.J.D., in preparation). This instrument has a baseline of 425 m, giving a lobe spacing of ~30 arc s at 5 GHz. At this frequency, the 4-arc min beam of the Mk IA telescope results in a typical confusion level of 1 mJy. The first flux density measurement of RS Ophiuchi was obtained on 13 February, only 18 days after the outburst. This first detection yielded a flux density of 23 ± 3 mJy and subsequent observations on 15 February revealed an increase in flux density[4].

Phase measurements made with the interferometer allow the position of the radio emission to be established to within ±2 arc s in right ascension and ±4 arc s in declination; within these limits the radio position was found to be consistent with the reported optical position of the nova. Figure 1 shows observations of the radio 'light' curve at 5 GHz for RS Ophiuchi between 13 February and 5 March. Further observations were not possible because of telescope scheduling considerations. The flux density calibration of these data is based ultimately on observations of 3C286, which is assumed to have a flux density of 7.30 Jy at 5.0 GHz (ref. 5), with the gain-elevation dependence of the interferometer being obtained from observations of many different radio sources. For all these observations, a referencing technique was used with the instrument observing a nearby calibrator source for a short time before moving to the field of interest. Using such a procedure, the flux density of the nova is accurately related to that of the source 1741–038 and this is reflected in the relative error bars in Fig. 1. However, the flux density of 1741–038 is not accurately known and so this has been related to 3C286. This latter calibration introduces a further systematic error of ~5% into the flux density scale.

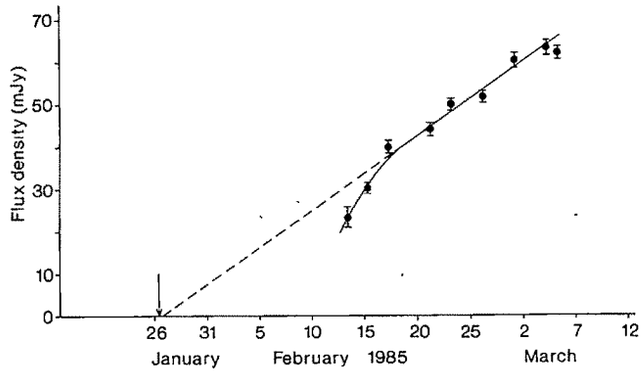


Fig. 1 Radio 'light' curve for the recurrent nova RS Ophiuchi measured at 5 GHz with left-hand circular polarization. The vertical arrow indicates the time of the optical outburst.

Figure 1 clearly shows two phases in the radio development of the nova. Initially there is a rapid increase in flux density at a rate of approximately 4 mJy per day until 18 February; this then gives way to a slower linear increase which projects back to the time of the initial outburst (t_0), giving a $(t-t_0)$ dependence with a constant of proportionality of 1.7 mJy per day. This $(t-t_0)$ dependence is characteristic of the classical radio novae observed by Hjellming *et al.*[6]. The initial rapid rise indicates that the radio emission 'turned on' about 14 days after the nova explosion, but after a further 10 days it reached the $(t-t_0)$ curve. Thus, although the radio emission was 14 days late in turning on, after ~24 days the curve proceeds as if the emission had started at t_0.

Analogies may be drawn between the radio development of RS Ophiuchi and that of Type II supernovae. In the latter case, the non-thermal radio emission is thought to arise from the interaction of an ejected shell with the pre-outburst wind of the late-type supergiant progenitor[7]. Similarly, RS Ophiuchi is thought to eject $\sim 10^{-5} M_\odot$ of material at $\sim 1{,}000$ km s^{-1} into pre-existing circumstellar material[8] which may be associated with a wind from the giant secondary of a binary system.

If we assume a distance of 2 kpc (ref. 9) and an average expansion velocity of 10^3 km s^{-1}, we calculate a brightness temperature of 10^7 K for the period of our observations. Initial measurements with the Very Large Array indicate a nearly flat spectrum (R. M. Hjellming, personal communication). Classical novae[6] exhibit thermal radio emission, typically with a temperature of 10^4 K, and during the optically thick, rising phase of the light curve have a spectral index of ~+1.5. These differences lead us to suggest a non-thermal origin for the radio emission in RS Ophiuchi. As the $(t-t_0)$ dependence is similar to that of a classical nova, albeit with a different constant of proportionality, these observations suggest that both the optical depth and dynamics of the shell after 24 days may be similar for the two phenomena, even though the radio emission mechanism may differ. The subsequent radio evolution of this system is clearly of considerable interest.

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Lower stratosphere trace gas detection using aircraft-borne active chemical ionization mass spectrometry

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Acetaldehyde and acetone are expected to be formed as trace gases in the free troposphere from photochemical conversion of non-methane hydrocarbons and to represent key precursors of peroxy-acetyl nitrate (PAN)[1–5]. PAN is important as a potential organic reservoir[1–5] for odd N and thus as a vehicle for odd N transport in the troposphere or even from the troposphere to the stratosphere. As PAN suffers from thermal decomposition in the lower troposphere, its transport into the stratosphere relies on its formation in the upper troposphere where temperatures are sufficiently low to prevent thermal decomposition. Neither PAN nor its key precursors, however, have been observed in the free troposphere and it is difficult, therefore, to assess the potential impact of non-methane hydrocarbons on upper tropospheric and stratospheric ozone[1–5]. An indication of the presence of acetone around the tropopause level was first obtained recently from ambient positive-ion composition measurements[6]. Here, we report on a search for these trace gases and other species with large proton affinities in the tropopause using aircraft-borne active chemical ionization mass spectrometry (ACIMS), a novel method for atmospheric trace gas detection.

The ACIMS method developed in our laboratory will be described in detail elsewhere[7,8] and only a brief outline is given here. Detection by ACIMS of molecules B possessing large proton affinities or gas phase basicities relies on their reaction with $H_3O^+(H_2O)_n$ ions in

$$H_3O^+(H_2O)_n + B \rightarrow HB^+(H_2O)_n + H_2O \quad (1)$$

Usually[8] proton transfer reactions (1), if sufficiently exothermic, occur on every collision and have a temperature-independent rate coefficient of $\sim 2\text{–}5 \times 10^{-9}$ cm^3 s^{-1} depending on the dipole moment of B. The ACIMS apparatus (Fig. 1) works like a flow reactor where ambient air is sampled by an air intake on top of the aircraft's fuselage and then the air stream is passed through a flow tube and directed to the sampling cone of a cryogenically pumped quadrupole mass spectrometer. Ultimately, the air stream leaves the aircraft's fuselage through an outlet tube. Precursor ions $H_3O^+(H_2O)_n$ are formed by a high-frequency glow-discharge ion source inside the flow tube, 70 cm upstream of the sampling cone that carries the inlet orifice through which ions enter the mass spectrometer. The time t_R for which ions reside in the reaction zone between the ion source and the sampling cone, being typically 17 ms, can be measured precisely by pulsing the ion source and measuring the ion arrival time. By measuring the flux ratio $F(HB^+)/F(H_3O^+)$ for precursor and product ions with the mass spectrometer, the [B] can be obtained using expression

$$[B] = \frac{1}{kt_R} \ln\left(\frac{F(HB^+)}{F(H_3O^+)} + 1\right) \quad (2)$$

which follows from integration of the kinetic equations for product and precursor ions.

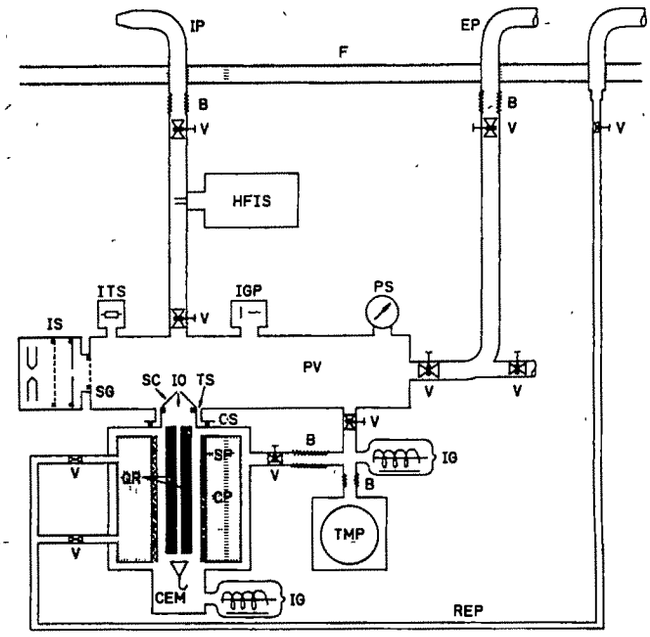


Fig. 1 Schematic representation of the aircraft-borne chemical ionization mass spectrometry apparatus. F, fuselage; IP, intake pipe; EP, exit pipe; V, valve; HFIS, high-frequency glow-discharge ion source; IS, electron impact ion source; ITS, temperature sensor; IGP, ion getter pump; PS, pressure sensor; SG, sampling grid; PV, probing vessel; SC, sampling cone; TS, Teflon seal; IO, inlet orifice; OS, O-ring seal; QR, quadrupole rods; CP, cryo-pump; SP, sorption panel; B, bellow; IG, ionization gauge; TMP, turbomolecular pump; CEM, channel electron multiplier.

Taking the above k and t_R and an ion flux ratio of 0.001 which can easily be measured, a conservative estimate for the typical detection limit of $\sim 3 \times 10^7$ cm^{-3} corresponding to a volume-mixing ratio of $\sim 4 \times 10^{-12}$ or 4 p.p.t.v. (parts per trillion by volume) at $\sim$11,000 altitude is obtained. The uncertainty in [B] is determined primarily by the uncertainty in k, which may be as low as ±30% in laboratory measurements. Typical uncertainties for t_R and the ion flux ratio are both ±20%. Taking a typical uncertainty for k of ±50%, an estimate of the overall uncertainty for [B] of ± ~57% is obtained. The precision of [B], which is not affected by the uncertainty in k, is ± ~28%.

The first flight of the novel ACIMS instrument was on 24 February 1984 over the FRG, reaching a maximum altitude of

Table 1 Concentrations and volume mixing ratios for trace gases measured in flight F5 at 11,300 m

Molecule	PA (kcal mol^{-1})	k (10^9 cm^3 s^{-1})	Concentration (10^{-8} cm^{-3})	VMR (10^{12})	Other data		Ref.
CH_3CN	187	3.2	2.0	36	2–4	20,000 m	10–12
					56	Ground	13
$(CH_3)_2CO$	193.6	2.9	1.4	25	≥20	11,300 m (F5 PACIMS)	6
					500	Ground	16
					15	9,000 m (Model)	1
NH_3	200.4	1.8	≤4.0	≤73	≤0.050	11,300 m (F5 PACIMS)	6
					600–2,000	11,000 m	14
					600	4,000 m	15
					500–20,000	Ground	1
CH_3CHO	185.4	2.3	≤0.11	≤2.0	3.0	9,000 m (Model)	1
					7.0	15,000 m (Model)	1

PA, proton affinities (see ref. 9); VMR, volume mixing ratios. References are for other data.

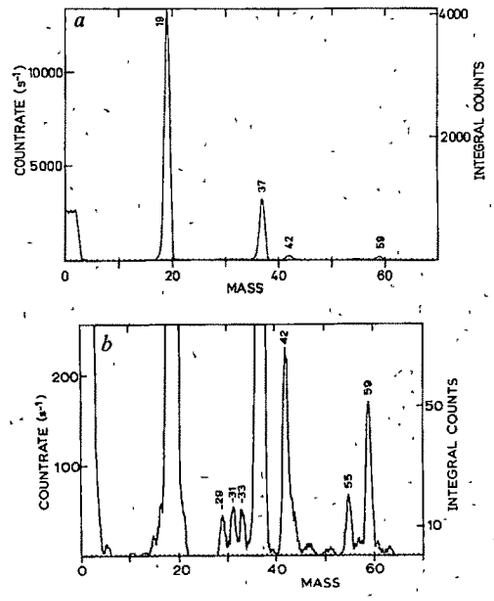


Fig. 2 *a*, Positive-ion ACIMS mass spectrum obtained at 4,300-m altitude. Panel *b* is enlarged in the *y*-direction for visualization of minor ion peaks. The signal at masses 0–4 does not represent a mass peak but is a reduced total ion signal occurring at very low radiofrequency-amplitude of the mass filter. Integral counts denotes the total number of ion counts per mass peak.

11,300 m, well above the local tropopause levels (9,750–10,360 m) and cloud-top levels (5,000–8,000 m). ACIMS measurements were taken only at 11,300 m and only for a short period of time (15.19–15.33 LT) and the remaining flight time was spent for ambient ion composition measurements[6].

A typical positive-ion ACIMS spectrum, obtained in 40 s at 11,300 m, is shown in Fig. 2. Besides the precursor ions $H_3O^+(H_2O)_n$ (masses 19, 37, 55), it contains several product ion peaks of which 42 and 59 are most prominent. Like H_3O^+, the most prominent member of the $H_3O^+(H_2O)_n$ family, these ions must be naked 'cores' of the type HB^+, with B possessing a large proton affinity (typically $> \sim 175$ kcal mol^{-1}). Therefore, the most probable candidates are protonated acetonitrile (42) and protonated acetone (59); no significant peaks attributable to protonated acetaldehyde (45) and protonated ammonia (18) occur.

Taking measured rate coefficients k (Table 1), concentrations (acetonitrile and acetone) and upper limits (ammonia and acetaldehyde) were obtained from equation (2). A compilation of our measured trace gas abundances is given in Table 1 together with other data. (Data for additional trace gases detected by the present ACIMS experiments are given elsewhere.)

Our present acetonitrile abundance of 36 p.p.t.v. at 11,300 m is similar to the average ground-level value of 56 p.p.t.v. obtained by Snider and Dawson[13] (Fig. 3), which suggests that acetonitrile has a sufficiently long lifetime to allow for efficient vertical mixing in the troposphere (typical timescale: several days). This is consistent with recent estimates of CH_3CN-lifetimes against reaction[18] with OH and washout[19] being about 1–2 years in the troposphere. In contrast, middle stratosphere acetonitrile mixing ratios as obtained from balloon-borne PACIMS (passive chemical ionization mass spectrometry) measurements[11,17] are much lower than our present value at 11,300 m, which is somewhat surprising in view of the relatively slow reaction of CH_3CN with OH, the only presently known CH_3CN-sink in the lower stratosphere. A one-dimensional photochemical model calculation of CH_3CN by Brasseur *et al.*[18] (also shown in Fig. 3) considering reaction with OH as the only CH_3CN-sink requires a tropospheric CH_3CN-volume mixing ratio of only 10 p.p.t.v. in order to reproduce the middle stratosphere observations. Compared to this value our present measured value of 36 p.p.t.v. and the average ground-level value of 56 p.p.t.v. are much larger.

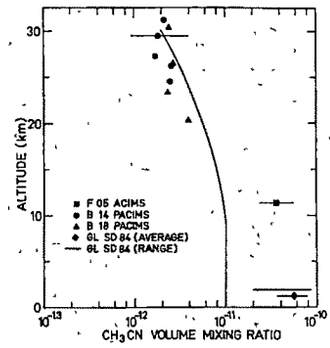


Fig. 3 Comparison of measured acetonitrile volume mixing ratios as obtained from aircraft-borne PACIMS[11,17] and ground-level (GL) condensation collection[13]; typical error bars for the various analytical methods are indicated. Also shown is a one-dimensional photochemical model calculation for CH_3CN by Brasseur *et al.*[18] considering reaction with OH as the only CH_3Cn-sink.

The reason for this discrepancy is presently not known. Possibilities include an additional lower stratosphere CH_3CN-sink or a non-uniform tropospheric CH_3CN-distribution with lower abundances in equatorial regions where tropospheric air primarily enters the stratosphere. However, it should be kept in mind that the stratospheric distribution of CH_3Cl, which is also removed by reaction with OH at a rate similar to CH_3CN and which is practically uniformly mixed in the troposphere, is not well reproduced by one- and two-dimensional model calculations. Usually stratospheric CH_3Cl is overestimated by the models[22]. Concerning additional CH_3CN-sinks in the lower stratosphere the possibility of CH_3CN-removal by ion processes may also be considered in view of the fact that stratospheric positive ions contain CH_3CN. Upon ion–ion recombination of such CH_3CN-containing clusters, CH_3CN may be destroyed by reaction or be incorporated into aerosols which may eventually result from ion–ion recombination[20,21]. However, very little is known about the products of ion–ion recombination and thus the possibility of CH_3CN-removal by ions remains speculative.

Compared with acetonitrile the situation for ammonia is entirely different. Here, the upper limit of 73 p.p.t.v. is much lower than the upper troposphere and lower stratosphere measurements yet made. Only two measurements at $\sim$11,000 m have been reported (2 p.p.b.v., March 1979; 0.6 p.p.b.v., August 1979)[14] using a remote infrared heterodyne radiometer technique. Our value is also much lower than the chemical filter data (0.6 p.p.b.v.) obtained[15] at $\sim$4,000 m. Again, more free tropospheric measurements covering a range of meteorological conditions are needed before conclusions on ammonia transport and removal can be reached.

We next discuss acetone and acetaldehyde. The present acetone abundance of 25 p.p.t.v. is higher than the model predictions of Singh and Hanst[1], being 15 p.p.t.v. (9,000 m) and 3 p.p.t.v. (15,000 m). By contrast, the upper limit described here for acetaldehyde of 2 p.p.t.v. is somewhat lower than the model predictions[1] (3 p.p.t.v., 9,000 m; 7 p.p.t.v., 15,000 m). If the kinetic data used by Singh and Hanst[1] are correct, PAN formation at 11,300 m from acetone would be at least 10 times more efficient than from acetaldehyde. A further implication is that the production of acetone would be much larger than predicted by the model (considering only propane as a precursor of acetone). Thus, either the propane level was higher than assumed or additional non-methane hydrocarbon species contribute significantly to acetone formation. It is also conceivable that the observed increase in acetone level is associated with jet aircraft exhaust, a possibility that deserves further attention. Alternatively, the model may underestimate the lifetime of acetone in the troposphere.

Clearly, more measurements, in particular height profiles, of acetone, and eventually also acetaldehyde, in the free troposphere and lower stratosphere are needed to elucidate the details of PAN formation in this part of the atmosphere. The ACIMS method has the potential to contribute significantly to an

improved understanding of the photochemistry of non-methane hydrocarbons in the free troposphere and lower stratosphere.

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An Antarctic ice core reveals atmospheric CO_2 variations over the past few centuries

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When snow is transformed into ice (firnification process) by sedimentation near the surface of an ice sheet, some of the atmospheric air is trapped in the inter-grain spaces which are progressively isolated from the surrounding atmosphere. The resulting material is an air-tight bubbly glacial ice. By analysing the air extracted from the bubbles found in the ice, it is possible to determine the air composition and thus its CO_2 content for the period during which the air was trapped. We aim here to provide the most direct evidence obtained so far for the background atmospheric CO_2 concentrations over the centuries preceding the recent anthropogenic perturbation due to the industrial revolution of the past century. This background level is important for assessing both the origin and the climatic response of the anthropogenic perturbation to the atmospheric CO_2 (see ref. 1). Our results, obtained from the analysis of the air trapped in the bubbles of an Antarctic ice core, indicate that the background level could have been as low as 260 p.p.m.v. (parts per million by volume) before the major anthropogenic influence and suggest that the so-called 'pre-industrial' CO_2 level was not constant over the few hundred years preceding the nineteenth century.

Although several parameters, such as snow melting at the surface of the ice sheet, may obscure the record, CO_2 results representing the atmosphere can be obtained with a good degree of confidence by choosing appropriate sites, cores and gas extraction methods (see ref. 2). In particular, the best conditions are found using unfractured ice cores taken in the dry snow zones where no melting occurs (the mean annual air temperature of these zones is generally below −25 °C). The ice record of atmospheric CO_2 against time is, furthermore, 'smoothed' because each ice layer contains bubbles which closed off at different times during the firnification. Because the firnification rate increases with the rate of snow accumulation at the surface, a low smoothing effect is expected in high-accumulation sites. For this study, the smoothing must be as low as possible to detect, with sufficient definition, the possible atmospheric CO_2 changes over a few centuries. In short, we are looking for a good core taken in a cold site without summer melting and with a high accumulation rate.

Such requirements were fulfilled by a 203-m-long ice core drilled in 1980–81 at the station D57 (68°11′ S, 137°33′ E) in East Antarctica. The ice from this core analysed for the present study was formed, according to an ice origin calculation, upstream from the drilling site in a zone where the mean annual surface temperature is ≤−32 °C (−32 °C at D57) and the present-day measured snow accumulation rate is 45 cm of ice equivalent yr^{-1} at D57 and highly variable upstream (M. Pourchet, personal communication). Taking into account the accumulation data and the conditions of gas enclosure, each air sample analysed could average between ~20 and 40 yr of atmospheric conditions.

For CO_2 measurements, we sampled eight depth levels between ~89 and 198 m below the surface (Table 1). For each level, between 16 and 50-cm lengths of the ice core were analysed after the ice samples (weighing between 23 and 46 g) had been cut into horizontal core layers ~2–4 cm thick. These ice samples were taken from the internal and unfractured part of the ice cores and cut in a cold room (at about −15 °C) with a band saw. The whole experimental procedure, including all the steps between the extraction of the gas by grinding the ice under vacuum at −40 °C and the gas chromatographic analysis, was performed using the Grenoble method described in ref. 3.

For each measurement, the analytical system is calibrated as described in ref. 3 and the experimental accuracy (with a 95% confidence limit) is evaluated from the standard deviation of the residuals corresponding to the calibration regression. This accuracy for the results presented here is in the range ±1.1–4.8%. Between 5 and 13 samples were processed for each depth level. The corresponding CO_2 results are shown in Table 2. The scattering of the CO_2 measurements for a given depth level is evaluated by calculating their standard deviation and lies in the range ±1.3–3.8% (Table 1). This scattering is smaller than, or

Table 1 D57 ice-core sample characteristics, mean CO_2 concentrations and mean ages of the gas

Depth below the D57 surface (m)	Length of ice core analysed (cm)	No. of samples analysed	Scattering of CO_2 measurements (%)	Mean CO_2 concentration (p.p.m.v.)	Mean age of the gas (AD)	
					a	*b*
89.3–89.6	24	7	2.4	288	1940	1890
102.4–102.6	19	7	3.8	266	1915	1830
124.8–125.0	17	6	1.3	261	1855	1710
133.0–133.7	16	5	2.8	259	1835	1670
141.3–142.0	50	13	2.6	264	1815	1630
161.6–161.8	16	7	2.0	268	1745	1520
181.1–181.3	17	6	1.3	272	1620	1420
197.2–197.5	24	6	3.4	271	1560	1340

The mean ages of the gas are calculated (see text) taking into account a constant rate of bubble enclosure between the densities 0.80 and 0.83 and ages of ice obtained from modelling the ice flow (*a*) and by assuming a constant accumulation rate of 18 cm of ice equivalent yr^{-1} (*b*).

similar to, the experimental errors and can consequently be attributed to them. The mean CO_2 concentrations measured for each depth level are shown in Table 1.

Our calibration gas has been compared with standards prepared according to the WMO 1974 provisional scale by the Scripps Institution of Oceanography[3] and the error due to the calibration gas on the absolute values of the CO_2 measurements is estimated to be $\leq \pm 1\%$. An absolute calibration of the complete procedure is, unfortunately, not possible because a standard ice enclosing bubble having a known composition is not available. Nevertheless, we recently simulated such a calibration by grinding artificial bubble-free ice surrounded by a volume of calibration gas equivalent to the volume of air trapped in the natural ice. The results indicate that the procedure does not change the CO_2 composition of the gas within experimental error. A similar conclusion has been reached after comparing CO_2 measurements on air from bubbles in polar ice performed in our laboratory and in the Physics Institute of Bern[3]. Nevertheless, more recent measurements made in Bern suggest that their results would have to be corrected by adding ~15 p.p.m.v. (ref. 4). A new comparison is in progress between the two laboratories to solve this uncertainty.

We will now evaluate when the air found in the bubbles was isolated from the free atmosphere. As pointed out above, the bubbles become isolated from the free atmosphere during the firnification process. Consequently, the gas at a given depth is younger than the ice itself. The bubble close-off process begins to be significant probably after the firn grains have been packed into the closest possible arrangement. This closest possible packing corresponds, according to Anderson and Benson[5], to a firn density of ~0.55. The enclosure process ends at the firn-ice transition, at a density of ~0.83. Recent volume measurements of the closed bubbles in the firn at Siple Station, Antarctica, by Schwander and Stauffer[6] indicate that 80% of the bubbles close off between the densities 0.795 and 0.83. Nevertheless, the observed occurrence of impermeable layers and the fact that the air, especially in the deeper part of the firn, could not be well mixed with the atmosphere, may isolate the air in the firn from the free atmosphere at shallower firn densities (and consequently at shallower depths). Furthermore, although the experimental method used by Schwander and Stauffer is the best available, we believe that this method risks breaking some bubbles near the sample surface. If this is the case, the method provides a minimum volume value of the closed bubbles, especially for the firn with relatively low densities. Also, the enclosure conditions could be different at D57 because the firnification parameters (temperature and snow accumulation rate) are not the same as at Siple Station. For these reasons we assume that: (1) the bubbles close off at a constant rate between the densities 0.80 and 0.83 which correspond, respectively, to depths of ~68 and 76 m below the surface at D57; and (2) the air can be isolated before the firn reaches the density 0.80. Taking into account the time of deposition of the snow at the surface of the ice sheet, that is, the age of the ice, and the time required to reach the densities 0.80 and 0.83, it is possible to calculate mean ages of the air samples we analysed. These gas ages are given in Table 1 and Fig. 1 for two different estimates of the age of the ice. They may be, in fact, older by several tens of years, as suggested by the arrows in Fig. 1, because the air can be isolated before the firn reaches the density 0.80. This uncertainty would be, in the case of D57, generally smaller than the uncertainty in the determination of the age of the ice.

Table 2 Results of the multiple CO_2 measurements performed at each depth level for the D57 ice core

Depth below the D57 surface (m)	CO_2 concentrations with experimental accuracies (p.p.m.v.)		
89.3–89.6	275.5±8	288.5±8	281.5±9
	289.5±8	292±7	294±9
	293±8		
102.4–102.6	251±7	256±6	266±3
	270±8	262±6	276±3
	279±3		
124.8–125.0	258.5±12	257.5±10	260.5±10
	267.5±10	261±8	261.5±10
133.0–133.7	258±8	247±9	262±9
	262±4	266±4	
141.3–142.0	252±7	255±7	254±7
	272±8	270±13	267.5±7
	264±10	270.5±11	263±6
	267.5±6	273±12	263±8
	263±4		
161.6–161.8	264.5±5	261.5±6	273±5
	263±8	272±11	276±7
	268±9		
181.1–181.3	274.5±5	277.5±10	268.5±9
	270.5±10	272.5±8	269±10
197.2–197.5	258±8	264.5±8	270.5±8
	278.5±8	284±7	271±7

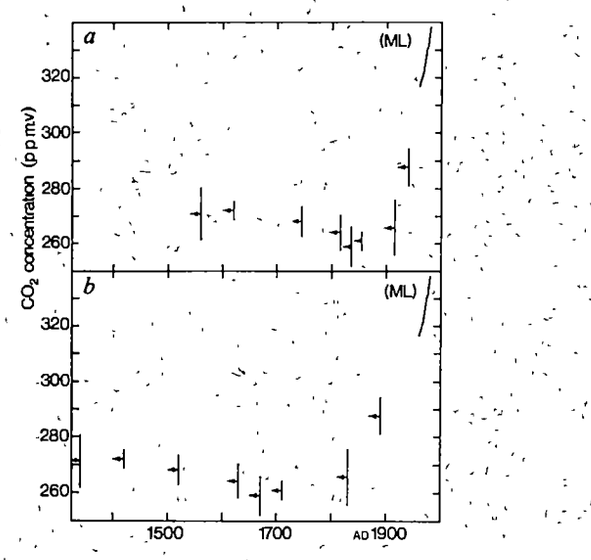


Fig. 1 Atmospheric CO_2 concentrations during the past centuries as deduced from the D57 ice-core analysis. The vertical lines indicate the scattering ($\pm 1\sigma$) of the measurements. The mean ages plotted were obtained using two different approaches (see text). *a*, Dating of the ice obtained by modelling the ice flow. *b*, Dating based on the Tambora horizon at a depth of ~48–50 m below the surface and on a constant snow accumulation rate for the whole period encompassed by the core. The arrows indicate qualitatively that the mean ages may be older if the air in the firn is isolated from the atmosphere before the firn reaches the density 0.80. ML, The atmospheric CO_2 record since AD 1958 at Mauna Loa[10].

For dating the ice, we have used two approaches. First, the depth–age relationship has been calculated (C. Ritz and D.R., unpublished data) from modelling the ice flow upslope from D57 by assuming a uniform vertical strain rate and horizontal ice velocities independent of the depth. The parameters required for the calculation include snow accumulation rate, horizontal ice velocity and ice thickness at the sites of snow deposition. The times required for the snow to reach the densities 0.80 and 0.83 have been obtained in a similar way. This dating depends essentially on snow accumulation data. Unfortunately, the accumulation rate pattern upslope from D57 is highly variable and poorly known. In this calculation, we take into account the limited accumulation rate data available: 45, 32, 4.4, 15, 34 and 28 cm of ice equivalent at D57 and 10, 15, 16, 20 and 33 km upslope from D57 respectively (M. Pourchet, personal communication). These data are representative of between 4 and 28 yr of present-day accumulation. The mean age of the gas corresponding to this approach is shown in Table 1 and Fig. 1*a*. The second approach takes into account two exceptionally marked sulphate horizons recorded at 48 and 50 m below the surface[7]. According to Legrand and Delmas (personal communication) (1) these two horizons are undoubtedly volcanic and have also been recorded in the Dome C and South Pole cores

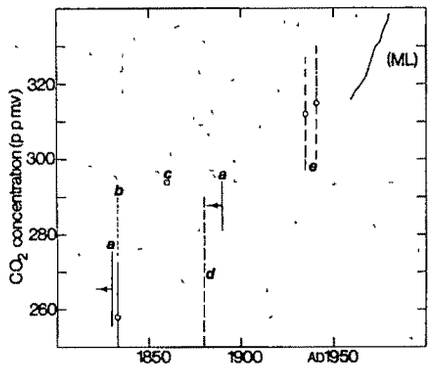


Fig. 2 Comparison between the CO_2 results from the D57 ice core and other estimates for the period since AD 1830. For the D57 results (*a*) only those ages in Fig. 1*b* for which we have most confidence for this time period are plotted. *b*–*e*, Estimates of past atmospheric CO_2 concentrations (discussed in refs 8 and 9) based on: *b*, the analysis of sub-surface ocean waters; *c*, a model calculation extrapolating from ML by assuming fossil-fuel combustion to be the only source of CO_2; *d*, the interpretation of some early chemical measurements of atmospheric CO_2; and *e*, preliminary analyses of archived solar spectra. ML is the atmospheric CO_2 record observed at Mauna Loa since AD 1958 (ref. 10).

which are independently datable with much more confidence than the D57 core; (2) the most likely candidate for one of these events is the Tambora eruption, AD 1815. This AD 1815 level at ~48–50 m below the surface would correspond to a mean accumulation rate of ~18 cm of ice equivalent yr^{-1} during the past 165 yr. We have tentatively calculated (Table 1) and plotted in Fig. 1*b* what would be the mean age of the gas if the accumulation rate remained constant at this value for the whole time period encompassed by the core. The two approaches give important differences (from 50 to 220 yr) in the mean age of the gas for the levels measured in CO_2. This clearly demonstrates the large uncertainties in the dating of the gas trapped in the D57 core. Nevertheless, the sulphate horizons found at 48 and 50 m below the surface are an important constraint for the dating of the upper part of the core and consequently, for the youngest part of the CO_2 record, we have more confidence in the timescale shown in Fig. 1*b*.

Whatever the dating uncertainties, this first detailed CO_2 record over the past few centuries from ice-core analysis indicates (Fig. 1) that the atmospheric CO_2 concentrations were at a minimum some time between AD ~1650 and 1850, with a mean value of ~260 p.p.m.v. This value must be compared with the ~295 p.p.m.v. calculated for the pre-industrial level (AD 1860 and before) by extrapolating observed CO_2 values from 1958 to the present and assuming that fossil fuels were the only sources[8,9]. The difference between the measured values from the D57 core and the results of this calculation strongly suggests that another important source has been active during the period preceding any significant use of fossil fuels, even if we have to correct systematically the measured values by +15 p.p.m.v. Taking into account the two youngest CO_2 levels plotted on Fig. 1*b* against the age that we favour for this part of the record, the main origin of this source may very well be the agricultural 'revolution' which began seriously to modify the biosphere during the past century.

Another interesting feature of the D57 core is, as shown in Fig. 1, that a mean decrease of the order of 10 p.p.m.v. may have occurred over a period of a few centuries before AD 1800. This fluctuation may reflect natural changes in the biospheric and/or oceanic CO_2 reservoirs which are possibly linked with the suggested worldwide Little Ice Age. This result suggests that the so-called 'pre-industrial' CO_2 level was not constant even over the few centuries preceding the important anthropogenic perturbation of the various CO_2 pools.

In the search for the atmospheric CO_2 level during pre-industrial times (before AD 1860, and on the scale of a few centuries), other kinds of records have been used, including the chemical measurements of atmospheric CO_2 performed during the second part of the nineteenth century, the $\delta^{13}C$ record of tree rings, and the chemical analysis of sub-surface ocean waters. A recent critical review[8,9] of the different types of record of the atmospheric CO_2 concentrations from pre-industrial times to 1958 concluded that CO_2 analysis of the ice cores currently seems to be the best, and most direct, method of determining the atmospheric CO_2 concentration before AD 1900. We can consequently consider that the D57 results presented here provide a most representative record of the atmospheric CO_2 background level for the 'pre-industrial' period.

Finally, the D57 record is also the first ice-core analysis to indicate clearly the increase in atmospheric CO_2 due to the burning of fossil fuels and probably also due to the anthropogenic influence on the biosphere over the nineteenth and twentieth centuries. Figure 2 shows this increase as recorded in the D57 core since AD ~1850, compared with other estimates discussed in refs 8 and 9.

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Structure of the ammonia synthesis catalyst

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The synthesis of ammonia from hydrogen and nitrogen over a reduced iron oxide catalyst is so well known[1–5] and so widely used (current world capacity is close to 100×10^6 tons per year) that it may seem surprising that the mode of operation of the catalytic remains enigmatic. That both H_2 and N_2 must first dissociate at the catalyst surface is beyond dispute, but there is debate as to the role of various promoters (Al, K, Ca) which greatly improve the catalytic performance when added to the precursor Fe_3O_4. Although it is widely accepted that regions of paracrystallinity exist within the catalyst, no one has questioned its overall crystallinity. In this work, which entails *in situ* X-ray diffractometry, we provide evidence that the active catalyst contains substantial amounts of a non-crystalline phase. Without promoter, reduction of Fe_3O_4 in the same conditions yields only crystalline α-iron.

Even though there have been more than 100,000 catalyst formulations for the synthesis of ammonia, the catalyst of choice still remains that selected for the original Haber–Bosch process,

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promoted iron. How do these promoters exert their influence? The likely role[6] of the alkali is to facilitate the dissociative chemisorption of nitrogen, the rate-determining step. The present consensus view[5,7] on the role of alumina is that it serves as a textural promoter by stabilizing the α-iron phase, and preventing it from sintering, after its formation by reduction in the reactant gas stream, from the fused oxide precursor. In particular, it has been argued[8] on the basis of *ex situ* X-ray diffraction studies, that individual spinel units ($FeAl_2O_4$) are built into the active α-Fe catalyst and that these units cause internal strain giving rise to the formation of so-called paracrystals. This paracrystalline state is characterized by slightly broadened X-ray diffraction lines, a fact confirmed recently in another study[9]. Note, however, that these investigations were performed with reduced catalysts which had been re-oxidized before the measurements. Model studies with iron single crystal surfaces revealed the highest activity for the (111) surface plane[10], and the highest rate of nitrogen chemisorption is exhibited by this plane[11]. The presence of potassium, however, was found to eliminate the activity differences between various crystal planes (without affecting their structures)[6]. In view of these findings, discussions as to which crystallographic faces of iron are chiefly involved in ammonia synthesis at real (that is, promoted) catalyst become less cogent. This will be underlined by the present results which shed new light on the structure of the catalyst in working conditions. They also prompt us to re-examine the accepted picture that the function of the Al_2O_3 promoter is to stabilize the α-Fe phase in the synthesis catalyst. The evidence suggests that the active state of the promoted iron catalyst could be non-crystalline. The catalyst possesses essentially no long-range atomic order in reaction conditions. Our results also show that a typical promoted catalyst precursor contains at least five distinct crystalline phases before reduction.

The experimental evidence has been acquired using, principally, *in situ* X-ray powder diffractometry, rendered possible by adapting a commercial cell[12], capable of repeated rapid and slow scans of 2θ (scattering angle) operating at temperatures of up to ~550 °C and pressures of up to 1 bar. This set-up, which readily permits data accumulation, is a more sensitive apparatus than earlier versions[13] and is capable of operation at temperatures higher than those explored, also *in situ*, by Mosesman[14]. Other measurements involving Mössbauer spectroscopy, physical adsorption, kinetic studies of ammonia formation rates at high pressure as well as a range of electron microscopic techniques all point to the catalytically active phase of iron being amorphous, in the sense that splat-cooled metals and alloys are amorphous. Full details of our work will be presented elsewhere. Here we concentrate on the results of the X-ray studies that have been carried out on typical industrial ammonia synthesis catalysts (provided by BASF (catalyst number MUC) and ICI).

The X-ray diffractogram for typical promoted BASF and ICI unreduced catalysts containing 2% Al_2O_3, 2% CaO and 0.5% K_2O shows predominantly *hkl* reflections for Fe_3O_4. However, a diffractogram which took ~100 h of recording time and involved three separate specimens with overlapped regions of 2θ (owing to suspected X-ray-induced radiation damage) shows the presence of a large number of weak diffraction peaks arising from minority phases. Analysis of such multiphase mixtures is not straightforward, and an unambiguous assignment using solely the diffractograms and the Joint Committee on Powder Diffraction Studies data file is not possible. When combined with knowledge of the known chemistry of Fe/Al and Ca/Fe oxides and previous information concerning the presence of CaO in promoted catalysts, the minority phases so identified were: $FeAl_2O_4$, $FeAlO_3$, CaO and $K_2Fe_{22}O_{34}$. Alternative assignments are possible, and could, for example, include $CaFe_3O_5$ and $KHCO_3$, but the presence of the bicarbonate can be safely discounted since there is no (analytically measurable) carbon present in the catalyst.

Unpromoted catalysts yielded diffractograms containing reflections from Fe_3O_4 only. When these unpromoted catalysts were heated in 1 bar H_2 (30 cm^3 min^{-1}) up to 500 °C, the resulting diffractogram, recorded at this temperature with the catalyst still exposed to the H_2 atmosphere, revealed (Fig. 1) peaks attributable to α-Fe.

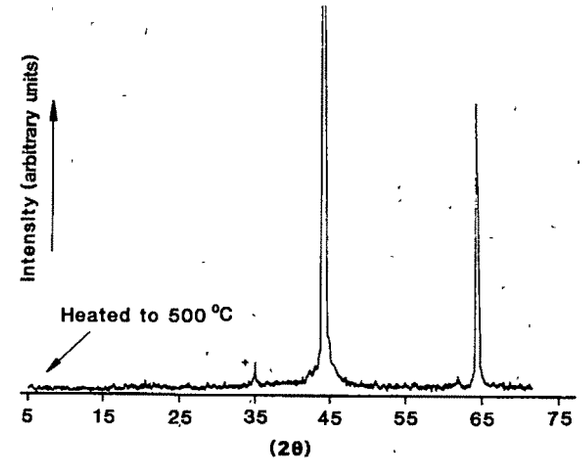


Fig. 1 Diffractogram (recorded at 500 °C) of unpromoted magnetite catalyst that had been heated in 1 bar H_2 up to 500 °C. The main peaks are attributable to α-iron. (The peak marked + is a spurious reflection from the high-temperature attachment.)

When the diffractograms were recorded with the promoted catalysts exposed to N_2 at a pressure of 1 bar and at temperature up to 500 °C, they were indistinguishable from the patterns of the starting material. Dramatic changes in the diffractograms were, however, seen when the promoted catalysts were heated in either 1 bar of H_2 or 1 bar of $3H_2:1N_2$ mixture (Fig. 2). At ~425 °C for the BASF catalyst and at ~460 °C for the ICI catalyst, very broad diffraction peaks (~10 times as wide as those previously reported[8,9] for paracrystalline iron) were obtained.

These diffraction patterns are very different from those expected for finely divided or paracrystalline α-iron. No intense reflections are observed, but instead there are a series of weak and broad features. The most surprising feature is a broad diffraction peak at 2θ ~25°. There are no allowed diffraction lines for αFe at this angle and hence it cannot be interpreted in terms of extremely finely divided crystalline iron. Experiments monitoring the weight loss during reduction confirm that the material is essentially fully reduced (±1%) during our experiments and so the features displayed must be caused by a phase

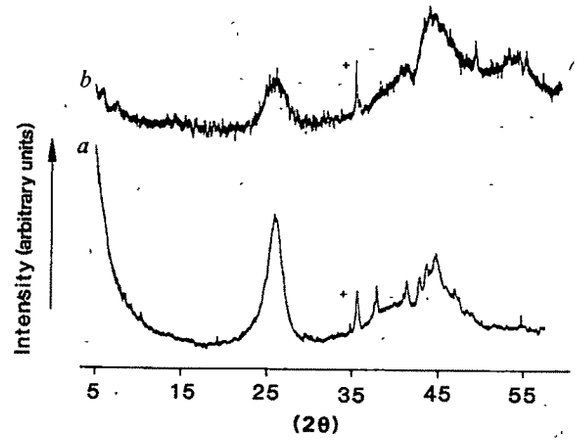


Fig. 2 Typical diffractograms of reduced promoted magnetite catalysts. *a*, Heated to 500 °C; *b*, subsequently cooled to 400 °C. These broad diffraction peaks are much wider than any previously reported (see text). A new rather broad peak (at 2θ ~25°) also appears irrespective of whether the reduction is carried out, at 1 bar, in either H_2 or $3H_2:1N_2$. The sharp peaks superimposed on the broad background arise chiefly from small particles of crystalline $FeAlO_3$. (The peak marked + is a spurious reflection from the high-temperature attachment.)

which contains predominantly iron. The shape of the peak at 2θ 25° seems to depend on the conditions used during reduction (the temperature and gas flow), but we will not consider this in detail here because the effects may be obscured by the deviations from parafocusing caused by the sample expansion, and by the tendency for the sample to fall off. The diffraction pattern shows a striking similarity to that of some glassy metal alloys such as Fe/Ni/B which display a broad diffraction feature at 2θ ~24° when doped with a small quantity of silicon[15]. The simplest explanation of the data observed here is the formation of an amorphous iron-containing phase which is stabilized by the incorporation of small quantities of dopants that may arise from the promoter additives or from residual oxygen.

It is also possible to propose more complex assignments for the data, based on the fact that the broad feature at 2θ ~45° arise from allowed diffraction peaks of αFe. Thus, microcrystalline particles of αFe (~20 Å diameter) may be present together with an additional new phase of iron giving rise solely to the peak at 2θ 25°. Evidence for this view is less compelling than the simpler explanation that the material is amorphous. Whilst the diffraction pattern is dominated by the 'amorphous' features, the presence of some residual crystalline phases is indicated by small but comparatively sharp peaks in the region of 2θ from 35° to 55°. These have been identified as arising for the most part from $FeAlO_3$. Through the viewing port of the X-ray camera, the promoted catalysts were seen to expand rapidly during the reduction in H_2 at elevated temperature, a fluffy black powder resulting after the expulsion of water. These changes occurred concomitantly with the changes in crystallinity, detected by the repeat-scan X-ray diffractometry.

The diffractogram in Fig. 2*a* was recorded over an 18-h period with the sample exposed to 1 bar H_2 at 500 °C. The essential features of this diffractogram are obtained with the catalyst exposed to $3H_2:N_2$ mixtures at elevated temperature (~425 °C) and are retained when the catalyst is cooled to room temperature in a non-oxidizing (H_2 or H_2–N_2 mixture) atmosphere. The catalyst spontaneously oxidizes on exposure to air even at room temperature and forms Fe_3O_4 indistinguishable from the non-reduced precursor. Other measurements (and all handling) were, therefore, carried out under a nitrogen atmosphere.

That this non-crystalline, reduced iron can be a catalytically active phase was confirmed by kinetic measurements ($3H_2:1N_2$ at total pressure of 9 bar in the temperature range 350–450 °C). At 400 °C, conversion rates were ~10 μmol of NH_3 per g of catalyst per s. This amorphous catalyst, nevertheless, retained some morphological texture, possibly because the coexisting microcrystalline $FeAlO_3$ there consist of numerous platelets (~200 Å dimensions); and, by transmission electron microscopy, we observed some mesopore structure. This is reminiscent of the small particles (~300 Å diameter) seen[16] by scanning electron microscopy. No Mössbauer signal could be obtained[17,18] in conditions which yielded clear signals from other iron-containing catalysts: this is what one expects from a catalyst that is more similar to a glass than a solid.

Our *in situ* X-ray diffractometric studies show that a promoted ammonia synthesis catalyst produced by reduction in hydrogen is largely non-crystalline interspersed with microcrystals of $FeAlO_3$ and other promoter phases.

Whilst more work remains to be done to elucidate the nature of the non-crystallinity, the precise effects of water vapour and the influence of $FeAlO_3$ during the reduction[7,9], it is clear that the degree of coordination of a surface Fe atom in the catalyst, and the apparently central role assigned to (111) faces, needs to be reconsidered.

If the main phase in the active promoted catalyst is not pure elemental iron and impurities such as promoter additives or residual oxygen in a total concentration of ~1% permit the formation of a disordered phase distinctly different from a material containing small particles of crystalline αFe, many of the numerous other catalyst formulations previously discarded could, in appropriate conditions of preparation, turn out to be very efficient.

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Liquid-phase polymer-based retention — the separation of metals by ultrafiltration on polychelatogens

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The importance of trace-element analysis in geological, biological, environmental and industrial processes has been increasingly recognized. Although modern instrumental methods of analysis have been applied, preliminary enrichment techniques are required to separate the components to be determined from the interfering constituents of the sample[1,2]. For the separation of inorganic ions contained in natural waters, industrial fluids or dissolved solid materials, solvent extraction, sorption, ion exchange, precipitation and other methods have been used. However, techniques based on a two-phase distribution or on heterogeneous reactions often cause problems because, in contrast with homogeneous reactions, diffusion controlled processes must be used. Until now, no simple, rapid method has been available for the preconcentration and separation of metals in the homogeneous aqueous phase. Here we demonstrate a technique for the enrichment and separation of metals in aqueous solution using soluble non-crosslinked polymers with chelating groups (polychelatogens)[3,4] called liquid-phase polymer-based retention (LPR).

A variety of water-soluble polymeric reagents with strong complexing properties have been synthesized and used, in combination with membrane filtration, to separate complex from non-complex metal ions in the homogeneous phase. The LPR method is based on the retention of metal ions by a membrane which separates macromolecules from low relative molecular mass compounds as the complexes form with the polymer from a sample solution.

The experimental arrangement is outlined in Fig. 1. The system consists of the membrane filtration cell, containing the polychelatogen solution, to which the sample is added. For separation and enrichment, the washing solution is passed to the cell from reservoir I under stirring. The filtrate contains all the metal ions which are not retained as macromolecular complexes by the polymer reagent. Large volumes of very diluted samples are first enriched in respect to metals of interest by passing the solution from reservoir II to the much smaller volume of cell solution. Thereafter, the additional separation process is carried out by adding washing fluid from reservoir I to remove non-bound metals from the cell.

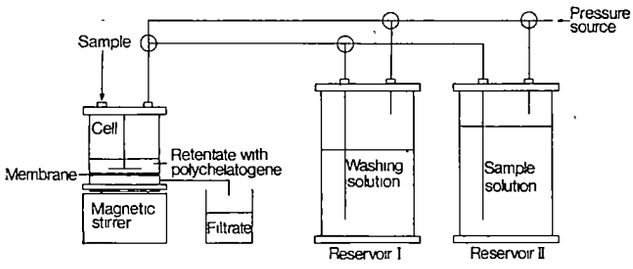


Fig. 1 The principle of metal separation with polychelatogens (from Chemische Fabrik Heyl) using membrane filtration. The sample solution is placed into the cell which contains an aqueous solution of polychelatogen (polymer reagent with chelating or other complexing groups). By adding the washing solution from reservoir I separation of metals is achieved by the selective retention of some of them in the retentate and the washing out of the others in the filtrate. Preconcentration of metals can be performed by passing the sample solution from reservoir II to the cell. The cell solution is then washed with the liquid from reservoir I.

The ultrafiltration cell is fitted with membranes, which retain molecules of molecular mass fractions greater than 10,000 (Berghof BM 10 or Amicon PM 10). Poly(ethyleneimine), poly(ethyleneimine-diacetic acid), poly(vinylpyrrolidone-*co*-vinylaminobutyric acid), poly(ethyleneimine-methylthiourea), and poly(acrylic acid-*co*-allylthiourea) act as polychelatogens in the cell as 1% aqueous solutions[3]. The average molecular mass of the polymer reagents is in the range 30,000–400,000 g mol^{-1} (ref. 3). Capacity studies of the polychelatogens have been reported previously[3]. The unfunctionalized poly(ethyleneimine), for example, binds 0.6–2.4 mmol metal per g polymer depending on the *p*H.

The initial concentration of metals in the cell solution (40 ml) or in reservoir II varies from 0.1 to 10 p.p.m.; the *p*H of the cell and reservoir solutions are adjusted to the same value. The system is pressurized (300 kPa), the cell solution stirred for 5 min and then washed with the reservoir fluid at a flow rate of 4–6 ml min^{-1}. The metal contents of filtrate fractions and cell solution are analysed by atomic absorption spectrometry. Other analytical methods in solution can be used, in principle, for metal analysis of the retentate; electroanalysis is also applicable[4].

The filtrate analysis data are used as the basis of the elution diagrams (Fig. 2) which show the metal concentration in the filtrate as a function of filtrate volume. If no metal can be detected in any filtrate fraction, complete retention has been achieved. When there is no retention, elution curves are obtained which can be approximated by the dotted curve in Fig. 2*b*. That curve is described by the exponential function:

$$C_f = C_0 e^{-V_f/V_0}$$

where C_f denotes the concentration of metal in the filtrate, C_0 the initial metal concentration in the cell, V_f and V_0 are the volumes of filtrate.

We have investigated the retention and separation of several metals using different polychelatogens. Typical results are shown in Table 1 for Co, Ni, Zn, Cd and Cu. Metal retention showed

Table 1 Retention of metal ions by poly(ethyleneimine) in the homogeneous phase at different *p*H values

	Retention (%)						
*p*H	Co^{2+}	Ni^{2+}	Zn^{2+}	Cd^{2+}	Cu^{2+}	Mg^{2+}	Na^{+}
2.1	1.2	<0.1	1.0	<0.1	86.3	—	—
3.2	16.3	21.3	0.5	0.7	>99.0	—	—
4 0	93.1	>99.0	—	98.1	>99.0	—	—
5.3	>99.0	>99.0	>99 0	>99.0	>99.0	<0.1	<0.1

Polymer concentration, 1%; initial metal concentration, 10 p.p.m.; filtrate volume was equal to 10 volumes of cell solution.

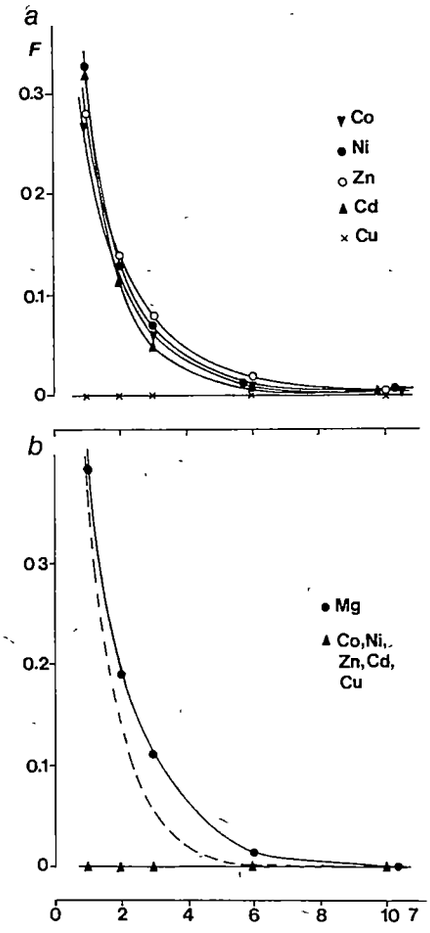


Fig. 2 Elution diagrams for some divalent metals separated by poly(ethyleneimine) using membrane filtration. Filtration quotient $F = C_f C_0^{-1}$ is plotted against the ratio $Z = V_f V_0^{-1}$ at *p*H 3.2 (*a*) and 5.3 (*b*); C_f and V_f, V_0 are concentration and volume of filtrate and cell respectively. Polymer concentration, 1%, $C_0 = 10$ p.p.m. Dotted curve, elution of an arbitrary metal which is not retained in the cell.

a strong dependence on *p*H, experiments were, therefore, performed at different *p*H values in the range 2–6. For example, >80% Cu was retained at *p*H 2.1 with $V_f = 10\ V_0$ (Table 1). Complete retention of Cu was achieved at *p*H 3.2, whereas the other four metals were washed out in the filtrate (Fig. 2*a*, Table 1).

The elution profiles of the metals Ni, Zn, Cd and Co are similar to the theoretical curve in Fig. 2*b*. At higher *p*H values, these metals are retained completely by poly(ethyleneimine); thus, they can be separated from other elements not bound to the polymer. For example, Mg and Na ions, which do not form stable complexes with this polymer, are washed out even at *p*H 5.3 (Table 1).

Other polychelatogens with different functional groups have proved to be effective for metal retention using membrane filtration. Poly(ethyleneimine-diacetic acid) can retain 90% Cu at *p*H 2.2 and >99% at *p*H 5. At this *p*H value, Cu is also complexed effectively by the *N*-methylthiourea derivative of poly(ethyleneimine). Moreover, this reagent allows the quantitative retention of Cd and Hg. Using the copolymer of acrylic acid and *N*-allylthiourea, Hg is even retained at *p*H 1.9. Poly(vinylpyrrolidone-*co*-vinylaminobutyric acid) showed a lower retention tendency than do the other polychelatogens studied.

To apply this procedure to the analysis of very diluted samples it is important to be able to preconcentrate the elements to be determined. The LCP technique allows preconcentration by using two reservoirs as depicted in Fig. 1. To demonstrate this, a model fluid containing metal ions and 3% of NaCl (concentration in seawater) was passed from reservoir II to the cell. The

volume of the sample fluid was 10 times higher than that of the cell solution.

After 10-fold preconcentration, the cell solution was washed with distilled water from reservoir I. At a 10-fold volume of filtrate referred to the cell fluid >98% of Hg, Cd, and Cu were retained by poly(ethyleneimine-methylthiourea), whereas poly(ethyleneimine) retained 75–95% of Cu, Ni, Zn, Cd and Cu. Note that the concentration of Na in the cell was as low as 3–5 p.p.m. after the washing. This means that the Na concentration decreased by 10^4 times and that a factor of 10^5 (relative preconcentration factor) for the metals retained is achieved when taking into account the first preconcentration step (absolute preconcentration). Another absolute preconcentration step (up to one order) can be applied by reduction of the cell solution volume using conventional ultrafiltration. In addition, it is an advantage for the analysis that the sample can be handled in the homogeneous phase

In conclusion, these polymeric reagents in combination with membrane filtration allow the enrichment and separation of trace elements in the presence of comparable and large excess quantities of sample constituents which could interfere with subsequent analysis.

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Evidence for an increase in cosmogenic ^{10}Be during a geomagnetic reversal

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Reversals in the geomagnetic field, which occur every few hundred thousand years, represent a dramatic change in the Earth's environment. Although there is no satisfactory theory for such reversals, it is generally accepted that the dipole field intensity decreases to <20% of its 'normal' value for a few thousand years during the change in direction[1]. Because the galactic and solar cosmic rays which impinge on the Earth's atmosphere are charged, a significant fraction (about half) of them are deflected by the geomagnetic field[2]. At the time of a reversal, this magnetic shielding is greatly reduced, and it has been suggested that the increased flux of high-energy particles could have effects on evolutionary[3] or climatic[4] processes. For example, the statistically significant coincidence in levels of some marine faunal extinctions and reversal boundaries in ocean sediments[5] could be caused, directly or indirectly, by the decreased geomagnetic intensity during the reversal. We report here evidence in marine sediments for an increase in cosmogenic ^{10}Be production in the Earth's atmosphere during the Brunhes–Matuyama reversal 730,000 yr ago. In addition to confirming an increase in cosmogenic isotope production, the results provide information on the magnitude and duration of the geomagnetic intensity decrease during such an event, and the depth at which remanent magnetism is acquired in marine sediments.

In previous work, we found no significant evidence for increased ^{10}Be (half-life 1.5 Myr) at a reversal boundary[6]. Among the explanations we gave for this negative result was that the sedimentation rate of the core studied (1.13 cm kyr^{-1}) was not high enough to retain a significant signal. We have thus looked at a core having a sedimentation rate of ~2.5 cm kyr^{-1}. This core, V16-58, was taken at 46°30′ S, 31°16′ E at a depth of 4,731 m. The material near the reversal zone is mostly diatomaceous lutite with thin interspersed layers of calcareous ooze[7]. The identification of the reversal level at ~1,129 cm as the Brunhes–Matuyama boundary, and the estimation of the sedimentation rate at this level are based on the biostratigraphy of Hays[7], with more recent studies putting the 425,000-yr extinction level of the radiolarian *Stylatractus universus*[8] at 420 cm (J. Morley, personal communication). The top part of the core has apparently suffered from a reduced sedimentation rate, hiatus or erosion.

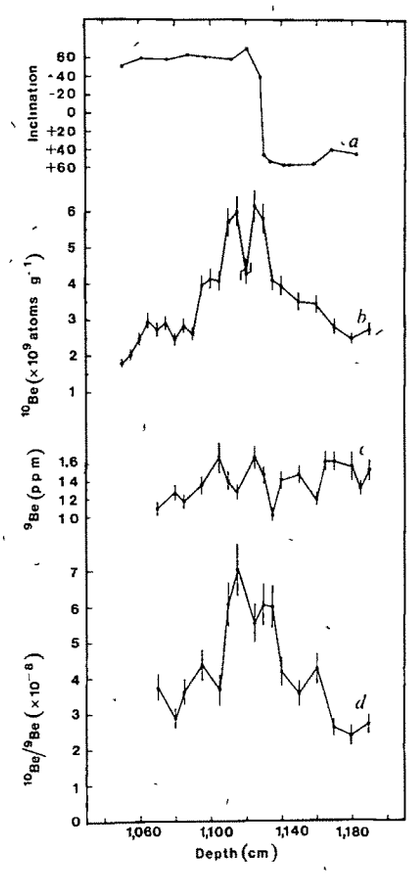


Fig. 1 *a*, Magnetic inclination; *b*, ^{10}Be concentration; *c*, ^{9}Be concentration; *d*, ^{10}Be/^{9}Be ratio as a function of depth in core V16-58.

Initial magnetic measurements, indicating a reversal level at ~1,129 cm, as shown in Fig. 1, were carried out using procedures described in ref. 9. After the ^{10}Be results became available, the core was resampled and the position of the reversal confirmed. A more detailed palaeomagnetic investigation around the reversal level is now in progress.

For the ^{10}Be measurements, samples of 0.3–1 g were dissolved in the presence of 0.5 mg of ^{9}Be carrier, and chemical purification carried out as described earlier[6]. The ^{10}Be measurements were made on a Tandetron accelerator mass spectrometer facility, using the procedure described in ref. 10. Several thousand ^{10}Be events were recorded for each sample, and the estimated uncertainties are due to instrumental variations, as deduced from replicate measurements of standards[10]. The results are shown in Fig. 1. The low value at 1,120 cm during the initial series of measurements was so surprising that we processed and measured a second portion of the same sample. The good agreement between the two results both confirms the low value for this sample, and gives us added confidence that our assigned uncertainties are realistic.

As can be seen in Fig. 1, the ^{10}Be concentration increases by a factor of ~2 for samples near the reversal level. Before

ascribing this to a production increase, it is useful to consider other possible factors which could effect a change in ^{10}Be concentrations. For example, a change in the biological productivity, possibly associated with the reversal, could give rise to a variable biogenic component (opal or calcium carbonate) in the sediments; this would probably give a variable dilution of the ^{10}Be. Another possibility is that some change in environmental conditions might lead to an 'enrichment' of beryllium compared with other components in the sediment. To examine these alternatives, we have measured the stable isotope ^{9}Be over the same interval as the ^{10}Be. The ^{9}Be was measured by flameless atomic absorption spectrometry. Uncertainties in the ^{9}Be concentrations are estimated from the reproducibility of the measurements (in all cases except one, the results are the average of at least two determinations). The decision to measure ^{9}Be was made after the completion of the ^{10}Be measurements. Unfortunately, this meant we did not have material remaining for some of the levels, including the critical minimum at 1,120 cm, in which ^{10}Be had been determined.

Although there are variations in the measured ^{9}Be, these do not parallel those of ^{10}Be. In fact, the ^{10}Be/^{9}Be ratio, also given in Fig. 1, shows a peak similar to ^{10}Be alone. Unfortunately, this normalization may not be completely satisfactory. Although the oceanic geochemistry of ^{9}Be is still poorly known, we believe[11,12], contrary to Measures and Edmond[13,14], that a significant fraction of ^{9}Be may be deposited in marine sediments without coming to equilibrium with ^{10}Be. In such a case, the ^{9}Be would be an imperfect tracer for ^{10}Be, although it still should compensate for a variable biogenic component.

While the ^{10}Be/^{9}Be evidence strongly supports the interpretation of the observed increase of ^{10}Be concentration as being due to increased production, it is not necessarily a better monitor of ^{10}Be production variations. However, because of the similarity in the two curves (except for the minimum at 1,120 cm), the conclusions that follow are relatively insensitive to which one is used.

We also note that both ^{10}Be concentration and ^{10}Be/^{9}Be are of potential interest as dating tools[11,15]. The strong variation in these parameters observed here demonstrates that caution must be exercised when dating by such procedures near reversal levels. In a preliminary study, Ku *et al.*[15] have also observed a high ^{10}Be concentration in a single sample near the Brunhes–Matuyama reversal level. However, they also found an abnormally low concentration in a sample nearby, so the origin of their variations is not clear.

The ^{10}Be (or ^{10}Be/^{9}Be) signal is not expected to reflect directly the geomagnetic palaeointensity profile for two reasons. First, the effect of the magnetic field on production rate is a function of geomagnetic latitude[2]. The degree to which mixing in the atmosphere and the ocean average out this variation is not yet established. However, the mid-latitude location of V16-58, in a region of significant input of stratospheric aerosols, means that its sediment probably should reflect a reasonably 'average' production effect. Second, once the ^{10}Be is incorporated in the sediment, it is subject to mixing processes by benthic organisms. To illustrate the potential effect of this bioturbation, we adopt a model[16] in which the sediment is assumed to be completely homogenized over a given depth, called the mixed layer, with no mixing below this depth. A more sophisticated model has been proposed which treats the mixing process explicitly by diffusion[17]. However, we do not have independent information on the appropriate diffusion constant for this core. For diffusion constants >100 cm^2 kyr^{-1} the uniform mixing assumption is a good approximation to the diffusion model.

Adopting a mixed-layer thickness of 10 cm, we calculated output profiles for several different assumed input profiles. Figure 2 shows an example of one of these which fits the observed ^{10}Be profile reasonably well. As can be seen in Fig. 2, the bioturbation has three effects. It attenuates the maximum increase in production rate; it tends to smooth out rapid variations; and it moves the ^{10}Be levels down by approximately the assumed mixing depth.

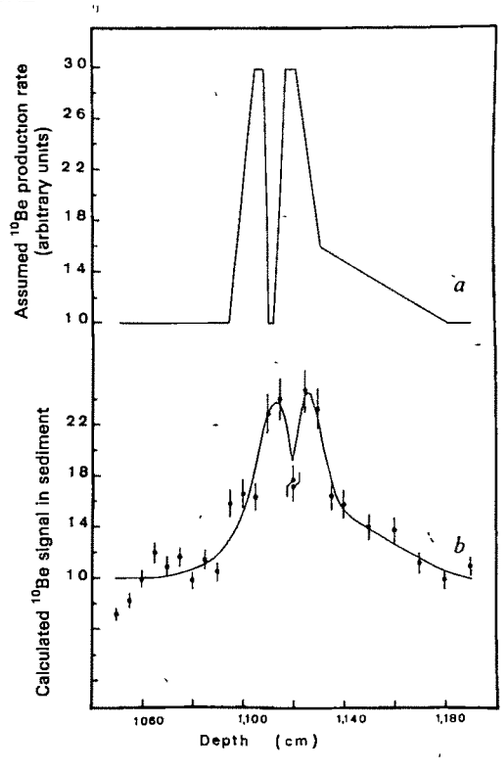


Fig. 2 *a*, Assumed ^{10}Be production rate profile; *b*, solid curve is calculated signal in sediment, using the bioturbation model described in the text, with a mixing depth of 10 cm. Points are experimental data from Fig. 1.

The input profile of Fig. 2 is, of course, not unique, and others which fit the experimental data equally well, or better, could be found. However, we do have some constraints. First, the maximum increase in production during the reversal cannot be significantly larger than the factor of 3 we have chosen, which corresponds essentially to a zero-intensity magnetic field[2]. Second, the total area under the input and observed curves must be the same. Thus, the input function in Fig. 2 should represent at least the major features of the actual production curve. In particular, the mean duration for the production increase (and thus magnetic intensity decrease) should be approximately correct. As can be seen in Fig. 1, the interval of increased ^{10}Be is significantly longer than the time taken for the reversal of inclination. Such a result is consistent with other evidence which suggests that the geomagnetic field intensity is low before and after the change in inclination[1,18]. From Fig. 1, the interval of increased production is ~30–60 cm, depending on what level of increase is chosen. Assuming a sedimentation rate of 2.5 cm kyr^{-1}, this interval corresponds to ~12,000–24,000 yr; this is similar to the time interval for the decreased magnetic field at another reversal, as estimated in ref. 18.

As can be seen in Fig. 2, the minimum at 1,120 cm implies a very significant decrease in production. However, the magnitude and duration of this decrease are obviously very strongly dependent on the parameters of the bioturbation model used. Thus, even if this decrease is due to an increased field intensity, the quantitative nature of the required change remains uncertain. It is interesting, however, that other authors have also found evidence for high intensities midway through reversals[19,20]. More definite conclusions along these lines will necessitate more detailed ^{10}Be and palaeomagnetic profiles, comparison between several cores and more reliable bioturbation modelling. With regard to the latter, although the bioturbation depth used here is fairly typical[16,17], we wonder whether it can be reconciled with the rather abrupt variations seen in ^{10}Be, ^{9}Be and calcium carbonate[7], over intervals as small as 5 cm. Piseas has recently made the same point regarding oxygen-isotope profiles in other cores[21]. Reduced bioturbation would mean that the actual ^{10}Be

production profile would approach more closely the observed ^{10}Be profile.

As we have discussed earlier[6], the location of the ^{10}Be peak compared with the reversal level can also provide information on the depth at which remanent magnetization is acquired in marine cores. In the present case, it can be observed that the midpoint in the ^{10}Be peak is ~10 cm above the change in magnetic inclination (or ~15 cm if we use the input profile of Fig. 2). Neglecting the residence time of ^{10}Be in the ocean[22], and assuming that the ^{10}Be midpoint corresponds to the change in direction, this would indicate that the magnetic remanence was 'locked in' at a depth of ~10–15 cm in the sediment (or slightly more, allowing for later compaction). This is consistent with the idea that the magnetic remanence is acquired at the base of the bioturbation zone.

We have presented the first direct evidence for increased cosmic ray radiation in the Earth's atmosphere during a geomagnetic reversal. Making some reasonable assumptions, the data also allow one to deduce significant information on the magnitude and duration of the magnetic intensity decrease during a reversal, and the depth at which detrital magnetic remanence is acquired in a marine sediment. It will be interesting to pursue this type of study at other reversal boundaries to determine whether there are significant differences in their intensity profiles, and, if so, whether they could be correlated with other features such as the severity of faunal extinctions. This work also demonstrates that ^{10}Be should be a potentially powerful tool for testing the global nature of various geomagnetic 'excursions' that have been observed during the Brunhes epoch[23].

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Re-dating the English art-historical tree-ring chronologies

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Precisely dated oak (*Quercus petraea* Liebl. and *Q. robur* L. and their hybrids) tree-ring chronologies that show consistent cross-dating from Germany to England to Ireland[1,2] have been labelled Type H[3]. In contrast, there have been difficulties in specifying the exact date ranges of some oak chronologies (termed Type A)[3] constructed from art-historical timbers in England[4,5] and the Netherlands[6,7]. It has been asserted that the oak planks and boards used in the English art-historical chronologies derived from England and Flanders[3,8]. The failure of these chronologies to date uniquely against Type H chronologies from the British Isles has led to suggestions that the timbers were imports, probably from the Baltic area[1,9], views supported by historical evidence[10,11]. It has also been suggested that the dating procedure used for these chronologies has an element of circularity which could result in an erroneous placement of the chronologies going undetected[12]. Evidence is presented here which indicates that the art-historical chronologies have been incorrectly dated, that they represent imports into England and Flanders and that failure to take account of exotic origins has led to the use of unsuitable estimates for missing sapwood.

In 1975, information on cold winters in the fifteenth century was derived from a section of art-historical oak tree-ring chronology, MC18, dated[13] to AD 1234–1550. This section of chronology had originally been designated[4] to the years 1230–1546 and subsequently reference chronologies related to MC18 (designated Refs1–4) were returned to this same '1546' dating[3,5,14]. These chronologies, all >270 yr in length, form an internally consistent group covering the period from the early twelfth to the early seventeenth centuries. At other times Refs1–4 have been moved 1-yr-back, equivalent to a '1545' dating[8,15,16] of MC18. The possibility of a circular argument in the dating of these art-historical chronologies has recently been discussed in an article which also gives documentary evidence for importation of oak planks and boards into England and Flanders from the Baltic during the later Middle Ages[12]. Since that publication, three new pieces of evidence relevant to these Type A chronologies have become available.

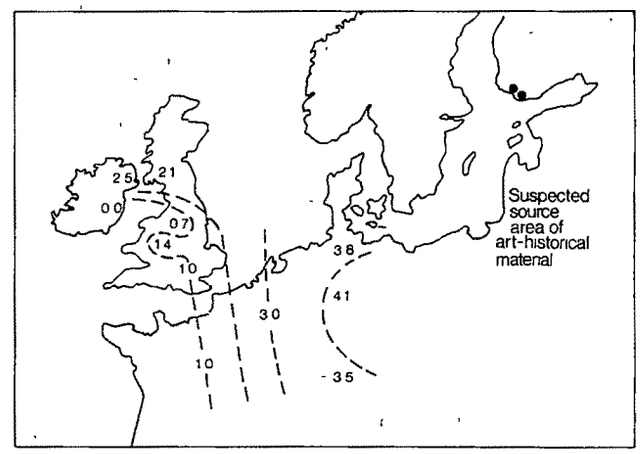


Fig. 1 Mean '*t*' values for the nine art-historical chronologies compared with 11 available Type H chronologies as in Table 1*c*, with the Type A chronologies in the 4-yr-forward or '1550' position. ●, The source of the Finnish oak sapwood values.

First, one of us (J.H.) acquired some medieval timbers from excavations at Ipswich. The mean ring pattern of three timbers was found to span the years AD 1128–1293 by cross-dating against the established, and absolutely dated, German Type H (after Huber[3]) chronologies of Eckstein (personal communication) and Delorme[17]. The correlation values between the Ipswich mean ring-pattern and these two chronologies, obtained using the Belfast CROS program[18], were '*t*' = 7.2 and '*t*' = 4.1 respectively for overlaps of 166 yr (normally '*t*' values >3.5 in association with overlaps of more than 100 yr are regarded as significant indicators of a likely tree-ring match). No significant

correlations could be found with any of the existing British Isles Type H oak chronologies. However, when this Ipswich ring-pattern was compared with the Ref4 Type A chronology[5], a significant correlation value ('t' = 5.3, overlap 166 yr) was obtained with Ref4 ending not in AD 1399 as published, but in 1403, that is, equivalent to the 4-yr-forward or '1550' dating used in 1975[13].

Almost immediately an exactly analogous situation was found by another one of us (M.B.). The mean ring pattern of two boards from a seventeenth-century painting of St Matthew was dated to the years AD 1326–1625 against the Type H German chronologies of Hollstein[19] and Becker[20] with 't' = 10.6 and 11.3 for overlaps of 300 yr in each case. When compared with the published Type A chronologies, significant correlation values were obtained at the '1550' position ('t' = 5.1 for Ref3, 't' = 4.6 for Ref2 with overlaps of 270 and 246 yr respectively).

Following these results, we re-examined the dating of the Type A chronologies. Most workers outside the art-historical field had assumed that more persuasive evidence must exist for the '1545' and '1546' chronology placements than for the '1550' placement and that all of the possibilities would have been explored by the art-historical workers. Table 1 shows the correlations obtained for all of the available Type A chronologies, namely MC18[4], Refs1–4[5] and variants NewRef1, NewRef2/3 and Ref2/3/5 (J. M. Fletcher, personal communication), when compared with the published Type H chronologies from Göttingen[17], Hamburg (ref. 21 and D. Eckstein, personal communication), South Germany[20], West Germany[19], Belfast[22], Scotland[23], Giertz[24], northern France (J. R. Pilcher, personal communication), English MC10 (J. M. Fletcher, personal communication), Sheffield[25] and Dublin[26] at the '1545', '1546' and '1550' positions.

Consistent with the Type A dating suggested by the Ipswich and St Matthew examples above, there is strong correlation between the Type A chronologies and the principal published German Type H chronologies at the '1550' position. There is much less agreement with chronologies from the British Isles at this position. There is no consistent agreement with the Type A chronologies placed at the '1545' or '1546' positions. Figure 1 shows how, at the '1550' placement, the mean correlation values for the Type A chronologies increase as they are compared with more easterly chronologies. The values are not high enough to support an origin for the art-historical timbers within Germany (see, for example, the correlations obtained for the St Matthew boards) but they clearly indicate a likely origin to the east of the German chronologies. This would be consistent with an eastern Baltic origin which has been suggested on historical grounds[12].

But why has this level of consistent correlation (against published chronologies) which indicates a '1550' dating been ignored and why was this same dating, used[13] in 1975, overridden? It is referred to as being "discarded on account of poor visual agreement"[5] and elsewhere the temporary use of the '1550' position is accounted for by a "4-yr periodicity in our oak chronology"[27].

In a '1550' re-dating of the art-historical chronologies, the time left between the last heartwood rings of surviving ring

Table 1 Correlation values obtained using the Belfast CROS program[18] for comparisons of nine art-historical chronology variants with precisely dated Type H chronologies

	MC18	Ref1	Ref2	Ref3	Ref4	Ref 1234	New Ref1	New Ref2/3	Ref 2/3/5	Mean
***a* 1-yr-back '1545' position**										
Göttingen	0.0	0 0	0 0	0 0	1.4	0 0	0 0	0.0	0.0	0.2
Hamburg	0.0	0.0	0.0	0 0	0 2	0.0	0.0	0 0	0.0	0.0
S. Germany	0.0	0.0	0.0	0.2	1 3	0 2	0.0	1.1	0 8	0 4
W. Germany	0.0	0.0	0.1	1.0	1.9	1.4	0.0	1 4	1.3	0 8
Belfast	1 0	2.7	1 5	0.0	0.5	1.4	1.8	1.8	2.4	1.5
Scotland	0.0	0.7	1.1	0.0	0.0	0 0	0.0	0 1	1.4	0 4
N. France	0 3	1.0	2.0	0.3	1.2*	1.4	0.1	1.6	1.6	1.1
England/Wales	0.4	1.0	3.3	1.7	†	2.1	0.7	2.4	2.2	1.7
Sheffield	0.0	0.6	1.5	0 9	†	1.1	0.0	1.1	0.3	0.7
MC10	0 0	0.6	1 5	2.8	†	1.9	0 2	2 1	1.2	1.3
Dublin	0 4	1 0	1 4	1.5	†	1.7	0.1	2.1	1.4	1.2
***b* '1546' position**										
Gottingen	0.5	0.4	0 1	0.0	2.2	1.7	1.4	0.0	0 7	0.8
Hamburg	1.0	1.7	1.0	0 0	2.9	2.5	2.0	0.1	1.1	1.4
S. Germany	2.1	1.8	1.3	0.8	3.8	3 2	2 5	0.0	1.3	1.9
W. Germany	0.7	1 0	1.4	0.0	2.3	1.5	1.4	0.0	0.6	1.0
Belfast	1.3	0.5	0.0	0.0	0.5	1.2	0.5	0.8	1.1	0.7
Scotland	0.1	0.0	0 0	0.0	0.6	0.0	0.0	0.0	0.0	0.1
N France	2.2	1 7	0.3	0.0	1.1*	0 8	2 4	0 0	0 0	1.0
England/Wales	0.0	0.0	0 0	0 1	†	0.0	0.1	0 0	0.0	0.0
Sheffield	0.0	0.0	0 0	0.0	†	0.0	0.0	0.0	0.0	0 0
MC10	0 0	0.0	0.0	0.0	†	0 0	0.0	0.0	0.0	0.0
Dublin	0.0	0.0	0.0	0.0	†	0.0	0 0	0 0	0.0	0 0
***c* 4-yr-forward '1550' position**										
Göttingen	3 3	1.8	4 5	4 6	3.7	5.3	3.3	4.2	6 1	4.1
Hamburg	3.4	2.7	2.7	2.4	4.8	5.9	3.5	2.8	5.6	3.8
S. Germany	3 2	2.0	4.2	4 7	2.6	4 8	2 8	4.0	3.4	3 5
W. Germany	2.2	2.0	2.7	4.1	2 4	4.1	2.4	3 5	3 2	3 0
Belfast	3.0	1 5	1 1	3.0	1.3	3.2	1.3	4.6	3.3	2.5
Scotland	1.8	2.2	0.0	1.5	3 6	4.0	1.7	1.0	2 7	2.1
N. France	0.0	0.5	2.0	1 5	0 0*	1 6	0 1	1 0	2.0	1 0
England/Wales	1.2	2 0	0 3	2.3	†	2.2	1.5	1.1	1.9	1 4
Sheffield	0.7	1 8	0.3	0.7	†	1.0	0.7	0.6	0.1	0.7
MC10	1.3	1.6	0.4	0 4	†	1 0	0.8	0.8	1.6	1.0
Dublin	0.0	0.3	0.0	0.0	†	0.0	0.0	0 0	0.0	0.0

Ref"1234" is the mean of the published[5] Refs1–4. 0.0 indicates zero or negative correlation

* An overlap of <130 yr. † An overlap of <65 yr. All other overlaps are >160 yr. *a*, With the art-historical chronologies at the '1545' position, *b* at '1546' and *c* at '1550'.

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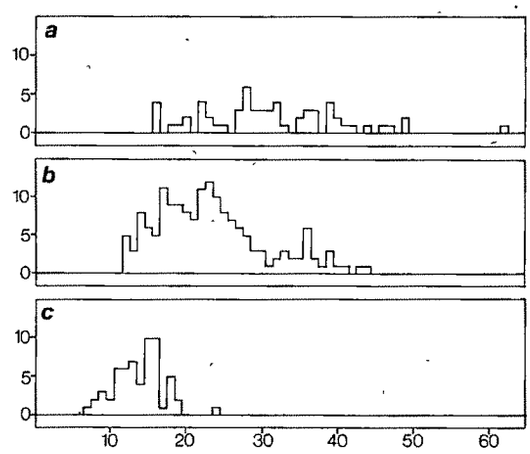


Fig. 2 Frequency of sapwood numbers in oaks from: *a*, England[12,28]; *b*, West Germany[29]; *c*, Finland.

patterns and the use dates for 'known age' paintings is shortened by 4 yr. In one of the examples used by Fletcher *et al.*[4] (Thomas, second Baron Wentworth, painted 1568), the '1550' chronology placement leaves only 20 yr between its last heartwood ring and 1568. This period has to account for both sapwood and seasoning factors. In the case of the First-Viscount-Montague panel (painted 1569) only 15 yr are left to account for possible missing heartwood, missing sapwood and seasoning. Such intervals would be unacceptably short for English panels given the numbers of sapwood rings observed on modern English oaks[12,28] and it may have been the erroneous belief in an English origin for the panels which made the possible '1550' dating unacceptable.

If our revised dating is accepted, it suggests that the number of oak sapwood rings in the area from which the panels derived must have been less than the numbers normally accepted in England. Sapwood on mature oaks tends to be shorter in Western Germany[29] and substantially shorter for Roman (mean 18, range 11–27) and Dark Age (mean 17, range 11–21) oaks in South Germany[20,30] We now have evidence for oaks from the eastern Baltic as the result of field work by members of the University of East Anglia Climatic Research Unit in 1984. The samples are 60 cores from 49 trees from two sites in southwestern Finland—Ruissalo and Solbole (all trees were *Q. robur*). The mean number of sapwood rings is 13.9 with a standard deviation of 3.2 and values range from 7 to 24. All trees were >120 yr in age. The mean number of sapwood rings from the Finnish sites is significantly smaller than the English sapwood values at the $P < 0.0001$ level (Fig. 2).

These results suggest that: (1) an eastern Baltic origin for the art-historical chronologies as suggested on historical grounds; (2) a movement of the art-historical chronologies forward to a '1550' placement; and (3) a very short sapwood interval are all compatible. When vindicated by subsequent work on Polish or Lithuanian timbers, these results make it inevitable that all of the art-historical datings derived using the '1545' or '1546' chronology placements, and the specifically tailored sapwood estimates favoured by some workers[15,31], will have to be revised. If the German art-historical dendrochronologists favour a '1548' chronology placement for their equivalent Netherlands II chronology[3], then all their dates will move forward in time by 2 yr. However, their estimated felling dates may have to be additionally revised in the light of the very short Baltic sapwood observations above.

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Patterns of mortality and age at first reproduction in natural populations of mammals

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There is great variation in the age at which females of different mammalian species first breed. Recent comparative analyses have focused on the relationship between age at first reproduction and body size[1–9], but differences in patterns of mortality experienced by natural populations are expected to have major effects on selection for age at first reproduction[10–14]. Here we show that the age at which females first reproduce is strongly correlated with expectation of life at birth, after the effects of body size have been removed, within and among species of mammals living in natural populations.

Some shrews give birth when they are only 7 weeks old[15], whereas female African elephants produce their first offspring at an age of some 15 years[16]; such differences in age at first reproduction are highly correlated with the species' differences in body size[1,2,8,9], suggesting that the time required to grow to adult size determines the age at which mammals become sexually mature[2,4]. Consistent with this idea, the timing of many physiological and reproductive processes (for example, cardiac cycle time and maximum recorded lifespan) scale in similar ways with body size, approximately to the 0.25 power[4,8].

However, body size might be a confounding factor in the sense that it influences one variable which in turn influences some other variable; both variables would then be correlated with body size. For example, species with high rates of mortality in the wild might be selected to mature earlier than those which are long-lived, but rates of mortality might be influenced by body size (larger species can defend themselves against predators more effectively and they can survive for longer periods without food[6]). Data are now available on mortality patterns,

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body sizes and age at first reproduction of females for 29 mammal species (representing 25 genera and 6 orders) from natural populations with stable age distributions[17]. The species range in weight from 20 g to 4,000 kg. In addition, equivalent data are available from six populations of a further species, the Columbian ground squirrel, *Spermophilus columbianus*[18,19]. These data can be used to test the hypothesis that differences in patterns of mortality are correlated with the ages of the females at first reproduction.

Six of the species in the sample belonged to two genera (*Spermophilus* and *Peromyscus*) and, to reduce taxonomic bias, analyses were performed at the generic level (that is, on the averages of logarithmically transformed congeneric species' values). All variables were logarithmically transformed before analysis. As a summary measure of mortality, we have used Millar and Zammuto's[17] estimates of mean life expectancy at birth in years (a comparison of the use of this versus other measures will be presented elsewhere). Genera with low mortality have a high expectation of life at birth. The correlation between age of females at first reproduction and life expectancy at birth is high and positive ($r=0.976$, $n=25$, $P<0.001$). This correlation, however, might result from independent correlations of each variable with body size, but this seems not to be the case since the two variables are more highly correlated with each other ($r=0.976$) than either is with body size ($r=0.894$ and 0.873 respectively, $n=25$ in both cases), and they remain highly correlated after the effects of body size have been removed by partial correlation ($r=0.892$, $n=25$, $P<0.001$). The latter relationship means that mammals with an unexpectedly long lifespan (relative to body size) start reproducing relatively late (again relative to body size). The relationship is demonstrated graphically in Fig. 1—note the taxonomic spread. Genera from each of the two best-represented orders, the Artiodactyla and the Rodentia, cover most of the ranges of relative age at first reproduction and relative life expectancy. The removal of size effects is also evident: values for squirrels lie near that for the elephant, and the value for mice near that for the warthog. A positive partial correlation between age at first reproduction and life expectancy at birth, with the effects of body size removed, also holds among populations of the Columbian ground squirrel ($r=0.874$, $n=6$, $P=0.05$).

Probably as a consequence of constraints imposed by seasonality, seven of the genera in the cross-species sample mature as 1-yr-olds (ranging in weight from the 0.1-kg *Tamias* to the 7.5-kg *Lynx*). When the analysis is repeated without these genera, the correlation between age at first reproduction and life expectancy (with body size effects removed) remains high and significant ($r=0.836$, $n=17$, $P<0.001$). Of course, differences in body size may still influence age at first reproduction. Elephants could not mature and produce offspring in the few weeks that it takes shrews or mice to mature and, indeed, the partial correlation between age at first reproduction and body size with the effects of life expectancy removed is positive, though barely significant ($r=0.390$, $n=25$, $P<0.1$).

To test for the possibility that gestation length, litter size, neonatal weight or litter weight was ultimately responsible for the correlation between age at first reproduction and life expectancy, data on these variables for the species in the sample were extracted from the literature[20–24], and the effect of each new variable was removed by partial correlation. With the effect of each variable removed, in no instance did the correlation between age at first reproduction and life expectancy fall below 0.9.

The positive relationship between age at first reproduction and expectation of life at birth, after removal of the effects of body size, calls for an explanation in terms of the costs and benefits of reproduction at different ages. Perhaps the most straightforward interpretation is that each bout of reproduction reduces subsequent reproductive success (either by further increasing mortality or by decreasing fecundity), and that older mothers produce larger litters (and/or more viable offspring) than do younger mothers[19]. As life expectancy at birth decreases

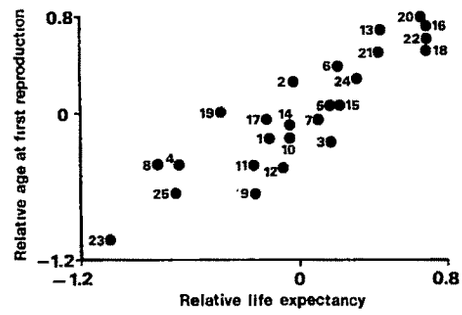


Fig. 1 Relative age at first reproduction plotted against relative life expectancy at birth for natural populations of mammals. Relative values refer to deviations from logarithmic regression lines of age at females' first breeding, or expectation of life at birth, on adult female body size. Numbers refer to different mammalian genera. Artiodactyla: 1, *Syncerus* (buffalo); 2, *Hippopotamus*; 3, *Aepyceros* (impala); 4, *Sus* (pig); 5, *Cervus* (deer); 6, *Ovis* (sheep); 7, *Hemitragus* (tahr); 8, *Phacochoerus* (warthog); 9, *Kobus* (kob); 10, *Connochaetes* (gnu). Carnivora: 11, *Taxidea* (American badger); 12, *Lynx*; 13, *Lutra* (river otter); 14, *Mephitis* (skunk). Lagomorpha: 15, *Sylvilagus* (cottontail rabbit); 16, *Ochotona* (pika). Perissodactyla: 17, *Equus* (zebra). Proboscidea: 18, *Loxodonta* (African elephant). Rodentia: 19, *Castor* (beaver); 20, *Tamias* (chipmunk); 21, *Spermophilus* (ground squirrel); 22, *Sciurus* (grey squirrel); 23, *Clethrionomys* (vole); 24, *Tamiasciurus* (red squirrel); 25, *Peromyscus* (mouse).

under such circumstances, selection will favour maturation at earlier ages. (This interpretation may also be consistent with an apparently similar relationship, pointed out by Lack[25], between age at first reproduction and patterns of mortality among families of birds.) At a more proximate level, nutrition levels may influence the correlations. High nutrition levels can result in early maturation[26,27] and decreased lifespan[28] within and among mammal species, for example, Columbian ground squirrel populations mature earlier and have shorter life expectancies when natural food resources are more abundant[18,19].

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An antiemetic is antidotal to the satiety effects of cholecystokinin

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Although cholecystokinin (CCK) has been proposed as a satiety agent[1–4], this property has been disputed by some who claim that the compound exerts its 'satiety' effects by inducing aversion[5–7]. We considered that if CCK-induced reductions in food intake occur through the mechanism of normal satiety, CCK-induced satiety and normal satiety should respond in the same way to a pharmacological challenge. We demonstrate here that the administration of an antiemetic to rats significantly attenuates the food intake reduction caused by exogenously administered CCK but does not increase normal consumption. The effects of endogenous CCK are therefore quite different from those of exogenous CCK, making any previous study equating exogenous CCK effects with natural satiety problematic.

Ninety-eight male Wistar rats (300–450 g) were housed and tested in individual wire mesh cages and maintained on a 12-h light/dark cycle. Tap water was available *ad libitum* and a maintenance diet of Purina rat chow was available for 2 h each day, beginning at 17.00 h. The rats were trained to drink a 1:1 Mazola corn oil/water solution emulsified with 1% lecithin (a diet expected to stimulate endogenous CCK release[8,9]) for 30 min each day after a 17-h period of food deprivation and were habituated to receive intraperitoneal (i.p.) injections of physiological saline. Rats were then assigned to one of four conditions matched by mean intake and standard deviation—saline–saline (S–S), antiemetic–saline (A–S), antiemetic–CCK (A–CCK) and saline–CCK (S–CCK). Each rat received two injections and was then given the emulsion to drink for the usual 30 min. The first drug for each group was given 30 min before the rat received his oil and the second drug immediately before oil presentation. The saline-treated rats received 0.9% saline solution (1 ml per kg, i.p.) and the antiemetic-treated rats received trimethobenzamide (5 mg ml^{-1} per kg, i.p.). (This drug and dose was used by Coil *et al.*[10] to show that antiemetics could be used to attenuate LiCl-induced aversions. Trimethobenzamide is an antiemetic which acts specifically on the chemoreceptor trigger zone and has been shown to be useful in alleviating nausea in humans.) Cholecystokinin octapeptide (Sincalide; Squibb) was administered i.p. at 5, 10, 20 or 40 Ivy dog units (IDU) per ml per kg.

The different CCK doses were tested in three successive sets of new naive rats, the initial oil and saline injection training being the same for each set. In the first set, after oil intake stabilization, 28 rats were divided equally into the four conditions, with the CCK rats receiving a dose of 20 IDU per ml per kg i.p.; in the second set, another 28 rats received CCK at 10 IDU per ml per kg, and in the final set, the extreme CCK doses (5 and 40 IDU) were given to one S–S group and one A–S group ($n = 42$). (Because the S–S and A–S controls were so stable in the previous two sets, only one S–S and one A–S control (seven rats in each) was run for each of the two CCK doses.)

One score was obtained for each rat by comparing the percentage consumpion on the experimental day with the mean consumption for the previous 3 days. Per cent mean intake scores were used rather than absolute intake scores, to minimize any potential differences in average drinking between the three sets of this experiment. The day of the experimental manipulation and the group of animals chosen for each set had no significant effect on the results. For the two control conditions (S–S and A–S), there was no significant difference in intake from one run to the next. An analysis of variance showed that the per cent mean intake between sets was not significantly different ($F = 0.49$, d.f. $= 2, 36$, $P > 0.05$). Furthermore, individual t-tests between an S–S and any other S–S condition and t-tests between an A–S and any other A–S condition showed no significant differences.

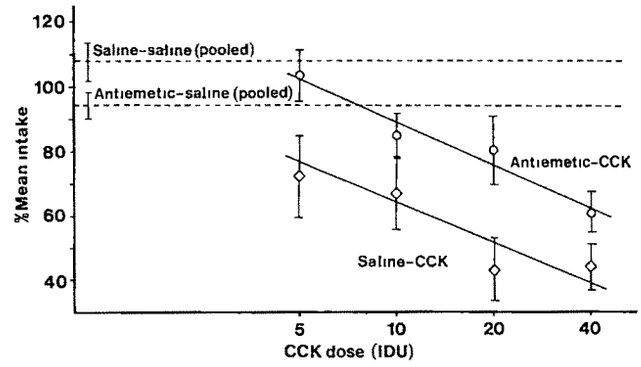


Fig. 1 Per cent mean intake (s.e.m.) of oil emulsion under four conditions of drug injections: saline–saline, antiemetic–saline, antiemetic–CCK and saline–CCK. The control S–S scores and the control A–S scores are pooled and are represented as dotted lines.

The pooled S–S group (from all three sets) consumed 108.1% of its normal amount on the experimental day (Fig. 1) and the pooled A–S group consumed 94.5% of its normal amount. Whereas neither the S–S group nor the A–S group showed significantly different consumption from normal (respectively $t = 1.30$, $n = 21$, $P > 0.05$ and $t = 1.30$, $n = 21$, $P > 0.05$), the A–S group showed marginally lower consumption than the S–S group ($F = 3.21$, d.f. $= 1, 36$, $P < 0.05$, one-tailed only). The antiemetic therefore did not increase normal consumption and, if anything, slightly reduced it.

The antiemetic attenuated the effect of CCK, results with the A–CCK group being significantly different from those of the S–CCK group ($F = 14.7$, d.f. $= 1, 48$, $P < 0.001$). The antiemetic (A–CCK) scores were higher than the saline (S–CCK) scores for all doses of CCK administered. Furthermore, whereas consumption in the S–CCK groups was always significantly different from their control (S–S) ($t = 4.90$, d.f. $= 12$, $P < 0.001$ for 40; $t = 4.01$, d.f. $= 12$, $P < 0.01$ for 20; $t = 2.84$, d.f. $= 12$, $P < 0.05$ for 10; $t = 2.59$, d.f. $= 12$, $P < 0.05$ for 5), that in the A–CCK groups was significantly different from their control (A–S) only for the highest CCK dose ($t = 2.67$, d.f. $= 12$, $P < 0.05$ for 40; $P > 0.05$ for 20, 10 and 5 IDU per ml per kg.

Because the antiemetic increased intake under CCK by about 26% for each dose of CCK used, if exogenous CCK were equivalent to natural satiety, normal intake with the antiemetic should have increased by 26% also. Instead, the antiemetic-treated rats consumed only 95% of their normal amount, rather than the expected 134%.

Clearly, exogenously administered CCK cannot be used as a model for natural satiety. Antiemetic administration increases eating after CCK injection, eliminating the supposed satiety effect of exogenous CCK. However, the antiemetic reduces or leaves unaffected normal eating when no CCK is given. The factors producing food intake reduction through CCK injection and the factors causing normal meal regulation therefore cannot be the same. Because the antiemetic is antidotal to CCK and because the antiemetic is not antidotal to normal satiety, it seems very likely that the factor causing food intake reductions after CCK administration is malaise and that malaise is not a factor producing normal satiety.

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Segregation of pathways leading from area V2 to areas V4 and V5 of macaque monkey visual cortex

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V5 and V4 are areas of macaque monkey prestriate visual cortex that are specialized for involvement in different aspects of visual perception, namely motion for V5 (refs 1–4) and colour vision, with other possible functions, for V4 (refs 2, 5–9). Thus, it is unlikely that they should be fed the same information for further processing, yet both receive a strong input from patches of the upper layers of V2 (refs 10, 11), the area immediately adjoining the primary visual cortex, V1. V2, however, seems to comprise functionally distinct subregions, which can be revealed by staining the tissue for the mitochondrial enzyme cytochrome oxidase[12–15]. Here we report that V4 and V5 are connected with separate cytochrome oxidase-defined subregions of V2, suggesting that cortical pathways dealing with motion and colour perception are segregated in their passage through V2, and reinforcing evidence for functional specialization in the visual cortex[16–18].

In primates, the primary visual cortex V1 (area 17 or striate cortex) receives most of the visual input to the cerebral cortex and sends parallel outputs to several of the visual areas[19] which, between them, comprise Brodmann's areas 18 and 19 of prestriate cortex. Because of this anatomical arrangement and the functional specialization in the prestriate areas[16,17], it was suggested that one function of V1 is to segregate different kinds of visual information relating to colour, form, motion and depth, and to distribute these to different prestriate areas for further processing[19]. Confirmation of this role of V1 has come from physiological studies in combination with cytochrome oxidase histochemistry. Tissue stained for this enzyme shows an array of 'blobs' in the upper layers of V1 in which are concentrated cells selective for wavelength but not orientation. In the more lightly staining 'interblob' regions, however, cells are normally not wavelength- but orientation-selective[15]. V2, the prestriate area bordering V1 (but occupying only part of area 18) receives a strong input from V1 (refs 20, 21) and, like it, contains several functional cell types[16,22] and projects to several other prestriate areas[23,24]. It, too, has a characteristic staining pattern for cytochrome oxidase, consisting of a series of stripes running orthogonal to the V1/V2 boundary[12–15]. These are best visualized in tangential sections through the flat mounted occipital operculum (Figs 1*c*, 2*a*) where in most cases the dense stripes are alternately thick and thin and are separated by lighter regions more uniform in size, the 'interstripes'. Two of these three sets of repetitive subregions receive specific projections from V1, the thin stripes from the blobs and the interstripes from the interblobs, but the specificity (if any) of the input to the thick stripes from V1 remains unknown[15].

Thus, the cytochrome oxidase-defined subregions of V1 and V2 are probably involved in segregated pathways of distinct functional characters and we sought to determine how the subregions of V2 project to the more uniformly specialized areas of prestriate cortex such as V5 and the V4 complex. These latter two areas differ profoundly in their functional characteristics: there is a total absence of wavelength-selective cells in V5, most cells being directionally selective[1–3,25], whereas V4 (which consists of at least two areas, each with probable subdivisions[6]) contains heavy concentrations of wavelength- and colour-sensitive cells but few that are selective for direction[5,8,19]. Thus, by relating the distributions of cells projecting to V4 or V5 with the pattern of cytochrome oxidase stripes, it might be possible to associate specific subregions of V2 with specific visual functions.

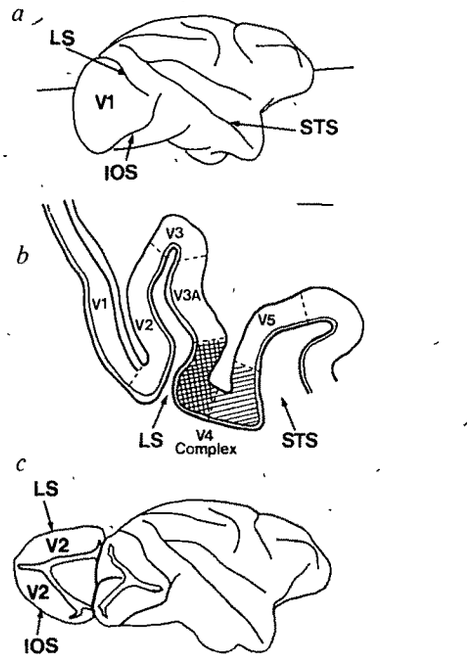


Fig. 1 Drawing of *a*, the surface of the left hemisphere of a macaque monkey brain, *b*, the lateral part of a horizontal section, taken at the level indicated in *a*, to show the locations of the three areas studied. The V4 complex (V4) consists of at least two areas with further subdivisions in each In this study, the injections were made in the posterior part of the V4 complex (cross-hatched) *c*, Illustration of how the occipital operculum is removed and showing the relation of V2 to V1. LS, lunate sulcus; STS, superior temporal sulcus; IOS, inferior occipital sulcus Scale bar, 2 mm.

Injections of wheat-germ agglutinin horseradish peroxidase (HRP) (0.1-0.3 μl of a 4% solution) were made into area V5 in three monkeys and is V4 in five monkeys. After 48-h survival, the animals were anaesthetized and perfused, following standard procedures, after which the brains were sectioned at 50 μm and pairs of adjacent sections stained for cytochrome oxidase[26] and reacted for HRP[27]. The cytochrome oxidase-stained sections were drawn at ×30 magnification under a drawing tube, with the boundaries of the cytochrome oxidase heavy zones and blood vessels marked. The adjacent HRP section, suitably magnified to compensate for its greater shrinkage, was superimposed according to the blood vessels and the positions of labelled cells and terminals added to the diagram. In this way, it was possible to determine the relation of the labelled cells to the cytochrome oxidase stripes in V2.

Where injections of HRP are made into V4 or V5, labelled cells and terminals in V2 are distributed in bands running orthogonal to the V1/V2 boundary and are very similar in disposition to the cytochrome oxidase stripes. The similarity is even more striking because, just as the cytochrome stripes show periodicities along their long axes[28], giving them a beaded appearance, so do the bands of labelled cells. Figures 2*b* and 3*a* show bands of label in corresponding locations in V2 following two separate V4 injections. In the former case the bands are quite narrow (0.7 mm) and well circumscribed, but in the latter they are broader (~1.5 mm) and rather more diffuse. The relationship of these bands to the cytochrome oxidase stripes is often difficult to determine because the pattern can be

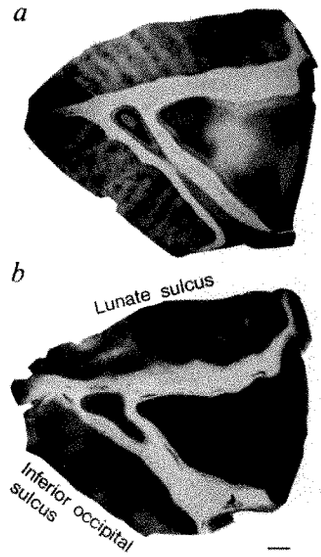


Fig. 2 *a*, Photomicrograph of a section taken through the flat-mounted occipital operculum and stained for cytochrome oxidase to show the thick and thin stripes and the interstripe zones in V2. The size of one cycle (centre-to-centre distance of two thin stripes) varies between 3.0 and 4.5 mm with a mean of 4.0 mm; the relative widths of the stripes also fluctuate, but are ~2:1:1.5 (thick:thin:inter). Not all macaque brains show such a clear pattern, especially in the lunate sulcus where the pattern is often complex and the thick and thin stripes are difficult to distinguish in individual sections. *b*, Dark-field photomicrograph of a similar section from a flat-mounted occipital operculum in an animal with an injection of HRP into V4. Note the striking similarity in the disposition of the labelled cells to that of the cytochrome oxidase stripes. The precise relationship of the two could not be determined in this brain, in which the cytochrome oxidase stain was unsatisfactory. Scale bar, 2 mm.

irregular, with alternate thick and thin stripes not easily discernible. Little could be learned from the cytochrome oxidase sections corresponding to the first case (Fig. 2*b*), in which the stain was technically poor; in the second case, where the staining pattern was irregular (Fig. 3*b*), superimposition of adjacent sections revealed that the labelled cells were centred over alternate stripes, and although overflowing into the interstripe zones, avoided the other set of stripes. That these were the thin stripes (those connected with the wavelength-selective blob of V1) was ascertained by tracing the pattern through a series of superimposed cytochrome oxidase sections, starting from those sections in which the stripes could be more clearly differentiated. This conclusion was reinforced by examination of another animal with a V4 injection, in which the two sets of stripes could be easily distinguished and in which a series of broad, if sparse, bands of label were again centred over thin stripes but avoided thick stripes (Fig. 3*d*). One further V4 injection again gave rise to the narrower bands of labelled cells and these were sited entirely in the interstripes. Four of these bands occupied 7.5 mm of cortex, a periodicity similar to that of the narrow bands of the brain in Fig. 2*b*, perhaps suggesting that these also might have a similar disposition with respect to the cytochrome oxidase stripes. This variation in the results obtained in V2 may reflect an inhomogeneity in V4 in relation to the distribution of wavelength and orientation selectivity[2,19]. The injection sites, although all in the posterior portion of V4, may have involved physiologically dissimilar regions.

Following injections into V5, labelled cells in V2 again appear in bands running orthognal to the V1/V2 boundary, but are now almost entirely confined to regions densely staining for cytochrome oxidase, which our evidence shows are the thick stripes. This was most clearly observed in the case of a brain sectioned horizontally (see Fig. 4*c*). The sections were drawn as described above and the information thus obtained was digitized along a

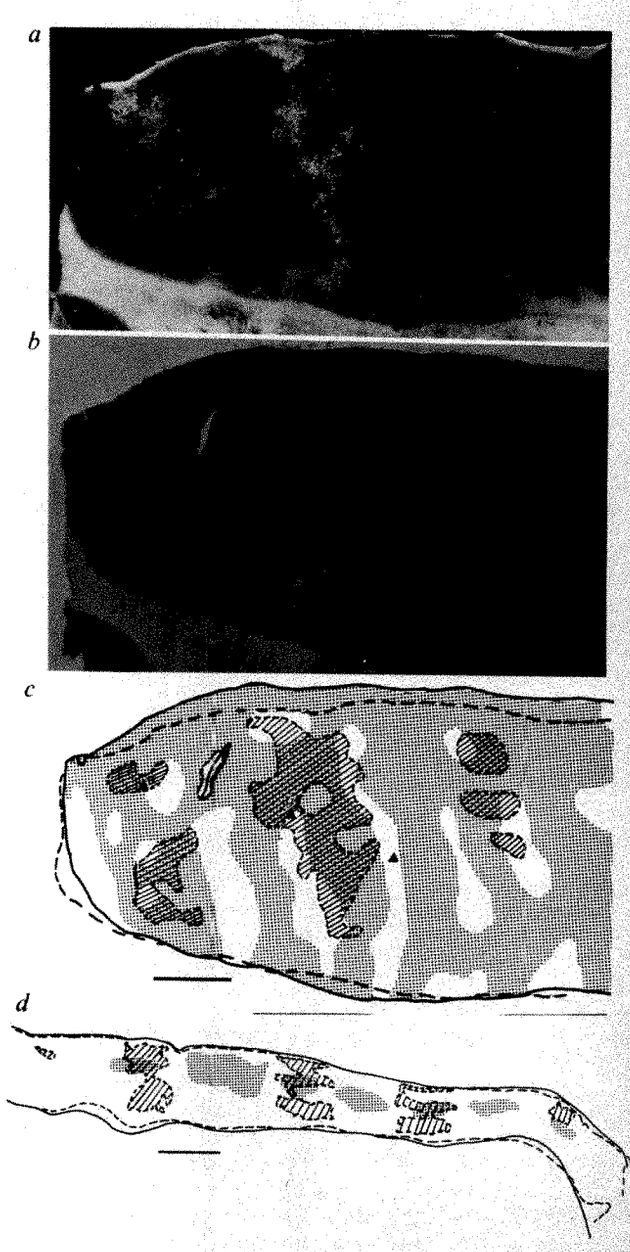


Fig. 3 *a*, Dark-field photomicrograph of the lunate sulcus from a flat-mounted operculum section to show the tangential distribution of labelled cells in V2 after an HRP injection into V4. *b*, Bright-field photomicrograph of a section next to the one shown in *a* and stained for cytochrome oxidase. *c*, Composite drawing, made from sections *a* (continuous outline) and *b* (interrupted outline) in which the two sections have been superimposed and areas of labelled cells (hatched) and dense stain (stippling) have been entered. The cytochrome oxidase distribution shown is also composite, drawn from superimposed images of section *b* and the other stained section next to *a*, a process that clarifies the pattern of stripes. Note that the labelled cells are centred over one set of stripes and skip alternate ones. We have not attempted to indicate more than one level of density for the distribution of either the labelled cells or of cytochrome oxidase, but it can be seen that both vary, not necessarily in parallel. For example, in the middle band of HRP-labelled cells (*b*) a roughly circular region of denser cytochrome oxidase staining coincides with a lower concentration of labelled cells. Unless traced over many sections, the stripes cannot, in this section, be identified as thick or thin. But *d* (a composite drawing made as for *c* but from sections from another brain with an HRP injection into V4), shows that the label is centred over the thin stripes and skips the thick ones. To align adjacent sections, as in *c* and *d*, we used blood vessels (three of which are indicated by triangles in *c*) and other landmarks to obtain the best fits, particularly because the shrinkage of the two sections is different in different directions.

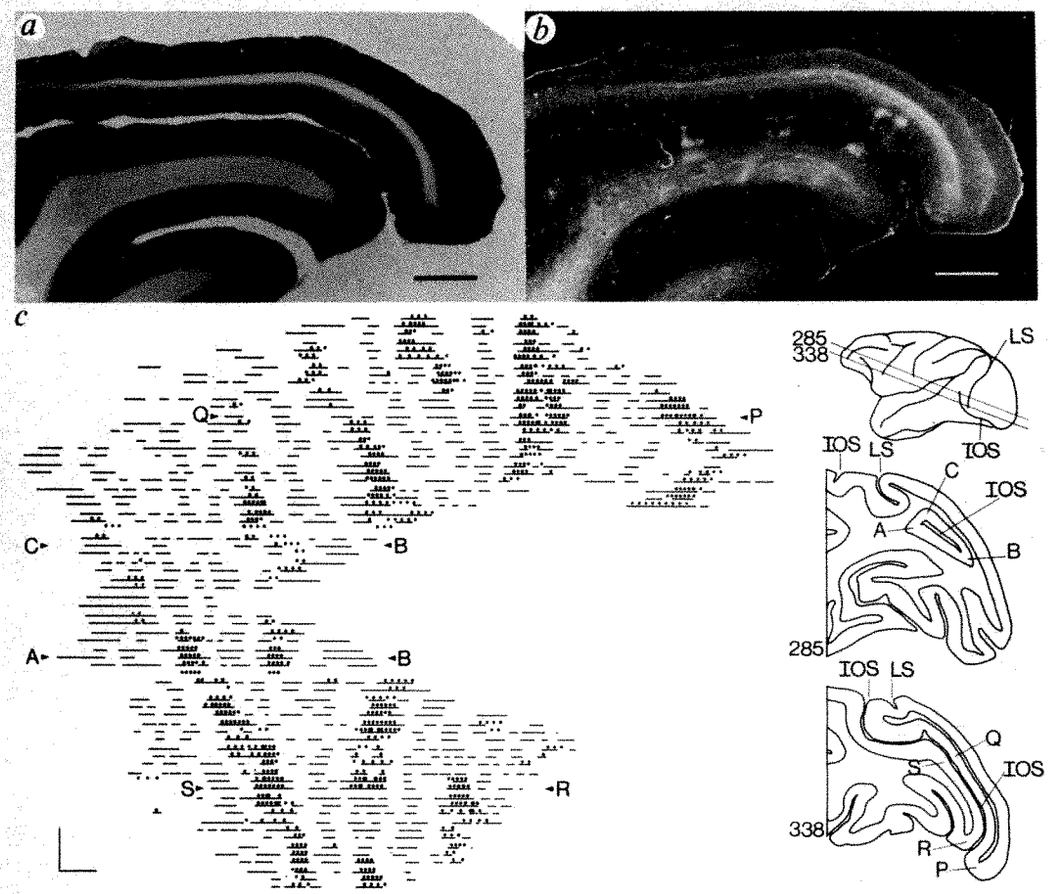


Fig. 4 *a*, Brightfield photomicrograph of a horizontal section taken through the part of V2 lying in the inferior occipital sulcus and stained for cytochrome oxidase activity. *b*, Darkfield photomicrograph, taken from an adjacent section, following HRP injection into V5. *c*, Computer-aided reconstruction made from many pairs of sections similar to *a* and *b*. The information in each pair of adjacent sections was incorporated into a single drawing (as in Fig. 3*c*) and this information was then digitized and entered in the computer. The distribution of cells is shown as dots, and regions densely stained for cytochrome oxidase as broken lines. The reconstruction is symmetrical about the fundus of the inferior occipital sulcus; each line represents one horizontal section, and the diagrams of sections 285 and 338 with the marked points, A, B, C, P, Q, R and S indicate how the overall configuration relates to the anatomy of the cortex. The cytochrome oxidase pattern is least clear where the stripes appear to be bifurcating, but elsewhere the appearance of alternate thick and thin stripes can be seen to emerge. LS, Lunate sulcus; IOS, inferior occipital sulcus. Scale bars: *a*, *b*, 2 mm; *c*, 1 mm for both axes.

'contour line' from the section and fed into a computer programmed to straighten and plot these lines to mimic the appearance that might have been obtained had the tissue been flattened and sectioned parallel to the cortical surface. The reconstruction reveals a number of irregularities, such as bifurcations in the stripes (which we have also observed in flat-mounted operculum cytochrome oxidase sections) but most of the stripes containing labelled cells are identifiably thick. This conclusion was in accord with the impression gained from those individual sections where alternating thick and thin regions of denser staining were visible, such as the lower bank of the inferior occipital sulcus in the section shown (Fig. 4*a*, the region between points 'R' and 'S' marked on its outline, labelled 338, in Fig. 4*c*).

In summary, we report here that alternating cytochrome oxidase stripes project to V4 and V5, our reconstructions suggesting that V4 receives input from the thin stripes and V5 from the thick, and that the interstripes also project to V4. Further evidence supporting functional distinctions between these subdivisions has come from our complementary physiological recordings (S.Z. and S.S., in preparation), in which we have made tangential penetrations running parallel to the stripes in the lunate sulcus. We have found that there is more variability in the responses recorded between penetrations than within any single penetration, and in several instances most of the units encountered in a single penetration, for a distance of over 1 mm, were either all orientation or all wavelength or all directionally selective. Where we have been able to associate penetrations with cytochrome oxidase stripes, we have found that interstripe cells are usually tightly tuned for orientation and are not wavelength-selective; most wavelength-selective cells, some of them double opponent, are found in or at the borders of thin stripes and are not orientation-selective. The frequency of occurrence of orientation selectivity is at an intermediate level in thick stripes, but these penetrations have the highest incidence of directional selectivity. Thick stripe cells, whether or not they are orientation-sensitive, generally respond equally well or better to spots of light moved in any direction than to long bars, unlike the orientation-selective cells of the interstripes, whose responses to spots are poor.

Thus, both anatomical and physiological evidence indicates that any operations performed in V2 must be performed separately for the different visual submodalities. This does not eliminate any interplay between the different pathways, given both local connectivities and that the outflow from V2, at least to V4, is not absolutely restricted to the territory of the thin stripes. But the segregation of pathways through V2 is much the more impressive and further reinforces the earlier evidence for functional specialization within the visual cortex[17].

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Note added in proof: We have since found that V3, which has no wavelength-selective cells, also receives from cells in the thick cytochrome oxidase stripes of V2.

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Complex–unoriented cells in a subregion of primate area 18

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In primates, both the primary and secondary visual cortical areas can be subdivided histologically by staining for the mitochondrial enzyme cytochrome oxidase[1–3]. In the primary visual cortex (area 17, the first cortical receiving area for visual information) these histological differences correspond to functional subdivisions[1–6], cytochrome-dark regions being concerned with information about colour and cytochrome-light regions concerned with form. Here we report that the second visual area, area 18, which receives its main cortical input from area 17 (refs 7, 8), similarly has functional subdivisions that correspond to the cytochrome oxidase staining pattern. In area 18 the segregation between form and colour is maintained, reinforcing our notion that form and colour information follow parallel pathways. The specific differences between cells in areas 17 and 18 suggest that a possible step in hierarchical information processing is spatial generalization, analogous to the difference between simple and complex cells.

In area 17 the cytochrome oxidase staining pattern consists of small dark-staining polka-dots (blobs) on a lighter-staining background[1,2]. Cells in the blobs are not orientation-selective and at least half are colour-coded, whereas most cells in the inter-blob regions are orientation-selective with only a small number colour-coded[6]. In area 18 the dark-staining regions are much coarser and form stripes, ~0.5–1 mm wide, which run perpendicular to the 17/18 border and extend the full width of area 18 to its anterior border (Fig. 1; refs 3–5). In some macaques and in most squirrel monkeys, the dark stripes are generally of two types, alternating thin and thicker, slightly lighter stripes. Although the thick stripes are not always easy to distinguish from the thin stripes by cytochrome oxidase staining alone, they differ anatomically in that they have a different laminar distribution of thalamic inputs[4]. Thus, area 18 seems to consist of at least three types of subregions: thin stripes, thick stripes and, in between, much lighter interstripes.

We have recorded from 1,019 cells in area 18 of 11 young adult female *Macaque fascicularis* monkeys and 7 adult female *Saimiri sciurius* (squirrel) monkeys. The recording apparatus was as described previously[6]. We made electrode penetrations perpendicular to the cytochrome oxidase stripes as nearly parallel to the surface as possible.

It was clear that area 18, like area 17, is physiologically heterogeneous but with a much coarser periodicity. Figure 2 shows the results from one macaque monkey, in which we recorded from 9 mm of area 18; almost all of the penetration appears in this single section. The figure indicates that we passed through four cytochrome oxidase-dark regions. Two of these stripes (the second and fourth) consisted exclusively of cells that entirely lacked orientation selectivity, whereas almost all the cells in the other two stripes and in the interstripe regions were orientation-selective. We found such long stretches of

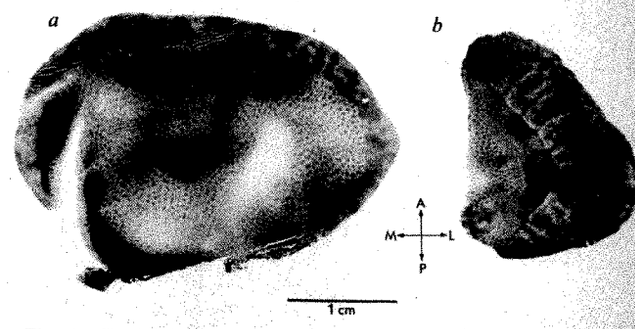


Fig. 1 Sections of monkey visual cortex stained for cytochrome oxidase. *a*, Section from a macaque monkey. Most of the section is in area 17 except for a 0.5-cm-wide strip of area 18 at the top. *b*, Section from a squirrel monkey. Area 17 is to the left with the fine polka-dot pattern and area 18 is to the right with a coarser-striped pattern. The alternation of thick and thin stripes is clearest near the top of the squirrel monkey section and is not apparent in the macaque.

unoriented cells in both macaques and squirrel monkeys (211 cells), always in cytochrome oxidase-dark regions. In the macaques it was not usually possible to say whether the dark stripes containing unoriented cells were thick or thin, but they usually alternated with stripes containing oriented cells, as in Fig. 2. In the squirrel monkeys the unoriented cells were clearly in the thin stripes. All these results are consistent with our earlier observation that the blobs in area 17 and thin stripes in area 18 are interconnected.

In macaques, about two-thirds of the unoriented cells showed colour opponency; in the squirrel monkey the proportion of colour-coded cells was much smaller. Of the colour-coded cells, a few had colour opponent centres and no antagonistic surrounds (for example, red-on centre, green-off centre, Type II; ref. 6), but most had some form of double-opponent colour coding (for example, red-on centre, red-off surround, green-off centre, green-on surround). Similar to blob cells in area 17, the unoriented cells in area 18 (whether or not they are colour-coded) responded more strongly as spot size was increased up to an optimum size beyond which the responses declined. What was different about many of these cells, compared with the blob cells in area 17, is that they responded to a spot of optimum size regardless of where it was positioned, over an area that was itself many times larger than the optimum spot. Thus, in these cells a spot large enough to cover the entire activating region was usually ineffective.

An example of one such colour-coded cell is shown in Fig. 3. It gave on responses to small red spots, off responses to small blue or green spots and no responses to white or to yellow spots (580 nm). In response to an optimal spot, 0.5° in diameter, the cell gives on responses to red and off to blue or green throughout an activating region 3.5° in diameter, maximum near the centre and falling off gradually with distance; increasing the spot beyond 0.5° resulted in a fall-off in responses (Fig. 3*b*, *c*). When we shone a small red and a small blue spot simultaneously in the same position (giving a yellow spot), the cell gave little or no response. We did not try spatially to separate red and blue spots with this cell (but in other similar cells, simulating with a small red spot and a small blue spot side-by-side within the activating region gave mixed on/off responses). This cell was located in layer 5 of a cytochrome oxidase-dark stripe in area 18 of a macaque.

We also observed cells, similar but not colour-coded, some giving on and others off responses regardless of wavelength over an activating region larger than the optimum spot size. Baizer *et al.*[9] have described 'spot cells' in area 18 that are similarly unoriented, not colour-coded and respond best to a small spot anywhere in a large receptive field. We term all of these cells, whether or not they are colour coded, 'complex-unoriented', because they differ from ordinary centre-surround cells in a manner analogous to the difference between oriented simple and complex cells[10]. (The distinction lies in the permissible

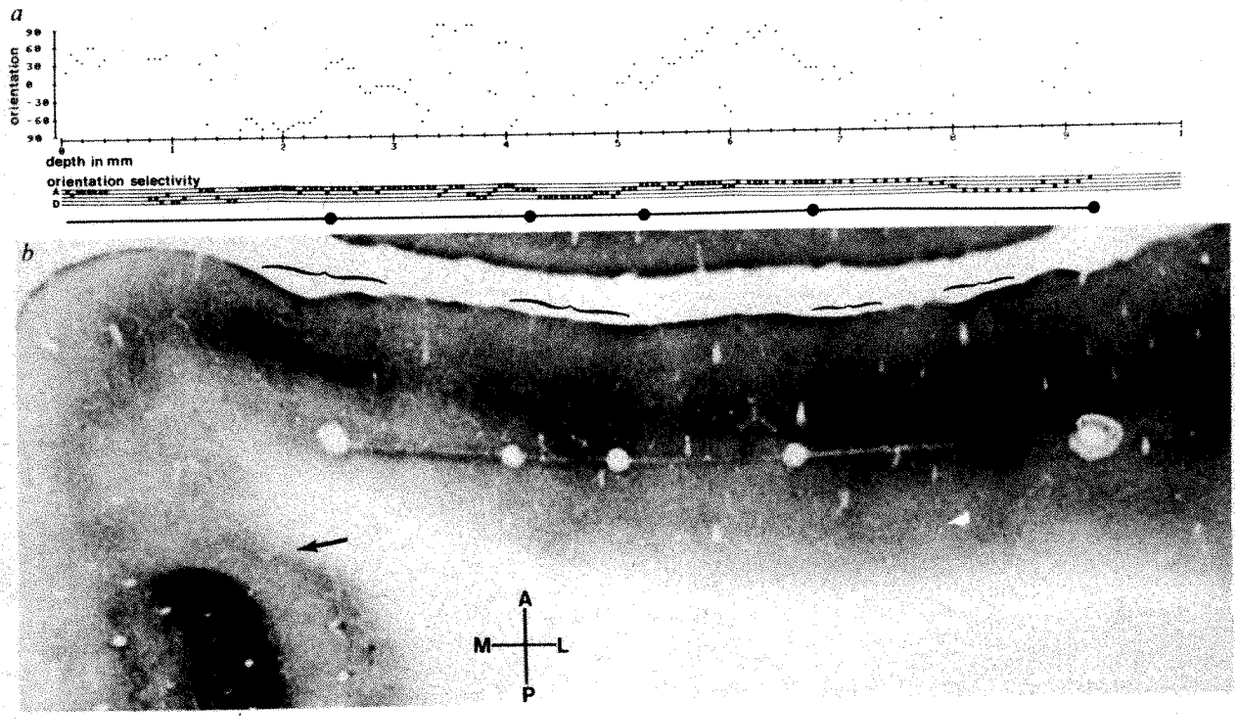


Fig. 2 *a*, *b*, Reconstruction of an electrode track in area 18 of macaque. *b*, Transverse section through area 18 just anterior and parallel to the area 17/18 border. Part of the border is visible at the left edge of the section (arrow). The space shown just above area 18 is the lunate sulcus; the midline is on the left. The electrode track, most of which can be seen together with five electrolyte lesions, runs from left to right. The section was stained for cytochrome oxidase[22] and the cytochrome-dark regions are indicated by brackets. *a*, From above downwards, preferred orientation (0°, vertical); orientation selectivity (A, well oriented, D, unoriented); and the location of the electrolyte lesions. Optimum orientation and orientation selectivity were judged subjectively by listening to the response of the cell to slits of light generated by a hand-held slide projector. Observations were made every 0·05 mm. Note the correspondence between two of the cytochrome-dark regions (between second and third lesions and just before the final lesion) and the absence of orientation selectivity. (At these points no orientation could be plotted.) The entire track after the first 0.5 mm was below layer 4.

variation in position; see ref. 10.) In both cases, although the size of the region of the visual field in which the higher level cell can be activated is larger (in our complex-unoriented cells, from two to several times the diameter), the optimum stimulus size for a given eccentricity remains the same, as if an 'or-gate' operation had been performed on many cells with similar receptive fields scattered over an area larger than each separate receptive field. Thus, although resulting in a loss of precision of localization, complexification need not lead to a loss of resolution or acuity, because at a given eccentricity the average optimum stimulus size, such as line width or spot diameter, shows no such increase. The increase in receptive-field size reported in several pre-striate areas[11–14] is presumably, at least in part, the result of such transformations. The occurrence of similar operations in different systems suggests that similar circuits are used, which would doubtless simplify the problem of building the cortex. Despite the frequency with which this operation seems to occur in the visual system, its role in information processing is still a matter of conjecture. (See page 285 of this issue.)

Some of the unoriented cells we found in area 18 were not complex and seemed the same as cells that we have observed in area 17. We are now trying to determine whether the different orders of unoriented cells in area 18 are segregated into different layers, as any sequence of unoriented cells tended to contain either ordinary or complex unoriented cells. In area 17, however, we have not seen complex-unoriented cells. The transformation of colour information in going from area 17 to 18 might be predicted to involve the acquisition of orientation selectivity, but this seems not to be so.

In the squirrel monkey it is clear that the regions in area 18 containing complex-unoriented cells are the same regions, the thin stripes that we previously found anatomically to be connected to the blobs in area 17. These blobs contain unoriented, often colour-coded cells[6]. In macaque monkeys the histological distinction between two types of cytochrome-dark stripes is often less clear, but, as shown in Fig. 2, only some of the stripes (usually alternate ones) are also characterized by colour-coded unoriented cells.

We have also studied the characteristics of the cells in the other stripes: cells in both the interstripes and the thick stripes show orientation selectivity and are not colour-coded. The light-staining interstripes in area 18 are reciprocally connected with the inter-blob regions of area 17, which contain oriented non-colour-coded cells[6]; the connections of the thick stripes are not yet known, but they clearly receive input from area 17 (see Fig. 30 of ref. 6). The most striking characteristic of cells in the interstripe regions is that over half of them are end-stopped[11], responding optimally to slits of a particular length and less well to longer slits. This suggests that the system that in area 17 carries information about the orientation of edges might next acquire information about their degree of curvature. In the thick stripes the cells seem to be concerned primarily with binocular disparity: many fail to respond to either eye alone but do respond if the eyes are aligned with a particular disparity, corresponding either to near, far or peaking at zero disparity. DeYoe and Van Essen[15], by injecting two different retrograde tracers into the pre-striate visual areas MT and V4 of macaques, have found that in area 18 alternating cytochrome-dark stripes project to MT and to V4. The results of Shipp and Zeki[16] in the accompanying paper confirm these observations. Given the high proportion of colour-coded cells reported in V4 (ref. 14), the V4-projecting stripes would be expected to be the ones to receive input from the blobs in area 17 and contain unoriented, often colour-coded cells: in a squirrel monkey these would be the thin stripes. Our finding that the thick stripes contain orientation-selective cells is consistent with their involvement with some system other than the colour system; they could correspond to the MT-projecting stripes described by DeYoe and Van Essen[15]. The segregation in area 17 of two systems, one apparently concerned with form

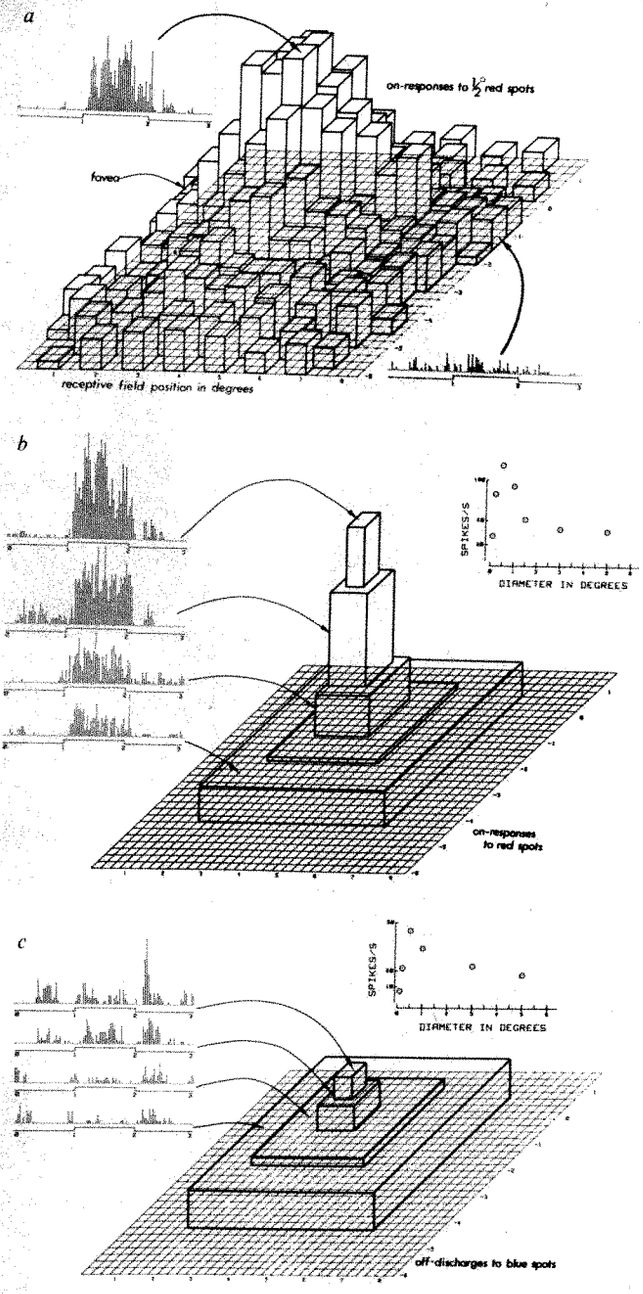


Fig. 3 Responses of a complex double-opponent cell in area 18 of a macaque monkey to red and blue spots of light, varying size and position of spot. The grid represents the visual field, marked off in degrees, with the fovea at (0, 0). The red and blue spots were produced by Kodak Wratten filters (29 and 47B) placed in front of a slide projector. *a*, Three-dimensional plot illustrating responses to red spots of optimal size, 0.5° in diameter. The plot is made over a region 8×8°, centred 5° lateral and 2.5° below the fovea. Responses were obtained over an area occupying 3×3°, outside which the bars represent spontaneous background activity. Bars represent means of five responses to stimuli of 1 s duration. The histogram for the area giving the peak response, shown in the inset on the upper left, represents 85 impulses during this 'on' period. The lower right inset shows one of the histograms for a region giving little or no response above the background firing rate. *b*, Diagram of on-responses to red light spots of various sizes. Each histogram is a mean of five stimuli. The three-dimensional graph illustrates the responses to the optimum size and to larger stimuli; the inset on the left shows the histograms obtained for four of these five spots. The upper right inset shows the entire curve of response versus spot size. Note that the 3.5 and 5° stimuli evoked responses that were barely visible above background firing levels, even though these covered the area over which 0.5° spots gave vigorous responses. *c*, Same cell, off-discharges to blue spots of different sizes.

and the other with colour, evidently persists into area 18 and beyond. That a similar segregation occurs in humans is apparent from reports of subjects with local cortical damage who experience loss of colour vision but retain normal form, movement and depth perception[17–21].

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Induction of regulatory T-lymphocyte responses by liposomes carrying major histocompatibility complex molecules and foreign antigen

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Regulatory (helper and suppressor) T lymphocytes become activated only when foreign antigen is presented to them on the surface of antigen-presenting cells (APC), together with class II major histocompatibility complex (MHC) molecules (heterodimers of polypeptides of 28,000 and 35,000 relative molecular mass)[1–4]. Once activated by a certain foreign antigen—MHC combination, T cells react to the same antigen only in combination with the same MHC molecule, a phenomenon termed MHC restriction of T-cell recognition (reviewed in refs 1, 5). Studies of the mechanisms involved in antigen presentation and MHC restriction have been hampered mainly by the virtual impossibility of inducing T-cell responses in the absence of APC. We describe here the production of synthetic lipid vesicles with inserted class II MHC molecules and a protein antigen coupled covalently to the lipid. These liposomes are shown to stimulate cloned helper T cells and T-cell hybridomas in an antigen-specific, MHC-restricted manner in the absence of APC. Thus, the recognition of foreign antigen together with class II MHC molecules seems to be the only signal required for the activation of antigen-primed regulatory T cells. Furthermore, 'processing' of antigen by APC is not essential for its recognition by T cells.

To determine the minimal requirements of T-cell activation, we constructed liposomes carrying a foreign protein antigen, or class II MHC molecules, or both, and tested whether these liposomes could activate antigen-specific class II-restricted T cells in the absence of APC. Class II MHC molecules were isolated from lipopolysaccharide-stimulated spleen cells. The membrane proteins were solubilized with 50 mM β-octyl gly-

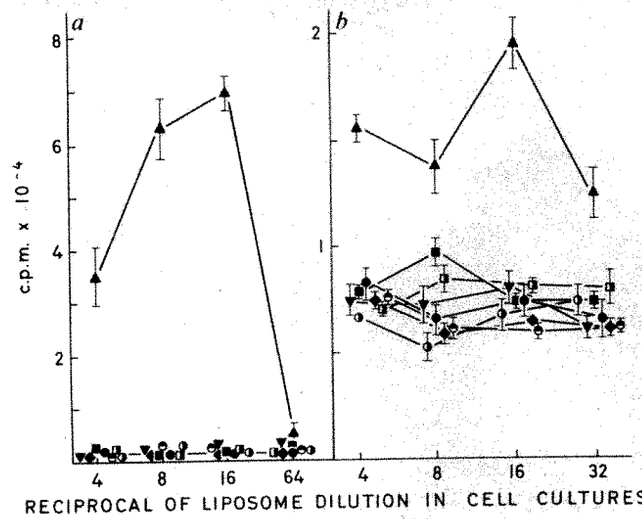


Fig. 1 Liposome-bound antigens induce the proliferative response of an ovalbumin (OVA)-specific, A^b-restricted T-cell clone (*a*) and IL-2 production by a bovine insulin (BI)-specific A^b-restricted T-cell hybridoma (*b*). Liposomes with the following proteins were used: ▲, *a*, OVA + A^b; *b*, BI + A^b; ■, *a*, OVA; *b*, BI; ●, *a* and *b*: A^b; ▼, mixture of liposomes with: *a*, OVA and A^b; *b*, BI and A^b; ◨, *a*, OVA + A^k; *b*, BI + A^k; ◑, *a*, BI + A^b; *b*, OVA + A^b; ◓, *a*, free OVA + A^b; *b*, free BI + A^b; ◆, liposomes without protein. Each point represents the arithmetic mean ± s.d. (vertical bars; not shown where too small to be represented graphically) of triplicate cultures. Control values (c.p.m. ± s.d.) for the proliferative response of OVA-7 (*a*) and for the IL-2 production by hybridoma 52H10F11 (*b*): feeder cells, *a* (C57BL/6), 654 ± 124; *b* (C57BL/6), 6,505 ± 522; antigen, *a* (OVA, 100 μg ml^{-1}), 1,821 ± 205; *b* (BI, 200 μg ml^{-1}), 5,623 ± 522; antigen + feeder cells, *a* (OVA 100 μg ml^{-1} + C57BL/6), 202,066 ± 12,019; *b* (BI 200 μg ml^{-1} + C57BL/6), 140,381 ± 1,727; control antigen + feeder cells, *a* (BI + C57BL/6), 657 ± 366; *b* (OVA + C57BL/6), 6,976 ± 611; antigen + control feeder cells, *a*, not done; *b* (BI + B10.A), 5,162 ± 542.

Methods. Clone OVA-7 of C57BL/6 origin (M. H. Schreier, unpublished data) was precultured with IL-2 and without feeder cells for 6 days, washed and distributed in 0.2-ml wells (3×10^4 cells per well), cultured in the presence of liposomes for 3 days (without IL-2 and feeder cells), and proliferation was measured by ^{3}H-thymidine incorporation[26]. Hybridoma 52H10F11 (BI-specific, A^b-restricted; B. Huber, unpublished data) was cultured (5×10^5 cells ml^{-1}) in the presence of liposomes for 2 days. Supernatants were then collected and tested for their capacity to induce proliferation of the IL-2-dependent cell line CTLL[27] (5×10^3 cells per well, 1 day's culture followed by a 16-h pulse with 2 μCi per well of ^{3}H-thymidine). Culture medium was α minimal essential medium supplemented with 10% fetal calf serum, antibiotics, L-glutamine and 3×10^{-5} M 2-mercaptoethanol. Liposomes were prepared by the dialysis method[7,28]. They consisted of dipalmitoylphosphatidylethanolanine (DPPE), 10% of which was SPDP-modified (the efficiency of modification was 1–1.5%). Liposomes were allowed to react with a large excess of SPDP-coupled OVA and BI, respectively (2.5 mg to 12.5 mM lipid). The antigen-coupled liposomes were purified by sucrose gradient centrifugation (5–45% flotation gradient, 100,000*g*, 46 h). The density of antigen on liposome was monitored by the position of the liposome band in the gradient. Class II MHC molecules were purified by affinity chromatography using monoclonal antibodies 17.227.R7 (ref. 29) for the A^b, and 10.2.16 (ref. 30) for the A^k molecule.

copyranoside in 10 mM Tris-buffered saline solution. Further purification was performed by affinity chromatography according to the procedure described by Turkewitz *et al.*[6], with the modification that β-octyl glycopyranoside was used as a detergent throughout. Monoclonal class II-specific antibodies used for the affinity columns were coupled with Sepharose-4B by the cyanogen bromide method followed by fixation with glutaraldehyde. The one-step affinity chromatography yielded highly enriched class II preparations with minor contamination by other proteins, as judged by SDS-polyacrylamide gel electrophoresis (data not shown). The class II molecules were inserted into liposomes made of synthetic lipids as described elsewhere[7,8]. Lipids in the liposomes were activated by thiolation with *N*-succinimidyl 3(-2-pyridyldithio)propionate (SPDP) via a disulphide bond as described previously[9,10]. The foreign protein antigens used were attached to these SPDP-activated lipids. The liposomes thus obtained carried class II MHC molecules inserted into the lipid bilayer by their hydrophobic transmembrane portion, and protein antigens covalently linked to the lipids. The vesicles were further purified by ultrafiltration, ultracentrifugation and in some experiments by flotation on a sucrose gradient. Flotation experiments were also performed to monitor the efficiency of reconstitution of MHC molecules into, and coupling of antigens to, the liposomes.

Figure 1 demonstrates that only the liposomes carrying both the priming antigen and the appropriate MHC molecules are able to stimulate proliferative response of an ovalbumin-specific, A^b-restricted T-cell clone, and interleukin-2 (IL-2) production by an insulin-specific, A^b-restricted T-cell hybridoma, in the absence of APC. Thus, the recognition of antigen + MHC molecules alone seems to provide a sufficient signal to induce these two kinds of responses of helper T cells. Note that, for a response to occur, the antigen and MHC molecules must be incorporated into the same liposome, indicating that the receptor structure of T cells recognizes these two moieties together. The finding that mixtures of antigen-bearing and MHC-bearing liposomes are not stimulatory, together with the results of other control groups of the experiments (MHC-bearing liposomes presented together with free antigen; antigen-bearing liposomes alone) makes it very unlikely that the liposomes are taken up and re-presented by residual APC or by the T cells themselves. Further evidence to rule out this possibility will be presented elsewhere (P.W., Z.A.N. and J.K., in preparation).

Our data extend previous results from several laboratories that demonstrated that secondary cytolytic T-cell responses and secondary mixed lymphocyte reactions can be stimulated by cell-free antigens, although in these experiments the possibility that APC contributed to the T-cell activation could not be excluded[11–18]. Our results also agree with a recent report by Malissen *et al.*[19] that the expression of transfected class II MHC genes seems to be the only requirement to convert a cell into an APC. The fact that intact protein molecules, when coupled to lipid, can activate T cells indicates that extensive processing is not required to render a protein immunogenic for T cells. Thus, although antigen degradation certainly takes place in macrophages and perhaps also in other cell types[20,21], and although the existence of T-cell clones with a preference for partially digested antigens has been demonstrated[22,23], the notion that regulatory T cells are capable of recognizing only degraded (or processed) antigen[24,25] is not generally valid.

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Developmental acquisition of Ca^{2+}-sensitivity by K^+ channels in spinal neurones

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A developmental change in the ionic basis of the inward current of action potentials has been observed in many excitable cells[1]. In cultured spinal neurones of *Xenopus*, the timing of the development of the action parallels that seen *in vivo*[2,3]. *In vitro*, as *in vivo*, neurones initially produce action potentials of long duration which are principally Ca-dependent; after 1 day of development the impulse is brief and primarily Na-dependent[3-5]. At both ages, however, both inward components are present[4-6] and the mechanism underlying shortening of the action potential is unknown. One possibility is that the outward currents change during development. Using the patch-clamp technique[7], we have recorded single K^+-channel currents in membrane patches isolated from the cell bodies of cultured embryonic neurones[3]. The unitary conductance of one class of K^+ channels was ~155 pS and depolarization increased the probability of a channel being open. Neither conductance nor voltage dependence seemed to change with time in culture; in contrast, the Ca^{2+}-sensitivity of this K^+ channel increased. In younger neurones, Ca^{2+}-sensitivity was greatly reduced or absent, whereas in more mature neurones, the activity of this channel was Ca-dependent. Such a change could account for the shortening of the action potential duration by increasing the relative contribution of outward currents.

Single-channel currents of *Xenopus* neurones were recorded at two stages of development in culture: early (7-11 h) and mature (22-43 h). All recordings were made from inside-out excised patches at room temperature. In most cases, K^+ was the predominant monovalent cation in both pipette and bath solutions. Channel activity during prolonged depolarization was characterized by bursts of openings interrupted by brief closures and followed by long closed intervals. The unitary conductance was estimated from the single-channel current/voltage (I-V) relationship. After 1-2 days in culture, a channel with a conductance of 156 ± 2 pS (mean ± s.e.m.) was observed in 15 patches. Unitary conductance was independent of membrane potential (±50 mV), of internal Ca^{2+} concentration ($[Ca^{2+}]_i$ = 0.5-100 μM), and of external Ca^{2+} concentration ($[Ca^{2+}]_o$ = 0-0.5 mM). Similarly, at early ages a channel with a unitary conductance of 154 ± 2 pS was observed in nine patches; conductance was independent of calcium concentration and membrane potential.

At both ages these channels selected primarily K^+ ions. Currents reversed direction at the K^+ equilibrium potential (E_K), both in symmetrical K^+ and when $Tris^+$ was partially substituted for internal K^+, shifting E_K to +17.5 mV ($n = 6$ patches at 1-2 days; $n = 6$ patches at 7-11 h). However, when recordings were made in saline solution with Na^+ as the predominant extracellular cation and K^+ primarily on the intracellular face (E_K = −75 mV), unitary currents reversed at an extrapolated potential of ~−50 mV ($n = 5$ patches, 1 day in culture). If the channel is assumed to be permeable to Na^+ as well as K^+, the relative Na^+/K^+ permeability, calculated from the Goldman-Hodgkin-Katz equation, was 0.08-0.1. An alternative explanation for the apparent imperfect selectivity is that the I-V relationship was nonlinear in the area of E_K.

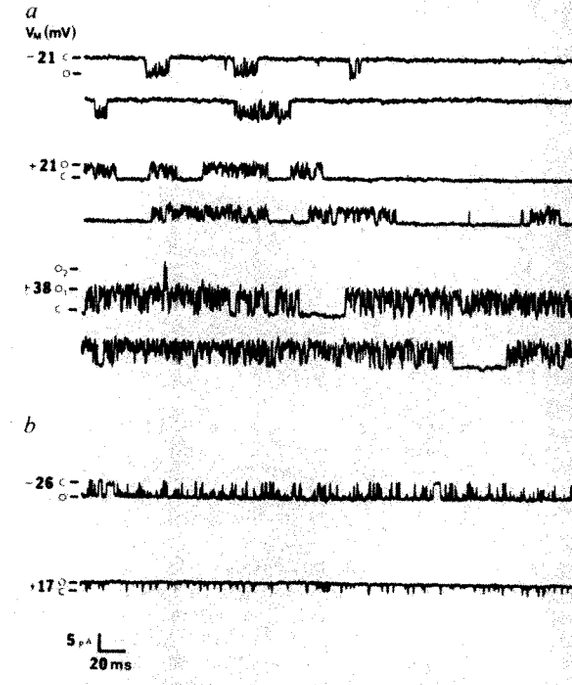


Fig. 1 Calcium- and voltage-sensitivity of K^+ channels in an 'inside-out'[7] patch excised from a neurone after 23.5 h in culture. *a*, Single-channel currents recorded with 1 μM Ca^{2+} at the intracellular surface. Openings occurred in bursts separated by long quiescent periods: O, open; C, closed. Burst durations increased and inter-burst intervals decreased with depolarization. Single-channel conductance was 158 pS. *b*, Increasing the $[Ca^{2+}]_i$ to 10 μM increased burst duration and decreased inter-burst closed intervals. At E_m = +17 mV, channels were almost continuously open. Same patch as in *a*. Multiple simultaneous openings occurred at depolarized potentials, indicating that a minimum of three channels were active in the patch (not illustrated). Activity of smaller-amplitude channels was not observed. During a burst there were many brief closures which were poorly resolved. Some of these apparent closures may have been only partial, to a lower-conductance substate. Membrane potential was varied by resetting the holding potential across the patch. Data were filtered at 4 kHz, sampled at 50-μs intervals, and recorded in 460.8-ms segments.
Methods. Neurones were cultured from dissociated *Xenopus* neural plates (Nieuwkoop and Faber stage 15)[16] as described previously[3,4], and single-channel currents were recorded at room temperature using established techniques[7,17,18]. Data were analysed for current amplitudes by computer[17,18]; the threshold for event detection was 50% of the mean amplitude. Burst duration and inter-burst intervals were measured by hand from a chart record of the data. Unless otherwise stated, the saline solution in the pipette and in the bath comprised (in mM): 150 KCl, 10 or 1 EGTA (results were indistinguishable), 5 HEPES, *p*H 7.8. Free internal Ca^{2+} concentrations of 0.5, 1, 10, 25 and 100 μM were achieved by adding $CaCl_2$ to the bath saline These concentrations were calculated assuming purity of the EGTA (but see ref. 19). Bath volume was 0.2 ml; bath changes were achieved by washing the patches with ≥25 vol. saline.

Embryonic *Xenopus* neurones exhibited a marked developmental difference in the effect of internal calcium on the activity of this channel. At 1-2 days, activity was sensitive to $[Ca^{2+}]_i$ (Fig. 1). Opening was not detected when free $[Ca^{2+}]_i$ was estimated to be $<10^{-10}$ M. Raising the $[Ca^{2+}]_i$ from 1.0 to 10 μM or from 0.5 to 25 or 100 μM substantially increased channel activity ($n = 12$ of 14 patches): both burst duration and the number of simultaneous openings increased, while the inter-burst intervals decreased. In the experiment shown in Fig. 1, raising the $[Ca^{2+}]_i$ from 1.0 to 10 μM increased the mean burst

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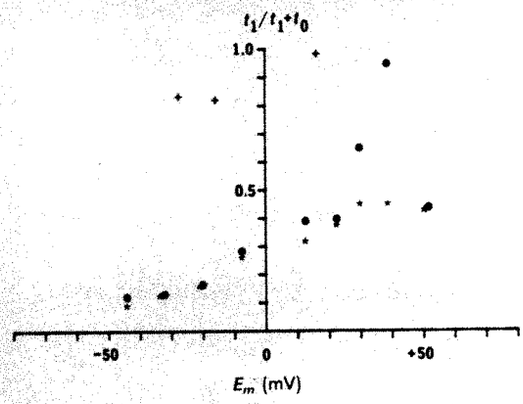


Fig. 2 The fraction of time for which one channel was open changed with membrane potential, $[Ca^{2+}]_i$, and the length of time after a change in membrane potential. This fraction was calculated as t_1/t_1+t_0 in which t_1 is the time for which a unitary level of channel activity was observed, t_0 the time in which no activity was observed. In 1 μM calcium, the fraction of time spent open increased with depolarization (●). Increasing the $[Ca^{2+}]_i$ to 10 μM increased this fraction several-fold (+). The fraction decreased during the recording in a time- and voltage-sensitive manner, presumably as a result of inactivation. In 1 μM calcium, the fraction was compared between the first 460.8 ms (●) and the section 2.9–3.4 s into the record (*); a large reduction was observed at depolarized voltages. At 38.1 mV, this reduction occurred within the first second of recording; at less depolarized voltages, the reduction was slower as well as smaller. The low initial activity seen at +50.3 mV probably results from inactivation during the ~1-s delay between setting the membrane potential and the start of recording. Ending depolarization, then re-stepping to positive potentials regenerated high levels of activity. Data are from the patch shown in Fig. 1.

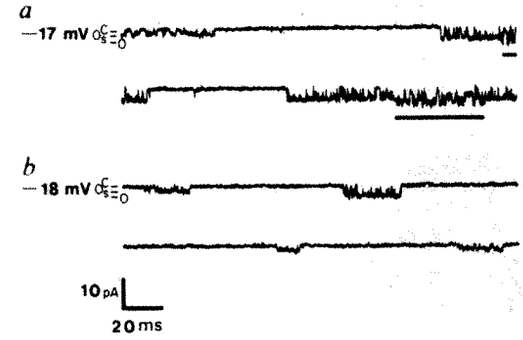


Fig. 3 Ca^{2+}-insensitive K^+ currents recorded from an inside-out patch of membrane excised from a neurone at 8.5 h. *a*, Single-channel currents at −17 mV were recorded in the absence of intracellular calcium; *b*, single-channel activity at −18 mV in 100 μM calcium. These records also illustrate the presence of a smaller-amplitude K^+ channel which was Ca^{2+}-insensitive at all ages and appeared to be independent of the ~155-pS channel studied here. O, opening of a 155-pS channel; O_s, opening of a smaller-conductance K^+ channel; C, closed. The dark bars denote regions of simultaneous opening of both the large- and small-amplitude channels. Data were filtered at 2.5 kHz and digitized at 100-μs intervals.

duration almost 15-fold; the mean inter-burst interval decreased ~30-fold (at membrane potential (E_m) = +17 mV). This resulted in a large increase in the fraction of total time for which a channel was open (Fig. 2). Different results were obtained from 2 of the 14 patches: in one case, channel activity appeared to be insensitive to change in the $[Ca^{2+}]_i$; in the other, few openings were observed until $[Ca^{2+}]_i$ was raised to 100 μM, but reducing the $[Ca^{2+}]_i$ subsequently failed to decrease the frequency of opening.

In contrast to the Ca^{2+}-sensitive activity observed in patches from mature neurones, channel activity in patches excised from early neurones was insensitive to changes in $[Ca^{2+}]_i$ ($n = 9$ patches). Currents were observed with free $[Ca^{2+}]_i < 10^{-10}$ M (Fig. 3), and increasing the $[Ca^{2+}]_i$ to 100 μM did not change the fraction of time for which a channel was open. In six additional patches no channel activity was observed in Ca^{2+}-free saline; raising the $[Ca^{2+}]_i$ to 100 μM did not induce activity. Extracellular calcium had no effect on channel activity of either early or mature neurones.

Activity at both ages was voltage-dependent. Figure 2 shows that the fraction of time for which a channel was open increased with depolarization. In addition, the mean inter-burst closed time declined and the mean burst duration increased, suggesting that the increase in open time of the channels was due both to an increased probability of opening to a burst and to a decreased probability of closing, once opened. In other patches, the fraction of time spent open showed sigmoidal increases with depolarization similar to that in Fig. 2; the half-maximal value was attained at ~+20 mV in all cases. The frequency of occurrence of bursts showed comparable voltage-dependence.

Most patches also possessed a lower-conductance K^+-selective channel: 94 ± 3 pS at day 1 ($n = 4$ patches); 93 ± 1 pS at 8.5–10.5 h in culture ($n = 4$ patches; Fig. 3). Opening of this channel was not correlated with that of the larger channel. The fraction of time for which the channel was open and the frequency of bursting were unaltered by changes in $[Ca^{2+}]_i$ but increased with depolarization. The half-maximum of the fraction of time spent open occurred at ~0 mV. Although lower conductances have been reported in other neural preparations[8,9], this channel may represent a delayed rectifier.

The Ca^{2+}-sensitive K^+ currents observed in these neurones at 1–2 days in culture appear to be similar to those of other preparations. Unitary conductances of 100–220 pS[8,10–12] at room temperature, calcium- and voltage-independence of the conductance[10], and calcium- and voltage-sensitive changes in bursting[10,13,14] have been reported, but inactivation was not prominent[10,13].

The results reported here suggest that the acquisition of calcium-sensitivity accounts in part for the developmental disappearance of the Ca^{2+}-dependent plateau of the action potential. The lack of age-dependent changes in unitary conductance, K^+-selectivity and voltage-dependence of activation suggests that the Ca^{2+}-sensitive and -insensitive channels represent a single class of channel, the gating mechanism of which is developmentally modified. Different sensitivities could be produced through mechanisms ranging from activation of a multigene family to post-translational modification. The infrequent presence of Ca^{2+}-insensitive channels in older neurones could indicate that these channels are inserted into the membrane in a form having reduced sensitivity and are then modified, or that a population of Ca^{2+}-insensitive channels persists into late stages of development. In view of recent results with the acetylcholine receptor[15], it would be of interest to see whether blockers of glycosylation or phosphorylation prevent the appearance of Ca^{2+}-sensitivity.

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Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain

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To date no nucleic acid has been found in the purified infectious agent which causes the spongiform encephalopathy known as scrapie. In an attempt to identify a unique scrapie virus-associated messenger RNA in tissues of infected animals, we have synthesized an oligonucleotide probe complementary to the mRNA sequence corresponding to the amino-acid sequence of the prion protein, PrP27–30 (ref. 1). We report here that, with this probe, a complementary DNA clone representing PrP27–30 was obtained from scrapie-infected mouse brain; the DNA sequence of this clone could be translated into a protein that matches exactly the published sequence of PrP27–30. The cDNA clone hybridized to a single 2.4–2.5-kilobase (kb) mRNA from both normal and scrapie-infected brain. Thus, the PrP27–30 mRNA is not uniquely associated with scrapie infectivity, suggesting that PrP27–30 may be a normal component of mouse and hamster brain.

Scrapie is a slowly progressive spongiform encephalopathy of sheep and goats[2] which can be transmitted to a variety of animal species including mice, hamsters and mink[3]. The pathology of scrapie is similar to certain devastating dementias of humans such as kuru, Creutzfeldt-Jacob disease and Gerstman-Straussler syndrome[4–6]. The replicating infectious agent which causes these diseases has many properties similar to those of a virus[7], but has not shown sufficient biophysical homogeneity to allow adequate purification for precise biochemical analysis[8,9]. Thus, the presence of a viral nucleic acid has not yet been demonstrated. In fact, in the case of scrapie, it has been proposed that the agent may consist solely of protein, and the term 'prion' has been coined to describe such proteinaceous infectious particles[10–12]. Recent studies have suggested that scrapie infectivity is associated with large characteristic fibril structures[13], termed scrapie-associated fibrils[14] or rods[15], which have a unique morphology when negatively stained and examined under the electron microscope but share some of the properties of amyloid which is a major feature of brain pathology in some scrapie models[16]. The proteins in these structures isolated from hamster scrapie have also been analysed serologically and biochemically and consist mainly of a polypeptide of relative molecular mass 27,000-30,000, PrP27-30 (refs 1, 13, 14, 17). The N-terminal 15 amino acids of this protein have been sequenced and show no homology with other known proteins[1]. Based on this amino acid sequence, we have synthesized a mixture of oligonucleotides for use as a hybridization probe to analyse mRNA populations derived from infected and uninfected animals and to identify unique scrapie virus-encoded mRNA species.

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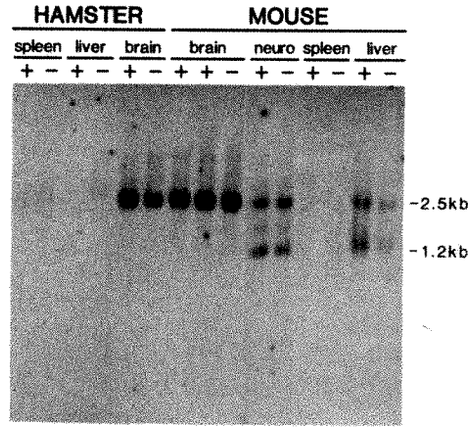


Fig. 1 Identification of scrapie-specific mRNA in infected (+) and uninfected (−) animals. Messenger RNA was isolated from RML outbred mice and Syrian hamsters who showed clinical neurological symptoms 120–150 days (mouse) or 65–75 days (hamster) after intracerebral inoculation of $10^{6.8}$ (mouse) or 10^9 (hamster) infectious units of scrapie. The mouse-adapted Chandler strain of scrapie was obtained from Dr W. J. Hadlow (RML). Hamster-adapted scrapie was obtained from Dr S. Prusiner, University of California School of Medicine, San Francisco. mRNA from uninfected, age-matched animals and from scrapie-infected and uninfected cells of a mouse neuroblastoma cell line (clone NA, obtained from Dr D. Lodmell, RML), was also isolated. Tissue culture cells, brain, spleen and liver tissue were homogenized in 5 vol. of 4 M guinidinium isothiocyanate and disrupted briefly in a blender[30]. RNA was pelleted by centrifugation through 5.7 M CsCl in 0.1 M EDTA at 35,000 r.p.m. for 16 h at 20 °C in a Beckman SW50.1 rotor. For brains, the RNA pellet was resuspended in 1% SDS, 10 mM Tris-HCl *p*H 7.4, 5 mM EDTA and extracted with 4:1 chloroform/1-butanol. The aqueous phase was brought to 0.3 M sodium acetate and RNA precipitated with 2.5 vol. ethanol at −20 °C. For liver and spleen, the RNA pellet from the CsCl spin was resuspended in 7.5 M guanidinium-HCl, 20 mM sodium acetate *p*H 7.0 and 1.0 mM dithiothreitol (DTT) and precipitated with 0.5 vol. ethanol at −20 °C. The pellet was resuspended in water, brought to 0.3 M with sodium acetate and precipitated with 2.5 vol. ethanol at −20 °C. For brain, liver and spleen, RNA pellets were resuspended in water, adjusted to 20 mM Tris-HCl *p*H 7.5, 1 mM EDTA, 0.5 M LiCl, 0.2% SDS, then passed through an oligo(dT)-cellulose column, washed with similar buffer, and poly(A) RNA was eluted with H_2O. Poly(A) RNA was brought to 0.3 M sodium acetate and precipitated with ethanol at −20 °C as before. RNA was stored at −20 °C as an ethanol precipitate. For analysis of mRNA, 3–6 μg of RNA was pelleted from the ethanol suspension, dried, redissolved in 0.01 M sodium phosphate buffer *p*H 7.4 containing 2 mM EDTA, 50% formamide and 6% formaldehyde, heated at 68 °C for 5 min and fractionated by electrophoresis in 1% agarose in 0.01 M sodium phosphate *p*H 7.4, 6% formaldehyde for 16 h at 20 V. Gels were then blotted onto nitrocellulose in 20× SSPE[26], and baked at 80 °C in a vacuum for 2 h. For hybridization to the oligonucleotide probe, filters were prehybridized in 6× SSPE, 0.1% SDS, 0.002 M sodium pyrophosphate, 100 μg ml^{-1} sheared herring sperm DNA and 1× Denhardt's at 37 °C for 3 h. Oligonucleotides were synthesized on a SAM-1 DNA synthesizer (Biosearch, San Rafael, California). To include all possible nucleotide sequences giving the published PrP27-30 amino-acid sequence, 32 different oligonucleotides were synthesized in a mixture as shown in Table 1; 0.1 μg of the oligonucleotide mixtures was labelled with 0.2 mCi of [γ-^{32}P]ATP in 100 mM Tris-HCl *p*H 7.5, 10 mM $MgCl_2$, 10 mM DTT using two units of polynucleotide kinase for 1 h at 37 °C. Non-bound radioactivity was removed by binding the oligonucleotides to a DE52 column in 0.1 M NaCl in TE (10 mM Tris-HCl *p*H 7.5, 1 mM EDTA), and eluting with 1.0 M NaCl in TE. The prehybridized filter was rinsed in 6× SSPE, 0.1% SDS, 0.002 M sodium pyrophosphate and the ^{32}P-labelled oligonucleotide was allowed to hybridize to the filter in this buffer at a concentration of 2×10^6 d.p.m. ml^{-1} at 37 °C for 18 h. Filters were then washed repeatedly in hybridization buffer without probe at 42 °C, and were exposed to film at −70 °C using a Lightning-Plus intensifying screen.

Table 1 Protein, mRNA and DNA sequences of PrP27–30, oligonucleotide probe and homologous cDNA clone

PrP27–30:					Gly	Gln	Gly	Gly	Gly	Thr	His	Asn	Gln	Trp	Asn	Lys	Pro	Ser	Lys	
Predicted mRNA			5′	GGN	CAR	GGN	GGN	GGN	ACN	CAY	AAY	CAR	UGG	AAY	AAR	CCN	UCN	AAR	3′
Oligonucleotide:										3′	GTR	TTR	GTY	ACC	TTR	TTY	GG	5′		
cDNA:	5′C	GGA	TGG	GGC	CAA	GGA	GGG	GGT	ACC	CAT	AAT	CAG	TGG	AAC	AAG	CCC	AGC	AAA-		
Predicted amino acids:		Gly	Trp	Gly	Gln	Gly	Gly	Gly	Thr	His	Asn	Gln	Trp	Asn	Lys	Pro	Ser	Lys-		
											10									
			CCA	AAA	ACC	AAC	CTC	AAG	CAT	GTG	GCA	GGG	GCT	GCG	GCA	GCT	GGG	GCA-		
			Pro	Lys	Thr	Asn	Leu	Lys	His	Val	Ala	Gly	Ala	Ala	Ala	Ala	Gly	Ala-		
					20										30					
			GTA	GTG	GGG	GGC	CTT	GGT	GGC	TAC	ATG	CTG	GGG	AGC	GCC	GTG	AGC	AGG-		
			Val	Val	Gly	Gly	Leu	Gly	Gly	Tyr	Met	Leu	Gly	Ser	Ala	Val	Ser	Arg-		
									40											
			CCC	ATG	ATC	CAT	TTT	GGC	AAC	GAC	TGG	GAG	GAC	CGC	TAC	TAC	CGT	GAA-		
			Pro	Met	Ile	His	Phe	Gly	Asn	Asp	Trp	Glu	Asp	Arg	Tyr	Tyr	Arg	Glu-		
			50										60							
			AAC	ATG	TAC	CGC	TAC	CCT	AAC	CAA	GTG	TAC	TAC	AGG	CCA	GT	3′237			
			Asn	Met	Tyr	Arg	Tyr	Pro	Asn	Gln	Val	Tyr	Tyr	Arg	Pro					
							70								78					

R = A or G; Y = C or T (or U); N = any base. mRNA from scrapie-infected mouse brain was used to synthesize cDNA using the conditions of Okayama and Berg[22] with an oligo(dT) primer. Reverse transcriptase was obtained from Seikagaku America, Inc., St Petersburg, Florida. mRNA:cDNA duplexes were extracted with phenol-chloroform, precipitated with ethanol, and the second DNA strand synthesized using the method of Gubler and Hoffman[23]. After extraction and precipitation as above, *Eco*RI sites were methylated with *Eco*RI methylase and DNA ends filled-in with the Klenow fragment of DNA polymerase. DNA was extracted and precipitated again and was ligated to *Eco*RI 8-mer linkers (New England BioLabs) using T4 DNA ligase. After 18 h at 12 °C, the reaction tubes were heated to 70 °C for 5 min to inactivate the ligase and linkers were digested for 4 h at 37 °C with *Eco*RI (12 units per μg cDNA). Small DNA fragments were removed by microdialysis for 1 h on Millipore VM 0.05-μm filters against *Eco*RI digestion buffer. *Eco*RI was again added for a second cycle of digestion, followed by microdialysis. DNA was then extracted, ethanol-precipitated and ligated to *Eco*RI-digested calf alkaline phosphatase-digested (Boehringer-Mannheim) λgt 11 DNA[24]. The ligation mixture was packaged using the Packagene system (Promega-Biotec, Madison, Wisconsin) and used to infect *Escherichia coli* strain Y1088 (ref. 25). Plaques were screened by blotting onto nitrocellulose filters[26] and were probed with ^{32}P-labelled oligonucleotide (see Fig. 1 legend). A positive plaque was picked off and purified by re-plating twice; DNA was isolated, digested with *Eco*RI and two insert fragments of 0.9 and 0.8 kb were found. The oligonucleotide hybridized only to the larger fragment. Both fragments were isolated on 1% low-melting point agarose (Sea Plaque; FMC Corp., Rockland, Maine), purified on Elutip ion-exchange columns (Schleicher & Schuell) and subcloned into M13 (mp18)[27] (BRL) and pEMBL8 (ref. 28). Complementary clones containing both the 0.8- and 0.9-kb fragments were identified by the C test[27], and the partial DNA sequence was determined using the dideoxy method[29]. The 0.8-kb fragment, which did not hybridize with the oligonucleotide, had a 60–70-base poly(dT) region at the junction with the M13 vector (sequence not shown), indicating that this DNA fragment was copied from the poly(A)-containing 3′ end of the mRNA. The 0.9-kb fragment, which hybridized with the oligonucleotide probe, had no poly(dT) sequence at either end, indicating that this fragment was derived from the 5′ end of the mRNA. The preliminary DNA sequence of one end of this fragment is shown. The amino-acid translation of this sequence shows exact homology with the published 15-amino-acid sequence of PrP27–30 (boxed). There is an open reading frame which continues as far as the sequence was read (237 bases from the *Eco*RI site of linkage to the M13 vector).

Messenger RNA populations were obtained from brains, livers and spleens of scrapie-infected mice or hamsters. The oligonucleotides hybridized with 1–3 bands in 9 of 15 samples analysed; however, in no case was there evidence for a scrapie-specific mRNA species (Fig. 1). In each case, uninfected cells or organs showed a band(s) similar to that seen in infected cells. The bands exhibited some 'organ specificity' as both hamster and mouse brain and mouse neuroblastoma cells showed a prominent 2.4–2.5-kb band which was not seen in mouse spleen. There was a weaker reaction with a similar-sized band in mouse and hamster liver. After longer exposure of the autoradiographs, a second band (1.3 kb) was seen in mouse liver and two other bands (2.0 and 1.2 kb) were seen in mouse neuroblastoma cells. However, when the blots were washed at higher temperatures (48 °C), most of these bands disappeared and only the 2.5-kb bands seen in the brain and neuroblastoma cells remained (data not shown).

In the hybridization conditions used, the oligonucleotides may have hybridized to mRNAs having rather poor homology. If a cDNA clone of the message for the PrP27–30 could be isolated, a more precise analysis should be obtained. Therefore, we used the oligonucleotides to screen a cDNA library made from scrapie-infected mouse brain. One clone was isolated, and its partial DNA sequence indicated that it could be translated into an animo-acid sequence identical to PrP27–30 at all 15 positions known (Table 1). Thus, this clone seemed to contain a sequence corresponding to the PrP27–30 protein message. Comparison of the predicted amino-acid sequence shown in Table 1 with a computer bank of known sequences has revealed to date no close homology with any protein tested, except PrP27–30. When the clone was used as a probe against various mRNAs, a prominent 2.4–2.5-kb band was seen in both normal and scrapie-infected mouse and hamster brain (Fig. 2); there was no evidence for any unique mRNA associated with scrapie infection. Furthermore, there was no reactivity with any mRNA in mouse spleen, which is known to contain significant infectivity[18–20]. Liver mRNA also failed to hybridize with the cDNA clone, indicating that PrP27–30 was not synthesized in this organ and reactivity of liver mRNA with the oligonucleotide probably detected a message unrelated to PrP27–30.

These results suggest that the published PrP27–30 sequence may not be specific for the infectious scrapie agent; our data imply that a similar protein exists in uninfected mouse and hamster brain. However, if expression of this protein is associated with scrapie disease[12], post-transcriptional regulation could conceivably result in increased levels of the protein in infected brain tissues. It is also possible that the messages detected in normal and infected tissues encode proteins which differ by point mutations or post-translational modifications; this might result in important biological variations similar to those encountered with certain cellular *onc* genes[21]. If this were the case, the normal homologue of the scrapie gene sequence would be endogenous in normal brains. It is interesting to note that PrP27–30 of mouse and hamster must be highly homologous as the hamster PrP27–30 protein sequence was identical to the translated sequence derived from mouse brain cDNA.

Another, to us more likely, interpretation of these results is that PrP27–30 is a normal protein of both mouse and hamster brain which is apparently synthesized in neurones such as the neuroblastoma cell line tested. This protein may be a component

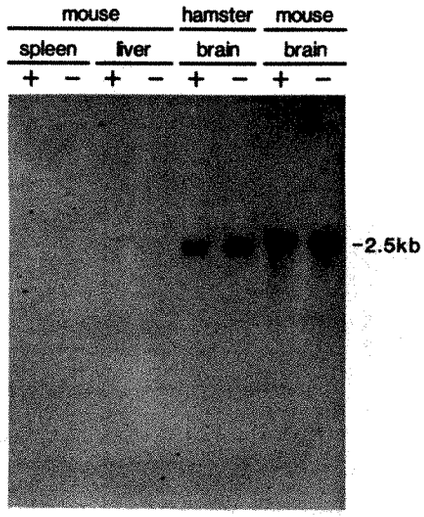


Fig. 2 Hybridization of the PrP27-30 cDNA clone with various mRNAs from hamster and mouse. Poly(A) RNA from various organs was electrophoresed in a formaldehyde agarose gel, blotted onto nitrocellulose, baked for 2 h *in vacuo*, then prehybridized in 2× SSPE, 0.1% SDS, 5× Denhardt's, 50 μg ml^{-1} denatured salmon sperm DNA at 65 °C for 3 h. Hybridization to the nick-translated (specific activity 5×10^7 d.p.m. μg^{-1}) 0.9-kb cDNA clone in the pEMBL8 vector was done in similar conditions for 40 h. Blots were washed three times for 30 min with 2× SSPE, 0.1% SDS and once with 0.1× SSPE, 0.1% SDS at 65 °C, and were exposed to film. +, Scrapie-infected; −, uninfected. When blots previously hybridized to the oligonucleotides (Fig. 1) were eluted and rehybridized to the cDNA clone, the bands detected migrated in an identical manner to the 2.4-2.5-kb bands detected by the oligonucleotides in mouse and hamster brain and neuroblastoma cell RNA; mouse and hamster liver and spleen RNA on these blots showed no hybridization with the cDNA probe (data not shown).

of the scrapie-associated fibrils which appear to be a consistent pathological feature of scrapie infection and disease; in this case, PrP27-30 would be concentrated during the isolation procedures used to purify the fibrils and the associated scrapie infectivity. It remains to be seen whether this protein has an active or passive role in the pathogenicity of scrapie infection.

We thank Gary Hettrick and Robert Evans for graphics assistance, and Helen Blahnik for preparing the manuscript.

Note added in proof: Since submission of this manuscript we have become aware of results by Oesch *et al.* (*Cell*, in the press) concerning cloning of PrP27-30-specific cDNA from scrapie-infected hamster brain. The DNA sequence of this hamster clone, kindly provided by Dr S. Prusiner, shows 84% base homology with the mouse cDNA sequence given in Table 1.

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Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1

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B-cell stimulatory factor-1 (BSF-1)[1], formerly designated B-cell growth factor, is a T-cell-derived factor required for entry into the S phase of the cell cycle by B cells stimulated with low concentrations of anti-IgM antibodies[2-5]. BSF-1 acts directly on resting B cells to prepare them to synthesize DNA more promptly on subsequent exposure to competent stimuli[6,7] and to strikingly enhance their expression of class II molecules of the major histocompatibility complex[8,9]. Previous studies have shown that murine BSF-1 can be separated physically from interleukin-2 (IL-2) and that the molecule has an apparent relative molecular mass (M_r) of ~15,000 and *p*I values of 6.4–6.7 and 7.4 (ref. 10). Here, we report the production of a monoclonal antibody to BSF-1, its use in characterizing BSF-1, and functional studies demonstrating that this molecule is distinct from IL-1, IL-2 and IL-3.

BSF-1 was obtained from 5-10-l batches of serum-free supernatants of cells of the EL-4 line, a C57BL/6 thymoma, which had been induced with 4β-phorbol 12β-myristate 12α-acetate (10 ng ml^{-1}, Sigma) for 48 h. Partially purified BSF-1 was used to immunize rats, and spleen cells from these animals used to obtain hybridomas (Fig. 1 legend). Subcloning of a selected hybridoma produced two lines, 18F10 and 11B11, both secreting rat IgG1 antibodies, which possessed identical properties and were used interchangeably (Fig. 1 legend).

Figure 1*a* illustrates the absorption assay for BSF-1 and demonstrates that 18F10 binds a major fraction of BSF-1 activity (9 U) when plates are coated with 40 ng of purified monoclonal antibody (Fig. 1*a*). Both 18F10 and 11B11 block BSF-1 activity when added directly to co-stimulation assays (Fig. 1*b*). As little as 8 ng of antibody per 200 μl culture almost completely inhibited the response of resting B cells to Bet-2, a monoclonal rat anti-mouse IgM[11], and 2 U of BSF-1. A rat IgG antibody to

Table 1 Binding to and elution from anti-BSF-1 affinity column

	Volume (ml)	BSF-1 activity U ml^{-1}	Total
Applied sample (TMS-CpG fraction)	5	5,700	28,500
Effluent	5	49	245
Acetic acid (0.8 M) eluate	0.4	74,000	29,600

A 5-ml sample of TMS-CpG-fractionated EL-4 supernatant was applied to an affinity column (2 ml packed volume). The effluent (5 ml) was collected and the column was washed with 20 ml of NaCl (1 M), 20 ml NP40 (0.5%) in water, 20 ml phosphate-buffered saline and 20 ml water. The column was then eluted with 20 ml of 0.8 M acetic acid. The eluate was lyophilized and reconstituted with 0.4 ml water. BSF-1 activity was measured in the anti-IgM co-stimulation assay using Bet-2.

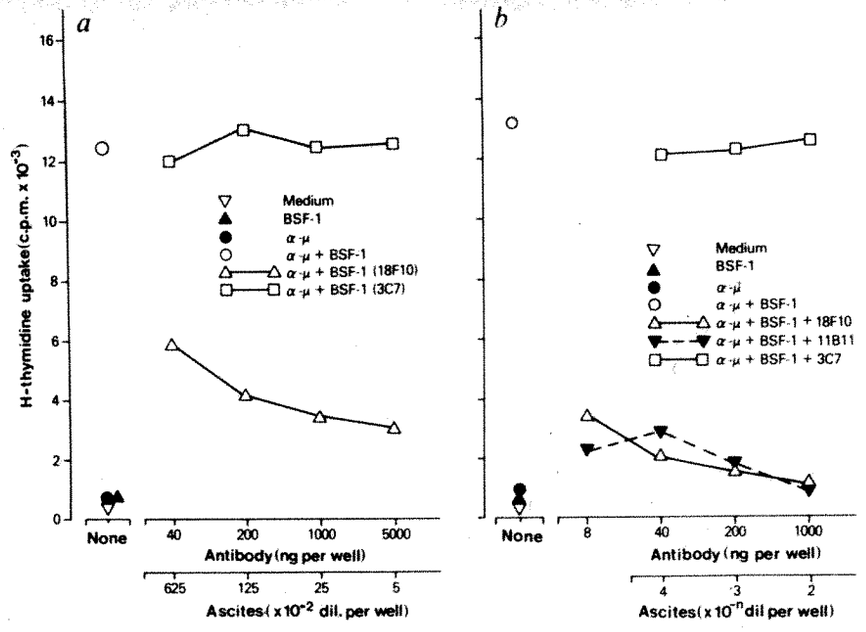


Fig. 1 Monoclonal anti-BSF-1 absorbs BSF-1 (*a*) and inhibits BSF-1 activity (*b*).
Methods. Partial purification of BSF-1, to be described in detail elsewhere (J.O. *et al.*, in preparation), involved adsorption to and elution from trimethyl silyl(TMS)-derivatized controlled pore glass beads (TMS-CpG; Sepralyte; Analytical Chemicals, Harbor City, California) followed by reverse-phase HPLC. This yielded a >2,000-fold increase in specific activity as measured in units (U) defined by the amount of BSF-1 required for half-maximal responses in the anti-IgM co-stimulation assay, using resting B cells as a test population[2]. Lew/Sn rats were immunized with 1.4×10^6 U of TMS-CpG-purified BSF-1 emulsified in complete Freund's adjuvant. Animals were boosted twice with 1.6×10^5 U of HPLC-purified BSF-1 in incomplete Freund's adjuvant, and challenged intravenously with 3.6×10^6 U of HPLC-purified BSF-1. Three days later, spleen cells were fused with the P3-NS-1/1-Ag4-1 line of the P3-X63-Ag8 myeloma. Hybridoma supernatants were screened for anti-BSF-1 activity by an absorption technique in which 24-well culture dishes were coated with ion-exchange chromatography-purified rabbit anti-rat immunoglobulin. Coated culture dishes were incubated with hybridoma supernatants for 4 h, washed and then incubated with a standard amount of HPLC-purified BSF-1 for an additional 4 h. BSF-1 activity remaining in solution was assessed by culturing samples with resting splenic B cells from BALB/c mice in the presence of purified goat anti-mouse IgM (5 μg ml^{-1}) or Bet-2. Uptake of ^{3}H-thymidine was measured after 3 days of culture. Of 72 fusion wells with growing hybridomas, 6 were selected for further study on the basis of this assay. Of these, cells from one fusion well which had reproducible absorptive activity at a high dilution were used in the remaining experiments. The cells from this well were subjected to a series of subclonings, the last of which yielded 18F10 and 11B11, both secreting rat IgG1 antibodies. ***a***, Absorption of BSF-1 activity. Tissue culture wells (Cluster 24, Costar) were incubated with 10 μg of purified rabbit anti-rat immunoglobulin, washed and incubated with various concentrations of purified 18F10 (anti-BSF-1) or with various dilutions of ascitic fluid containing 3C7 (anti-IL-2 receptor). After washing, HPLC-purified BSF-1 (9 U) was added to wells, allowed to incubate for 4 h and supernatants were tested for residual BSF-1 activity in the anti-IgM (α-μ) co-stimulator assay. Bet-2, a monoclonal α-μ, was incubated with purified B cells (1×10^5 per 0.2 ml) in the presence of absorbed supernatants and uptake of ^{3}H-thymidine measured 3 days later. As controls, ^{3}H-thymidine uptake with medium alone, α-μ, BSF-1, or α-μ and BSF-1 are shown. ***b***, Inhibition of BSF-1 activity. Purified B cells were incubated with Bet-2 and BSF-1 (2 U) in the presence of various amounts of purified 18F10 or 11B11 (anti-BSF-1) or of various dilutions of ascitic fluid containing 3C7 (anti-IL-2 receptor). Uptake of ^{3}H-thymidine was measured 3 days later.

Table 2 Anti-BSF-1 does not block responses to IL-1, IL-2 or IL-3

Response of B cells to anti-IgM and BSF-1					
Stimulant	0	11B11		3C7	
		10^{-3}	10^{-4}	10^{-3}	10^{-4}
None	425	497	403	296	176
αIgM + BSF-1	21,020	1,295	3,425	25,160	18,640
Response of thymocytes to PHA and IL-1*					
	0	18F10	3C7	50C1	
None	660	580	441	336	
PHA (2 μg ml^{-1})	1,395	1,651	1,120	1,313	
PHA + IL-1 (50 U)	90,943	87,877	19,451	68,788	
Response of HT-2 cells to IL-2*					
	0	18F10	3C7	50C1	
None	143	—	—	—	
IL-2 (0.3 U ml^{-1})	22,913	19,290	5,300	23,983	
Response of DA-1 cells to IL-3*					
		0	11B11	50C1	
None		40	—	—	
WEHI-3 supernatant (1:5)		22,073	29,590	21,686	

^{3}H-thymidine uptake in response to anti-IgM and BSF-1 (10 U ml^{-1}) was measured at 3 days of culture and responses to IL-1, IL-2 and IL-3 were measured at 2 days of culture and recorded here as c.p.m. Assays were performed as described in ref. 14 for IL-1, ref. 15 for IL-2 and ref. 16 for IL-3, except that in the last case the concentration of the fetal calf serum (FCS) was 2% rather than 10%. 18F10 and 11B11 are rat monoclonal antibodies to BSF-1; 3C7 is a rat monoclonal antibody to the IL-2 receptor; 50C1 is a rat monoclonal anti-dinitrophenyl antibody; 10^{-3} and 10^{-4} refer to dilutions of ascitic fluid. 3C7 inhibits the proliferation of thymocytes in response to phytohaemagglutinin (PHA) and IL-1 because of the involvement of IL-2 in that response; it also inhibits the proliferation of HT-2 cells in response to IL-2, as the IL-2 receptor is critical to such proliferation.
* Ascitic fluid dilution of 10^{-2} used in these experiments.

the IL-2 receptor (3C7, generously provided by Drs G. Ortega, E. Shevach and T. Malek[12]) had no inhibitory activity, indicating that the response of resting B cells to anti-IgM and BSF-1 does not depend on IL-2, and providing a control for nonspecific inhibition.

To characterize BSF-1 more fully, 1.2×10^5 U HPLC-purified BSF-1 was labelled with ^{125}I by the chloramine-T method. A sample of this material was incubated with affinity-purified monoclonal antibody and immunoprecipitated with Sepharose beads to which rabbit anti-rat immunoglobulin had been coupled. SDS-polyacrylamide gel electrophoresis (PAGE) of dissolved immunoprecipitates yielded two major bands which migrated with apparent molecular sizes of 19,000–20,000 and 14,000, respectively (Fig. 2*a*). Two-dimensional gel electrophoresis (isoelectric focusing followed by SDS-PAGE)[13] indicated that the 19,000–20,000-M_r species had a *p*I of 6.7; the material of lower M_r was not detected on the two-dimensional gel (Fig. 2*b*). The *p*H range examined was 4.6–7.1 and it is possible that the 14,000-M_r molecule had a *p*I outside this range and migrated off the gel.

HPLC-purified BSF-1 was subjected to SDS-PAGE, then individual lanes were transferred to nitrocellulose paper for protein blotting[14] or cut into fragments for extraction and assay of BSF-1 activity (Fig. 3). ^{125}I-labelled antibody bound to two bands, migrating with mobilities of 19,000–20,000 and 14,000, respectively. Analysis of extracted material showed that the bulk of the BSF-1 activity co-migrated with the 19,000–20,000-M_r species.

The antibody could also be used for purification of BSF-1 from complex mixtures (Table 1). An affinity column was prepared by conjugating a mixture of 18F10 and 11B11 to CnBr-activated Sepharose 4B (1–2 mg antibody per g Sepharose). TMS-CpG-purified EL-4 supernatant (2.85×10^4 U BSF-1) was applied to a 2.0-ml column; 200 U appeared in the effluent and

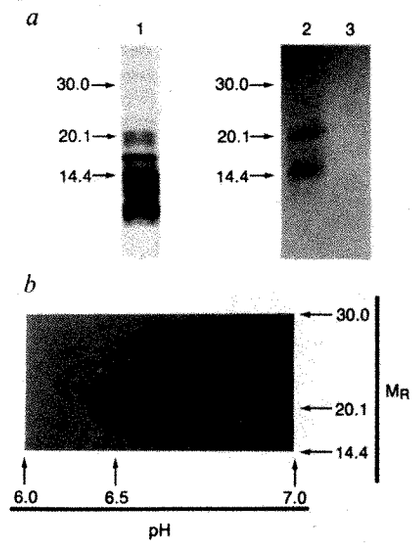


Fig. 2 SDS-PAGE (*a*) and two-dimensional gel electrophoresis (*b*) of immunoprecipitates of HPLC-purified ^{125}I-labelled BSF-1. *a*, SDS-PAGE of HPLC-purified ^{125}I-BSF-1 and of immunoprecipitates prepared with anti-BSF-1 or with normal rat serum. Samples were electrophoresed in 20% polyacrylamide at a constant voltage of 150 V. Autoradiographs were prepared using an intensifying screen and Kodak X-Omat film. Lane 1, SDS-PAGE of HPLC-purified ^{125}I-BSF-1. Lane 2, SDS-PAGE of a sample prepared by overnight incubation of HPLC-purified ^{125}I-BSF-1 with affinity-purified 18F10 and 11B11 (100 μg), absorption with Sepharose 4B, and then binding to rabbit anti-rat immunoglobulin coupled to Sepharose 4B. The sample was dissolved by boiling in 0.1% SDS in 2 mM dithiothreitol, 1 mM EDTA. Lane 3, SDS-PAGE of a sample prepared identically to that in lane 2 except that normal rat serum (10 μl) was used in place of 18F10 and 11B11. *b*, Two-dimensional electrophoresis of a replicate sample of the immunoprecipitate applied to lane 2 of the SDS-PAGE in *a*. The dissolved sample was placed in a buffer containing 9.5 M urea, 2% NP40, 2% ampholines (1.6%, *p*H 5–7; 0.4%, *p*H 3–10), and 5% β-mercaptoethanol in water and applied to an electrofocusing gel. After focusing, the sample was subjected to SDS-PAGE. The *p*H range analysed was 4.6–7.1. Only a portion of the autoradiograph is illustrated here. M_r values given are $\times 10^{-3}$.

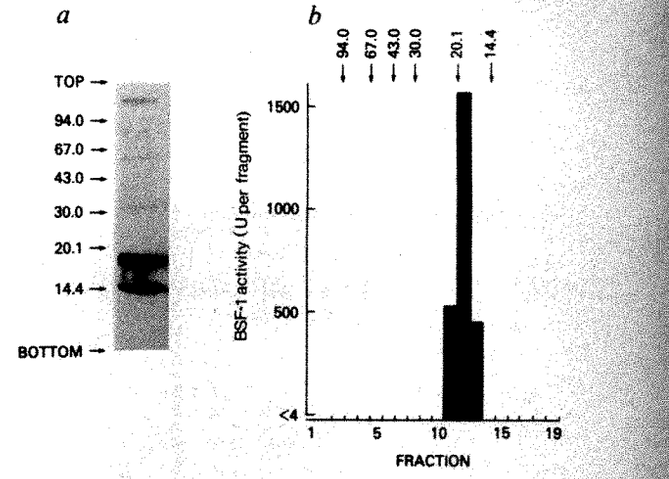


Fig. 3 Protein blotting (*a*) and BSF-1 activity of HPLC-purified BSF-1 separated on SDS-PAGE (*b*). *a*, 2.8×10^4 U of HPLC-purified BSF-1 in 20 μl was separated by SDS-PAGE as in Fig. 2*a*. Low-M_r markers ($\times 10^{-3}$; Pharmacia) were included in these lanes and in parallel lanes. The contents of this lane were transferred to nitrocellulose paper, using a Transblot Cell (Bio-Rad) and developed with ^{125}I-labelled 11B11. *b*, 2.8×10^4 U of HPLC-purified BSF-1 in 20 μl was separated by SDS-PAGE. The lane was cut into 0.5-cm fragments which were disrupted in 20% FCS-containing medium by cycles of freezing and thawing; extracted material was assayed for BSF-1 activity and is shown in units per fragment. Low-M_r markers were included in the separated sample and in adjacent lanes; the latter were stained with Coomassie blue to determine the location of each marker.

elution with 0.8 M acetic acid yielded 2.96×10^4 U. When BSF-1 was applied to a control column, prepared by conjugating normal rat immunoglobulin to Sepharose 4B, essentially all applied BSF-1 activity appeared in the effluent.

The antibody has also been used to test the relationship of BSF-1 to several other well-defined lymphokines. Table 2 indicates that although 18F10 ascites at a dilution of 10^{-4} causes marked inhibition of BSF-1 activity, the activities of IL-1, IL-2 and IL-3 tested, respectively, in a thymocyte assay[15], on the growth of HT-2 cells[16] and in the stimulation of an IL-3-dependent T-cell line, DA-1 (ref. 17), are not inhibited even at dilutions of ascitic fluid of 10^{-2}.

We found a discrepancy in the apparent size of the active BSF-1. Previous studies, based on separation of EL-4 supernatants by phenyl Sepharose chromatography and characterization on SDS-PAGE, had indicated respective M_rs for BSF-1 of 15,000–16,000 and 11,000 (ref. 10). The present results indicate that two major molecular species bear an antigenic determinant that reacts with the monoclonal anti-BSF-1 antibody. The larger, 19,000–20,000-M_r species, has BSF-1 activity, whereas the activity of the smaller species remains unresolved. The BSF-1 used in the present studies was prepared from the same cell line used in the previous work, the antibody seems to bind to virtually all material possessing BSF-1 activity in EL-4 supernatant, and the co-stimulation assay used is essentially identical. Gel filtration studies of a human BSF-1-like molecule have revealed a M_r of 17,000 which fell to 14,000 on treatment with 4 M urea[3], raising the possibility that changes in apparent M_r result from the nature of the purification procedure used. Nonetheless, the relationship between the active 19,000–20,000-M_r species and the 14,000-M_r species remains to be established.

Results indicating that BSF-1 is distinct from IL-1, IL-2 and IL-3 confirm earlier but less direct work[2–5,10]. This, together with the absence of interferon activity in BSF-1 preparations, indicates that BSF-1 is unique.

Thus, our results demonstrate the value of the monoclonal antibody to BSF-1 as a tool for the molecular characterization and purification of this lymphokine. Indeed, it had previously been proposed that this stimulant be designated BSF-p1, the 'p' reflecting the provisional assignment of the designation in view of the still-partial characterization of the molecule[1]. The development of a monoclonal antibody and our more precise characterization now allows us to refer to this as BSF-1.

The finding that the monoclonal antibody is a powerful inhibitor of BSF-1 activity in the anti-IgM co-stimulation assay may allow the role of BSF-1 to be examined in a wide variety of *in vitro* and *in vivo* immune responses, by incorporating the antibody in culture or by treating animals with anti-BSF-1. Such studies are now in progress.

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Anti-idiotypic T cells suppress rejection of renal allografts in rats

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Kidney allografts between inbred rats differing at the major histocompatibility complex (MHC) are normally rejected, usually within 10 to 12 days. In many strain combinations, however, permanent graft acceptance can be induced by either immunological enhancement[1,2] or a short course of immunosuppressive chemotherapy[3–5]. In both cases, prolonged graft survival is accompanied by the appearance in the spleen of a population of suppressor cells[6]. When transferred to a syngeneic host, these cells abrogate or strikingly diminish the rejection response elicited by a renal allograft of the same genotype as the original kidney donor. We have now examined the properties of these suppressor cells and have detected a subpopulation that proliferates *in vitro* when stimulated by irradiated syngeneic T blasts reactive to MHC alloantigens of the kidney donor strain. Comparable proliferation, however, is not induced either by syngeneic blasts reactive to a third strain or by polyclonal syngeneic blasts. These results support the hypothesis that this subpopulation is anti-idiotypic, with specificity for the idiotypes carried by syngeneic T cells stimulated by the kidney allograft. Such anti-idiotypic cells could function as suppressors.

Long-lived immunologically enhanced kidney allografts were induced as follows: AS ($RT1^l$) recipient rats were given 50×10^6 donor strain spleen cells intravenously 12 days before transplantation, together with three injections of AS anti-donor antiserum on days −11, 0 and +1. This procedure induces donor-specific hyporesponsiveness in the AS recipient, enabling it to accept a semi-allogeneic $(AS \times AUG)F_1$ kidney graft, and subsequently a fully allogeneic homozygous AUG graft that survives and functions indefinitely. At least two mechanisms seem to be involved in long-term allograft survival. First, the strong immunogenicity characteristic of a primary MHC-incompatible graft is reduced as donor strain dendritic cells are lost from the grafted tissue[7] and second, the graft recipient progressively develops increasing hyporesponsiveness to the donor genotype, which can be adoptively transferred in a population of splenic T lymphocytes[8].

Long-term kidney allograft survival can also be induced with the use of immunosuppressive drugs, of which cyclosporin is one of the most effective currently available[3–5]. In the rabbit, and in many rat strain combinations, indefinite graft acceptance can be achieved following a limited course of drug treatment for 2–4 weeks[4,6,9]. Recent evidence suggests that long-term graft survival after pharmacological immunosuppression may depend on similar mechanisms to those responsible for the maintenance stage of immunological enhancement[6]. Similar mechanisms may also apply in clinical organ transplantation, because patients show diminishing requirements for immunosuppression during the later post-transplant period. Consequently, the properties of such putative suppressor cells, in particular their specificity, are of considerable interest.

The experimental protocol to investigate cell specificity *in vitro* is shown in Fig. 1. Strain AS ($RT1^l$) rats carrying accepted $(AS \times AUG)F_1$ or AUG ($RT1^c$) kidneys (immunological enhancement), or AUG or WAG ($RT1^u$) kidneys (pharmacological immunosuppression) were boosted with 10×10^6 spleen cells of kidney donor genotype on day −6 to maximize the number of suppressor cells present in the spleen. On day 0, the spleens of these animals were excised, and cell suspensions prepared in culture medium (RPMI 1640 supplemented with 2mM L-glutamine, 100 units penicillin and streptomycin per ml, 5% heat-inactivated fetal calf serum v/v). These spleen cells, at 2×10^6 viable lymphocytes per ml, formed the responder population for mixed leukocyte cultures.

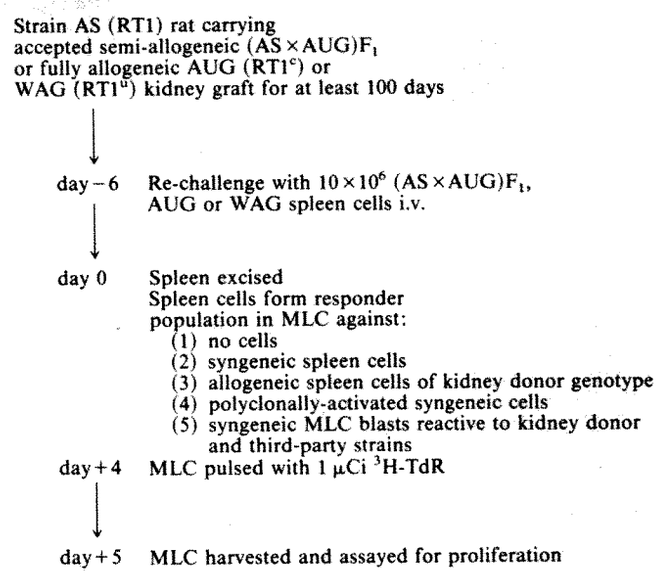


Fig. 1 Model of *in vitro* culture system.

Stimulator cells were prepared as follows. Activated blasts of the same strain as the graft recipient (AS, $RT1^l$) were generated *in vitro* by polyclonal activation or in mixed leukocyte culture (MLC). Polyclonal blasts were collected 3 days after stimulation with concanavalin A (Con A, 1 $\mu g\ ml^{-1}$), whereas blasts generated in MLC against either the kidney donor strain (AUG or WAG) or against an unrelated third party strain were restimulated (2^0 MLC) with the same antigen on day 10 of culture, and were harvested 3 days later. Normal spleen cells of both recipient and donor strains were also prepared. Activated blasts and normal cell suspensions were X-irradiated (3,000 rad) before use.

Responder (R) and stimulator (S) populations were then mixed at R:S of 1:1 in the presence of 2-mercaptoethanol (2-ME, 5×10^{-5} M), and were maintained *in vitro* for 5 days (optimal time-course determined from previous experiments, data not shown). The cells were pulsed with ^{3}H-TdR 1 $\mu Ci\ ml^{-1}$ for the final 18 h of culture.

Results of cell proliferation in response to the different stimulating populations are presented in Tables 1 and 2. Essentially similar results were obtained using spleen cells from animals with long-surviving kidney allografts irrespective of the method used to ensure protection of the graft. Table 1 presents spleen cell responses of rats with enhanced grafts and Table 2 those of rats that had received immunosuppression consisting of cyclosporin (10 mg per kg per day by gavage for 14 days post-transplant). In each case, spleen cells from animals with long-surviving kidney allografts showed a diminished response to stimulator cells of kidney donor genotype when compared to spleen cells from naive control animals. However, they demonstrated marked proliferation in response to syngeneic 2^0 MLC blasts reactive to the kidney donor strain. Significantly, this proliferative response was not elicited by polyclonal syngeneic blasts, or by 2^0 MLC blasts reactive to a third party strain, indicating that cells from the kidney graft recipient were not

Table 1 Proliferative responses of spleen cells from individual AS ($RT1^l$) rats carrying long-surviving immunologically enhanced (AS×AUG)F_1 or homozygous AUG ($RT1^c$) kidneys

Source of responder cells	Expt	Stimulator cells: No cells	Normal AS spleen	Normal AUG spleen	Con A-stimd AS blasts	AS blasts generated in 2° MLC, reactive to: AUG	WAG	DA
Strain AS rat carrying (AS×AUG)F_1 kidney	1	2.1±0.1	2.0±0.0	4.5±0.8	3.4±0.3	7.3±1.1	3.5±0.5	—
	2	1.1±0.4	1.2±0.1	3.6±0.2	3.2±0.4	6.4±0.9	3.0±0.1	—
Control, naive AS	1	1.7±0.2	2.0±0.3	9.2±0.4	3.2±0.2	2.6±0.4	3.1±0.1	—
	2	1.3±0.2	1.3±0.1	6.9±0.4	3.1±0.7	2.7±0.4	2.5±0.4	—
Strain AS rat carrying AUG kidney	3	3.7±0.5	3.9±0.6	5.0±0.9	5.4±1.3	14.4±1.1	—	4.6±0.7
	4	2.2±0.3	2.8±0.1	5.3±0.7	4.4±0.3	9.1±0.5	3.9±0.4	3.2±0.1
	5	1.8±0.1	1.8±0.3	4.5±0.3	—	9.0±0.7	—	3.6±0.2
	6	2.4±0.7	2.5±1.0	5.0±0.4	—	12.0±1.8	—	3.9±0.1
Control, naive AS	3	1.7±0.1	2.3±0.3	16.9±2.1	4.3±0.7	3.4±0.7	—	3.7±0.6
	4	2.7±0.2	3.0±0.1	9.9±1.3	3.9±0.6	2.9±0.1	3.4±0.2	3.4±0.4
	5, 6	2.0±0.4	2.1±0.2	9.5±0.6	—	3.1±0.3	—	2.4±0.4

Values represent proliferative activity in MLC (c.p.m.±s.e.×10^{-3}). Responder and stimulator cell preparation as described in the text. Culture volumes = 200 μl. Six replicates per culture.

Table 2 Proliferative responses of spleen cells from individual pharmacologically suppressed AS ($RT1^l$) rats carrying long-surviving AUG ($RT1^c$) or WAG ($RT1^u$) kidneys

Source of responder cells	Expt	Stimulator cells: No cells	Normal AS spleen	Normal AUG spleen	Normal WAG spleen	Con A-stimd AS blasts	AS blasts generated in 2° MLC, reactive to: AUG	WAG	DA
Strain AS rat carrying AUG kidney	7	1.4±0.0	1.8±0.3	5.1±0.3	8.2±0.4	4.3±0.6	9.4±0.9	4.2±0.3	3.8±0.1
	8	0.9±0.1	0.7±0.0	4.3±0.6	7.8±0.3	—	7.6±1.0	3.5±0.2	1.6±0.2
Strain AS rat carrying WAG kidney	7	1.9±0.4	2.3±0.1	8.0±0.7	4.5±0.2	4.2±0.5	4.0±0.8	8.5±1.0	3.3±0.2
	8	0.6±0.1	0.9±0.2	8.5±0.8	4.5±0.4	—	3.3±0.1	7.4±0.9	2.0±0.1
Control, naive AS	7	1.6±0.2	2.0±0.5	8.7±1.1	8.3±0.8	3.8±0.7	4.0±0.4	3.4±0.1	3.1±0.6
	8	0.8±0.2	0.8±0.3	7.4±0.6	6.8±1.1	—	2.9±0.7	3.0±0.4	1.7±0.1

Values represent proliferative activity in MLC (c.p.m.±s.e.×10^{-3}). Responder and stimulator cell preparation as described in the text. Culture volumes were 200 μl, with six replicates per culture.

merely being nonspecifically stimulated by activated syngeneic cells. The slight increase in ^{3}H-TdR uptake by responder cells in the presence of these non-specific stimulators may possibly be attributed to secretion of interleukins by activated stimulator cells.

These data suggest that a population of T lymphocytes contained within the spleens of rats bearing accepted renal allografts can specifically recognize and respond to the idiotypic determinants present on syngeneic cells reactive to the kidney donor genotype. Other explanations, for example a response against retroviruses carried by syngeneic blasts, are unlikely in view of the specificity of the response. Likewise the notion that the spleen cells from AS rats with long-surviving AUG kidneys proliferate in response to highly immunogenic alloantigen presented by or adsorbed to the surface of the AS anti AUG blasts seems improbable in view of the negligible response of naive AS spleen cells to such blasts. Further, there is a marked diminution in responsiveness of spleen cells from the 'suppressed' AS rats when cultured with AUG stimulator cells. Although not proven at the clonal level, this anti-idiotypic subpopulation is a strong candidate for the cell which mediates suppression of kidney allograft rejection *in vivo*.

Suppression of skin-graft rejection induced by autoimmunization with MLC blasts and attributed to anti-idiotype responses was reported by Andersson *et al.*[10]; however, it has proved difficult to confirm their experiments, which were based on a concept of shared idiotypes between T and B lymphocytes—an idea still unproven in the context of responses to MHC alloantigens. Dorsch and Roser[11], Stockinger[12], and Stephenson and Roser[13] have presented evidence that neonatally induced tolerance is mediated by suppressor cells with anti-idiotypic specificity, and Kimura and Wilson[14] have similarly concluded that resistance to graft-versus-host disease induced in F_1 rats injected with sublethal numbers of parental lymphocytes is due to anti-T cell idiotype responses. Our experiments show that a population of cells capable of transferring suppression powerful enough to protect a homozygous MHC incompatible kidney graft from rejection also contains a subpopulation of T cells which have anti-idiotypic specificity *in vitro* for syngeneic clones directed against the MHC antigens of the graft. Taken together, this evidence suggests that anti-T cell idiotype responses may underlie many states of unresponsiveness to MHC incompatible tissues, and it raises the question as to whether the same mechanisms are involved in self tolerance.

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Developmental regulation of a cloned adult β-globin gene in transgenic mice

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At different stages of mammalian development, distinct embryonic, fetal and adult haemoglobins are synthesized in erythroid cells, a process termed haemoglobin switching. The cellular and molecular mechanisms controlling haemoglobin switching have been intensively studied, but remain poorly understood[1]. To study the developmental regulation of globin gene expression, we have produced transgenic mice[2–6] in which cloned globin genes are present in erythroid cells throughout development. Recently, we reported that adult mice in several transgenic lines carrying a hybrid mouse/human adult β-globin gene, expressed the gene in a correct tissue-specific manner[7]. This finding raised the question of whether an exogenous globin gene could also be subject to appropriate stage-specific regulation. We report here that the hybrid β-globin gene, like the endogenous adult β-globin genes, is inactive in yolk sac-derived embryonic erythroid cells and is expressed for the first time in fetal liver erythroid cells. Our results indicate that a stage-specific pattern of expression can be conferred by *cis*-acting regulatory elements closely linked to an adult β-globin gene. They also suggest that the embryonic and adult β-globin genes in the mouse are activated (or repressed) by distinct *trans*-acting regulatory factors present in embryonic, fetal and adult erythroid cells.

The timing of expression of the various β-like globin genes during normal mouse development has been well characterized. The first erythroid cells are produced in the blood islands of the yolk sac between days 7 and 11 of gestation, and are released into the embryonic circulation where they persist until day 15–16 (refs 8, 9). Before day 12, these 'primitive' nucleated erythrocytes synthesize the embryonic β-like globin chains y and z[10–12], encoded by two genes termed *y* and *βh1* (refs 13, 14); at around day 12, the primitive erythrocytes begin to produce small quantities of the adult β-globin chains[15,16] in addition to the y and z chains. The next site of erythropoiesis is the fetal liver, from which smaller, non-nucleated erythrocytes are released, beginning at ~day 12 and continuing until birth[17–19]. In fetal liver erythroid cells, all of the β-like globin chains are encoded by the adult β-globin genes, and the *y* and *βh1* genes are inactive[9–11]. At about day 16, erythropoiesis begins in the spleen and bone marrow, where it continues after birth[8]. The 'adult' erythroid cells produced in these organs display a pattern of globin gene expression similar to that observed in the fetal liver erythroid cells[9–11]; thus, the mouse has no separate 'fetal' globin

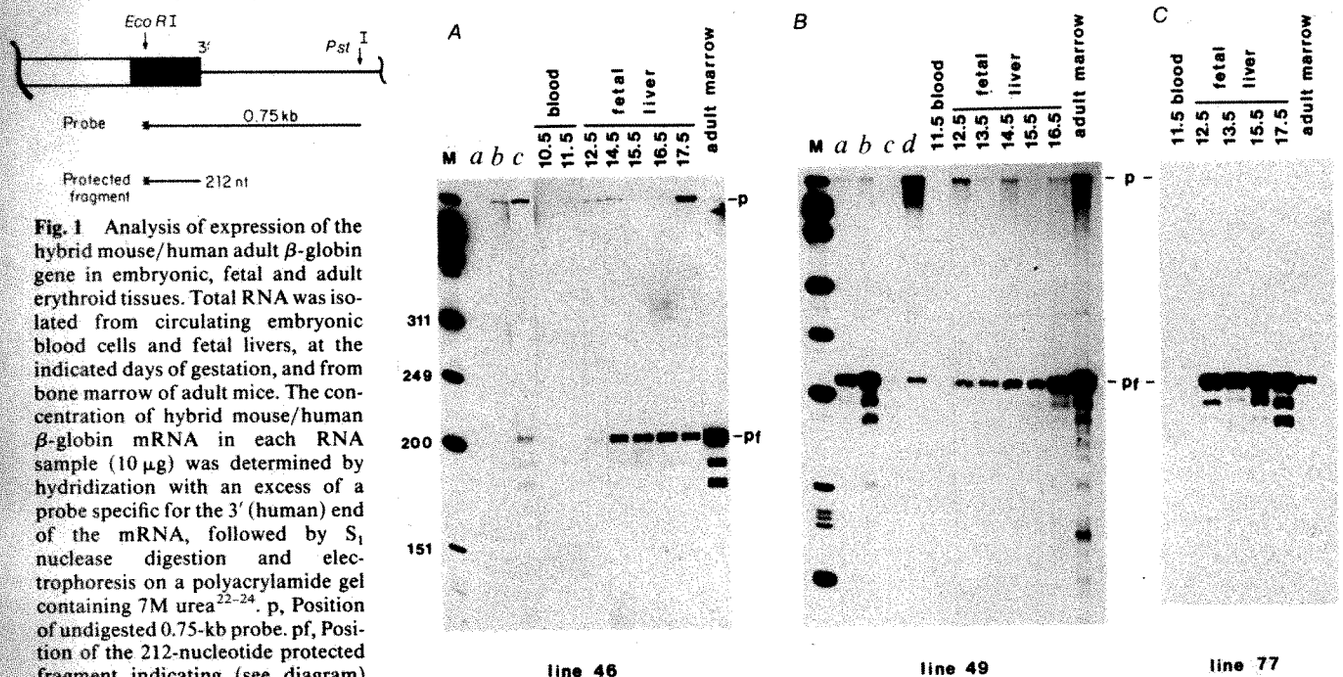


Fig. 1 Analysis of expression of the hybrid mouse/human adult β-globin gene in embryonic, fetal and adult erythroid tissues. Total RNA was isolated from circulating embryonic blood cells and fetal livers, at the indicated days of gestation, and from bone marrow of adult mice. The concentration of hybrid mouse/human β-globin mRNA in each RNA sample (10 μg) was determined by hydridization with an excess of a probe specific for the 3′ (human) end of the mRNA, followed by S_1 nuclease digestion and electrophoresis on a polyacrylamide gel containing 7M urea[22–24]. p, Position of undigested 0.75-kb probe. pf, Position of the 212-nucleotide protected fragment indicating (see diagram) correctly terminated human or hybrid mouse/human β-globin mRNA (the bands of lower relative molecular mass (M_r) were not reproducibly observed and are believed to be S_1 nuclease artefacts). It has previously been shown that the hybrid mRN4 has correct 5′ termini[1]. M, M_r markers (ΦX174 DNA digested with *Hin*fI). *A*, Transgenic mouse line 46: lanes *a–c*, positive control hybridizations with human cord blood RNA samples containing 1.4, 7 and 14 pg, respectively, of β-globin mRNA. *B*, Transgenic mouse line 49: lanes *a–d*, human cord blood RNA containing, respectively, 14, 70, 1.4 and 2.8 pg of β-globin mRNA. *C*, Transgenic mouse line 77. The steady-state level of endogenous β-globin mRNA in both fetal liver and adult bone marrow RNAs, expressed as a fraction of total RNA, is $\sim 10^{-3}$ (Fig. 2 and ref. 7). The approximate steady-state levels of the hybrid β-globin mRNA, determined by comparison with human β-globin mRNA standards, were: line 46 fetal livers (days 14.5–17.5), 3×10^{-6}; line 46 bone marrow, 1.5×10^{-5}; line 49 fetal livers (days 12.5–15.5), 5×10^{-7}; line 49 bone marrow, 10^{-5}; line 77 fetal livers, 2×10^{-5}; line 77 bone marrow, 2×10^{-6}. Lines 46 and 49 each contain one copy of the hybrid gene per diploid genome, while line 77 carries three copies[7].

Methods. Homozygous transgenic males from lines 46 and 49 were mated with normal C57BL/6J females and the resulting hemizygous transgenic embryos were dissected out at various stages of gestation. The day on which the copulation plug was observed was designated day 0. In line 77, which carries the foreign gene on the X chromosome, hemizygous transgenic males were mated with homozygous transgenic females to produce 100% transgenic (hemizygous male or homozygous female) embryos. Embryos in their extraembryonic membranes were first washed in phosphate-buffered saline (PBS) containing 10 U ml^{-1} heparin (PBS/H) to remove maternal blood. The membranes were then removed, the embryos were bled from the umbilical cord into PBS/H, and the cells were collected by centrifugation. Blood cells or whole fetal livers were pooled from 5–10 embryos at the same stage of gestation, and total RNA was isolated by homogenization in 4M guanidine thiocyanate and centrifugation through CsCl[34]. RNA concentrations were determined from the A_{260} and confirmed by electrophoresis on denaturing formaldehyde/agarose gels[35], which also showed that the RNA preparations were undegraded, as judged by the ribosomal RNA profiles. Preparation of the probe, hybridization and S_1 nuclease analysis were performed as described elsewhere[6,7]. The concentration of β-globin mRNA in the human cord blood RNA was determined by R_0t analysis (data not shown) to be 1%, in agreement with published estimates of the globin mRNA content of reticulocytes[36].

genes. This contrasts with the pattern of haemoglobin switching in humans, in which the adult β-globin gene encodes only ~5% of the β-globin chains in fetal liver erythroid cells (the majority being encoded by the fetal ^{A}-γ and ^{G}-γ genes) and becomes maximally active after birth[20].

The transgenic mice used in this study carried between one and three copies of a hybrid mouse/human β-globin gene that included the 5′ portion of the mouse β^{dmaj} adult globin gene and 1.2 kilobases (kb) of 5′ flanking DNA, joined to the 3′ portion of the human β-globin gene and 2 kb of 3′ flanking human DNA[7,21]. All of the plasmid vector DNA, except for 350 base pairs, had been removed from the gene before microinjection into mouse zygotes. Adult mice in four different transgenic lines were found to express the foreign globin gene exclusively or predominantly in erythroid tissues[7]. Here we examine the developmental regulation of the hybrid β-globin gene in three of the lines, numbered 46, 49 and 77.

Circulating blood was collected from transgenic embryos at day 10.5 or 11.5 post-coitum (p.c.), a stage at which essentially all of the blood cells are primitive erythrocytes derived from the yolk sac. Whole livers, consisting largely of fetal erythroblasts, were dissected from 12.5–17.5-day p.c. fetuses. In addition, bone marrow was obtained from the femurs of adult transgenic mice. Total RNA was isolated from each of these tissues, and the level of hybrid mouse/human β-globin messenger RNA was measured by hybridization with a ^{32}P-labelled probe specific for the human portion of the mRNA, followed by digestion with S_1 nuclease, electrophoresis on polyacrylamide gels and autoradiography[21–24].

No hybrid mouse/human β-globin mRNA was detected in blood cell RNA from 10.5- or 11.5-day p.c. embryos, in any of the three transgenic lines examined. In contrast, the foreign mRNA was detected in fetal livers at all stages examined, as well as in the adult bone marrow (Fig. 1). For comparison, the expression of the endogenous adult β-globin genes in the same tissues was monitored by hybridization with a probe specific for mouse adult β-globin mRNA (Fig. 2). As expected, the endogenous adult mRNA was detected only in trace amounts in circulating blood of 11.5-day p.c. or earlier embryos, but was present at a high level in the fetal liver and adult marrow. To ensure that the circulating embryonic blood cells analysed were indeed erythroid cells containing globin mRNA, the RNA preparations were hybridized with a probe specific for the mouse embryonic y-globin mRNA. The y-globin specific probe hybridized strongly with RNA from 11.5- or 12.5-day p.c. embryonic blood, but not with fetal liver RNAs (Fig. 3). We conclude that the mouse/human hybrid β-globin gene in three different transgenic mouse lines is inactive in embryonic blood cells, and is first expressed in the fetal liver erythroblasts. This pattern of switching is identical to that of the endogenous adult β-globin genes.

Several aspects of the regulation of the exogenous globin gene differed significantly from that of the endogenous adult β-globin genes. First, the steady-state level of the hybrid β-globin mRNA did not exceed 2–3% (per gene copy) of the level of endogenous β-globin mRNA (see Fig. 1 legend) in either fetal liver or adult bone marrow cells[7]. Second, when the relative levels of expression of the foreign gene in fetal and adult erythroid tissues were compared, two different patterns were observed, each somewhat different from that of the endogenous adult β-globin genes. The endogenous adult β-globin mRNA was present at approximately equal levels in fetal liver and adult bone marrow RNA preparations, in each of the transgenic mouse lines (Fig. 2). In mice of lines 46 and 49, the hybrid β-globin mRNA was present at a 5–20-fold lower level in 12.5–15.5-day p.c. fetal liver RNA than in adult bone marrow RNA (Fig. 1). This low fetal/adult expression ratio is characteristic of the adult β-globin gene in humans[20], suggesting a possible influence of human β-globin sequences in the hybrid gene. However, in the third transgenic line examined (77), the hybrid β-globin mRNA level was 10-fold higher in fetal liver than in adult bone marrow (Fig. 1), which resembles neither the mouse nor the human pattern.

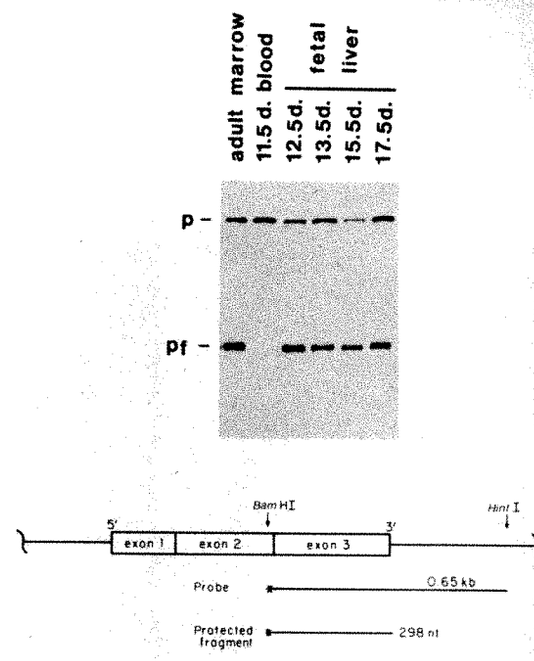


Fig. 2 Analysis of endogenous adult β-globin mRNA in embryonic, fetal and adult erythroid tissues. RNA samples from embryonic blood, fetal liver and adult bone marrow (0.5 μg of each) from transgenic mice in line 77 (the same RNA preparations as analysed in Fig. 1c) were analysed by hybridization with an excess of a probe specific for mouse adult β-globin mRNA, followed by S_1 nuclease digestion and electrophoresis on a polyacrylamide gel containing 7 M urea. The probe was a *Bam*HI–*Hin*fI fragment derived from a mouse β^{dmaj} globin complementary DNA clone[37], and hybridization to either β^{dmaj} globin mRNA or β^{s} globin mRNA (found in C57BL/6J mice) protects a labelled 298-nucleotide (nt) fragment from S_1 nuclease digestion (see diagram). p, Position of undigested 0.65-kb probe; pf, position of 298-nt protected fragment.

Methods. The *Bam*HI–*Hin*fI fragment of a mouse β^{dmaj} globin cDNA clone was 3′ end-labelled with ^{32}P to a specific activity of 6×10^{7} c.p.m. μg^{-1}, strand-separated on a polyacrylamide gel and eluted[24]; 2 ng of the probe was incubated with each mouse RNA sample at 45 °C in 80% formamide buffer[7], then digested with S_1 nuclease[22,23]. Control hybridizations with varying amounts of mouse blood RNA (data not shown) confirmed that the probe was indeed present in excess over the β-globin mRNA, and that the hybridization signals observed (298-nt protected fragment) were proportional to the concentration of mouse β-globin mRNA in each RNA sample.

Similarly, in a fourth transgenic line, 75A (ref. 7), preliminary data (not shown) indicate that the hybrid gene is expressed at a much higher level in the fetal liver than in adult erythroid tissues. We have recently produced several new transgenic mice, carrying either the human β-globin gene or the cloned murine β^{dmaj} globin gene, and analysis of these lines may help to elucidate the factors influencing the fetal/adult expression ratios of the mouse and human adult β-globin genes.

The main conclusion from the data presented here is that an adult β-globin gene, on a 5-kb DNA fragment, contains the information to remain inactive in primitive erythroid cells which are synthesizing embryonic haemoglobins, and yet to be specifically activated later in development in fetal and adult erythroid cells. This represents the first demonstration that a cloned globin gene can be subject to stage-specific regulation during the transition from embryonic to fetal erythropoiesis. It has already been established that globin genes introduced with limited amounts of flanking DNA can be specifically activated during the *in vitro* differentiation of erythroid cell lines[21,25–28] and preferentially expressed in erythroid rather than non-erythroid cells both in tissue culture[29,30] and in transgenic mice[7]. However, the possibility that globin gene switching requires much more DNA

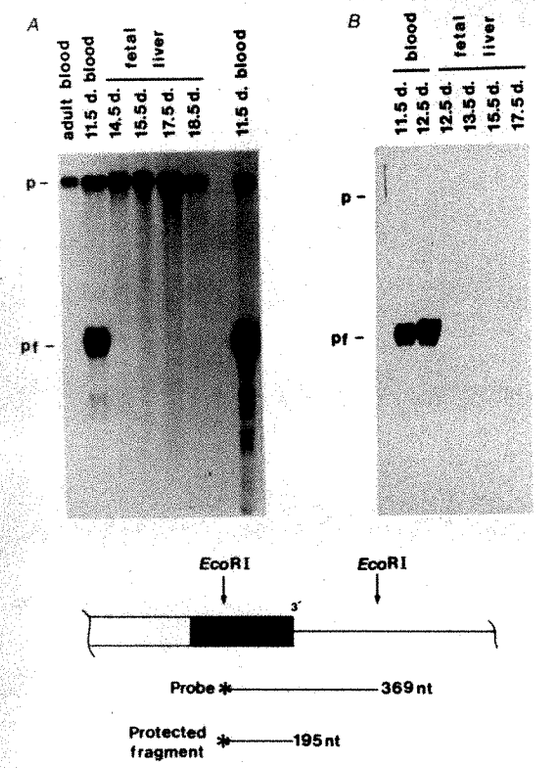


Fig. 3 Detection of endogenous γ-globin mRNA in embryonic blood cells. RNAs from embryonic blood and fetal livers of mice in lines 46 (*A*) and 77 (*B*) were analysed for the presence of mRNA from the mouse embryonic γ-globin gene; 0.1 μg of each RNA sample was hybridized with 3 ng of a 3′ end-labelled, strand-separated probe derived from the 3′ end of the mouse γ-globin gene[13]. The hybrids were digested with S_1 nuclease and analysed by electrophoresis on a polyacrylamide gel containing 7 M urea. p, Position of undigested probe (369 nt); pf, position of the 195-nt protected fragment. The lane marked 'adult blood' is a control hybridization with whole blood RNA from an adult mouse. The two lanes in *A* marked '11.5-day blood' show hybridization with two blood cell RNA preparations from different batches of 11.5-day-old embryos.

Methods. A pBR322 plasmid containing the 6-kb *Hin*dIII mouse DNA fragment including the γ-globin gene[13] was digested with *Eco*RI, 3′ end-labelled with ^{32}P, and the 369-nt fragment shown in the diagram was purified on a strand-separating polyacrylamide gel. The probe had a specific activity of 5×10^7 c.p.m. μg^{-1} and hybridization was performed at 45 °C.

sequence information was suggested by several lines of evidence, such as the observation that deletions several kilobases 3′ to the human γ-globin genes can block their normal postnatal inactivation (reviewed in ref. 31).

Our data do not exclude the possibility that more distant sequences in the β-globin cluster have an important role in regulation of the adult β-globin gene. Indeed, their absence from the microinjected globin gene is one of several factors that may be responsible for the low level of expression observed in the transgenic mice, as discussed previously[7]. However, the ability of a single adult β-globin gene, removed from the context of an intact β-globin gene cluster or 'domain'[32,33], to be nevertheless switched-on at the appropriate developmental stage, argues that distant sequences in the β-globin domain are not required in *cis* for this particular regulatory event to occur. Our results suggest a model in which the switch from embryonic to adult β-globin in the mouse is mediated by the presence of distinct *trans*-acting factors in embryonic as opposed to fetal and adult erythroid cells, which interact with DNA sequences closely linked to the embryonic and adult genes. Whether these regulatory factors are positive or negative, and the number and location of the DNA sequences with which they may interact, are questions that can be addressed by constructing hybrid genes between embryonic and adult β-globin genes, or between globin and non-globin genes[26,27] and examining their developmental regulation in transgenic mice.

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Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation

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The t(11;14)(q13;q32) chromosome translocation has been reported in diffuse small and large cell lymphomas and in chronic lymphocytic leukaemia (B-CLL)[1,2] and multiple myeloma[3]. Because chromosome band 14q32 is involved in this translocation, as well as in the t(8;14)(q24;q32) translocation of the Burkitt tumour[4], interruption of the immunoglobulin heavy-chain locus was postulated for this rearrangement[5,6]. We have cloned the chromosomal joinings between chromosomes 11 and 14 and also

between chromosomes 14 and 18, in B-cell tumours carrying translocations involving these chromosomes[6,7], and suggested the existence of two translocated loci, *bcl-1* and *bcl-2*, normally located on chromosomes 11 (band q13) and 18 (band q21) respectively, involved in the pathogenesis of human B-cell neoplasms[6,7]. The results indicate that in the leukaemic cells from two different cases of CLL, the breakpoints on chromosome 11 are within 8 nucleotides of each other and on chromosome 14 involve the *J*4-DNA segment. Because we detected a 7mer–9mer signal-like sequence with a 12-base-long spacer on the normal chromosome 11, close to the breakpoint, we speculate that the t(11 ; 14) chromosome translocation in CLL may be sequence specific and may involve the recombination system for immunoglobulin gene segment (*V–D–J*) joining.

Our previous analysis of the breakpoint between chromosomes 11 and 14 in one case of B-CLL (CLL 271) indicated that it occurred in the joining (*J*) region[6] of the heavy-chain locus[5] on chromosome 14. Chromosome 11-derived DNA sequences close to the breakpoint in the CLL 271 cells were then used to detect rearrangements of the homologous DNA sequences in neoplastic cells of a patient with a diffuse B-cell lymphoma with the t(11 ; 14) chromosome translocation (LN87) (ref. 6). These initial results indicated that the chromosomal breakpoints in these two cases were close to each other. In the present study we have examined the structure of the chromosome breakpoint in CLL 271 cells and have compared it with another case of B-CLL (CLL 1386).

Using the chromosome 11-derived DNA probe near the chromosomal breakpoint on the 14q$^+$ chromosome from CLL 271 (Fig. 1; ref. 6), we screened DNAs from several human B-cell neoplasms for rearrangements of the homologous DNA sequences and detected rearrangement in sample CLL 1386. Frozen CLL 1386 cells stored in liquid nitrogen were defrosted, stimulated to divide with B-cell mitogens[8] and karyotyped. CLL 1386 cells were found to have the characteristic t(11 ; 14) (q13 ; q32) translocation (Fig. 2).

Southern blots of *Bcl*I, *Hin*dIII and *Sst*I digested DNAs derived from CLL 271, CLL 1386 and LN87 were then hybridized with probes *a* or *b* (see Fig. 1*B*). We detected germline restriction fragment(s) in normal human DNA, whereas an additional fragment, depending on the restriction enzyme and probe used, was found in CLL 271, CLL 1386 and LN87 DNA (Fig 1*A*).

The results shown in Fig. 1*A* indicate that the breakpoint in CLL 271 is between the *Sst*I^1 and *Hin*dIII^2 sites and the breakpoint of LN87 is between the *Hin*dIII^2 and the *Sst*I^2 sites. Digestion of DNA from another case (CLL 1386) with *Sst*I did not result in the appearance of a rearranged DNA fragment, so the chromosome 11 breakpoint may be mapped between the *Hin*dIII^1 and the *Sst*I^1 sites. However, CLL 1386 DNA shows only a germline *Hin*dIII^1/*Sst*I^1 fragment (data not shown). Because the breakpoint of CLL 1386 is mapped close to the *Sst*I^1 site in the *Sst*I^1/*Hin*dIII^2 fragment, the result of digestion of CLL 1386 DNA with *Sst*I (shown in Fig. 1*A*) may reflect co-migration of a rearranged fragment with a germline DNA fragment.

We attempted to clone the joining between chromosomes 11 and 14 on the 14q$^+$ chromosome of CLL 1386 cells to compare it with that in CLL 271 cells, which we had already isolated[6]. The DNA of CLL 1386 cells was partially digested with *Sau*3A restriction enzyme and DNA fragments of 15–23 kilobases (kb) were collected by size fractionation using sucrose density gradient centrifugation. These were ligated to the λ phage vector EMBL3A DNA (ref. 9) and packaged *in vitro*. By screening 200,000 recombinant clones with probes *a* and *c* (Fig. 1), we obtained five independent clones. Two of the five clones also hybridized with a probe specific for the heavy-chain locus joining (J_H) segments (pHj)[6], indicating that they contained the joinings between chromosomes 11 and 14 on the 14q$^+$ chromosome and also that the breakpoint on chromosome 14 was close to the J_H region. The restriction maps of the region surrounding the 11 ; 14 chromosome joinings in CLL 1386 cells and of the corresponding normal chromosome 11 and the breakpoint in CLL 271 cells

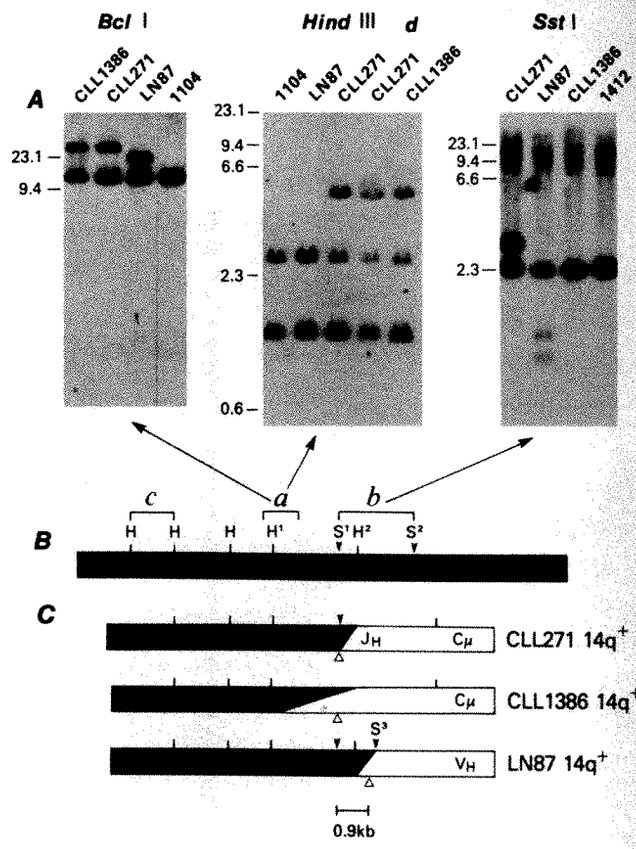


Fig. 1 Southern blot hybridization of CLL 271, CLL 1386 and LN87 DNA with human chromosome 11-derived probes (*A*) and the maps of chromosome 14q$^+$ in CLL 271, CLL 1386 and LN87 (*C*) and of normal chromosome 11 (*B*).
Methods. *A*, DNAs derived from neoplastic cells of CLL 271, CLL 1386 and LN87 were cut with the restriction enzymes shown, run on 0.7% agarose gels and transferred to nitrocellulose filters. The filters were hybridized with nick-translated probe *a* (pRc8SmR)[6] or *b* (shown in *B*). Hybridizations were carried out in 50% formamide/4 × SSC at 37 °C and finally washed with 0.2 × SSC at 65 °C. DNA 1104 and 1412 were obtained from T-cell lymphomas and used as germline control for chromosome 11. The size is shown in kb beside each panel. *B*, *C*, Structure of normal chromosome 11 in *B* is deduced by analysing the chromosome 11 sequence-containing recombinant clones in Fig. 3 obtained from CLL 271 and CLL 1386 genomic phage libraries. Filled box and open box show chromosomes 11 and 14, respectively. The cleavage sites by restriction enzyme *Sst*I and *Hin*dIII are shown by S (▲) and H (|), respectively. All restriction sites are not shown. Open triangles represent the joining sites between chromosomes 11 and 14 on chromosome 14q$^+$ which are determined as described in the text.

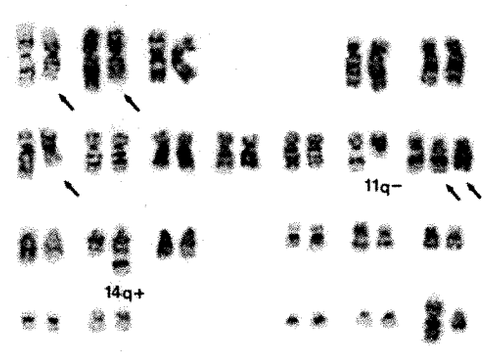


Fig. 2 Representative Giemsa-banded karyotype from CLL 1386 interpreted as: 47, XY, del (1) (p11p21) (q22q25), del (2) (q31), del (6) (q13–15), t(11 ; 14) (q13 ; q32), inv (12) (p13q13), +inv (12) (p13q13). Abnormalities other than the t(11 ; 14) translocation are indicated by arrows.

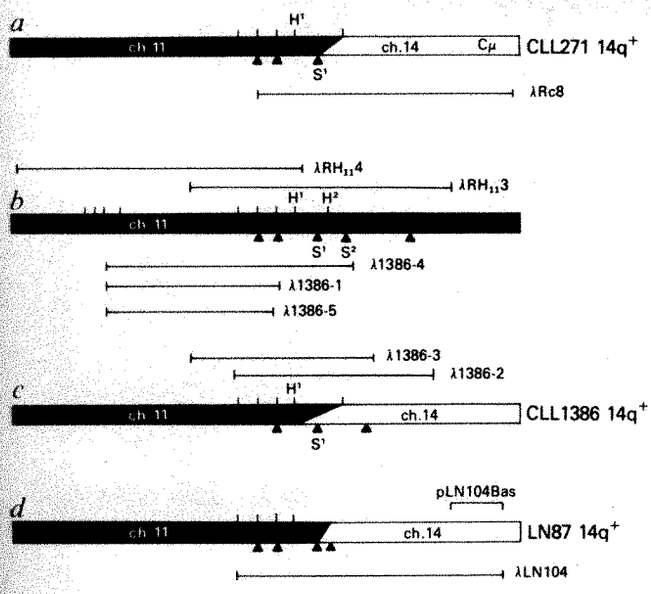


Fig. 3 Restriction maps of the regions surrounding the breakpoints on chromosome 14q$^+$ in CLL 271 (*a*), CLL 1386 (*c*), LN87 (*d*) and of normal chromosome 11 (*b*). Filled and open boxes represent chromosomes 11 and 14, respectively. Horizontal lines represent the insert DNAs of representative recombinant clones. λRc8 has been described previously[6]. λ RH11-3 and -4 were obtained by screening the CLL 271 library with chromosome 11 sequences. λ1386s were obtained from the CLL 1386 library as described in the text. λ1386-1, 4 and 5 all contain only DNA sequences of chromosome 11. The cleavage sites of restriction enzyme *Sst*I and *Hin*dIII are shown by H (|) and S (▲), respectively.

are shown in Fig. 3. As more detailed restriction mapping showed that the chromosome 11 breakpoints in CLL 271 and CLL 1386 are both very close to the *Sst*I[1] site (data not shown), we determined the nucleotide sequences surrounding these breakpoints, and the corresponding segment of normal chromosome 11 from CLL 271 cells. The sequences obtained are shown in Fig. 4, and indicate that the first 31 bases following the common *Sst*I[1] site are identical between the normal chromosome 11 and the 14q$^+$ joining region of the two CLLs. The sequences first start to diverge between normal chromosome 11 and the 14q$^+$ segment of CLL 1386. Then, just 8 bases further, the DNA sequences of the normal chromosome 11 and that of the 14q$^+$ joining region of CLL 271 diverge.

Because the breakpoint on chromosome 14 in CLL 271 cells was mapped in the J_H region of the immunoglobulin heavy-chain locus[6], we expected to find DNA sequences of the J_H region beyond the breakpoint. As shown in Fig. 4, beyond the breakpoints of both CLL 271 and CLL 1386 cells, the DNA sequences are almost identical to the *J*4 segment of the heavy-chain locus[10], although there are some unidentified sequences between the breakpoints on chromosome 11 and chromosome 14 of both cases. Addition of a few extraneous nucleotides during variable-diversity-joining (*V-D-J*) segment joining has been described[11].

Thus the breakpoints of CLL 271 and CLL 1386 on both chromosome 11 and chromosome 14 are very close to each other. Although the short sequence of 9 bases between the breakpoints on chromosomes 11 and 14 in CLL 271 is of unknown origin, the comparable sequence in CLL 1386 between chromosome 11 and 14 sequences on the 14q$^+$ chromosome shows some similarity to the corresponding region on the normal chromosome 11. Therefore, this sequence in CLL 1386 cells seems to have been generated by disturbance of normal chromosome 11 sequences during the translocation event. This may move the breakpoint on chromosome 11 in CLL 1386 slightly closer to chromosome 14 sequences. DNA sequences 3′ to *J*4 coding sequences are identical in the 14q$^+$ chromosomes of CLL 271 and CLL 1386, although these sequences show a considerable number of base changes from the published DNA sequences 3′ to the *J*4 segment[10].

Note also that the breakpoints on chromosome 14 in both our cases of CLL are in the region where the *D* segment is joined to the J_H segment during normal *V-D-J* joining to produce an active immunoglobulin gene. This surprising finding suggests the involvement in the t(11;14) translocation of a recombinase responsible for *V-D-J* joining. This putative recombinase is thought to recognize conserved DNA sequences called '7mer-9mer', located close to *V*, *D* and *J* segments of immunoglobulin genes in germline DNA[12]. It has been proposed that the 7mer-9mer sequence with a 12-base-long spacer is paired with a 7mer-9mer sequence with a 23 ± 1-base-long spacer during *V-D-J* joining[12]. Interestingly, as shown by brackets in Fig. 4, DNA of normal chromosome 11 has a sequence very similar to this conserved 7mer-9mer signal sequence, very close to the breakpoint. This 7mer-9mer sequence on chromosome 11 is separated by a 12-base-long spacer, whereas the 7mer-9mer sequence of the *J*4 segment is separated by a 23-base-long spacer. These two sets of sequences can be drawn into a secondary structure, as shown in Fig. 5. Although the complementarity between the 7mer of chromosome 11 and the *J*4 segment is not very striking (3 bases in 7), this complementarity does not seem to be absolutely necessary even for normal *V-D-J* joining. The 7mer sequence on chromosome 11 is perfectly palindromic, which is one of the characteristics found in most 7mer sequences[12]. The homology to the consensus 9-mer is much stronger (8 of 9 residues). Thus, the t(11;14) chromosome translocation in two separate cases of CLL seems to be sequence specific, suggesting that the recombination system for *V-D-J* joining is involved, and that the translocation may be mimicking normal *V-D-J* joining. This also suggests that the putative recombinase can function even between two independent chromosomes.

The restriction map of the DNA region encompassing the breakpoint of the t(11;14) translocation in a diffuse B-cell lymphoma (LN87) and of the corresponding normal chromosome 11 are also shown in Fig. 3. The recombinant clone contain-



Fig. 4 DNA sequences of the joining sites between chromosomes 11 and 14 in CLL 271, CLL 1386 and of corresponding normal chromosome 11. Identical nucleotide sequences are shown by vertical lines. The boxed region indicates the *J*4 coding segment of the immunoglobulin heavy-chain gene[10]. The DNA sequences shown by brackets on chromosome 14 indicate the conserved sequence 7mer-9mer (see text). DNA sequences were obtained from the *Sst*I[1] (S[1]) site shown in Fig. 3 to the chromosome 14 sequence by using the chemical degradation method of Maxam and Gilbert[14] with a modification of the A reaction[15].

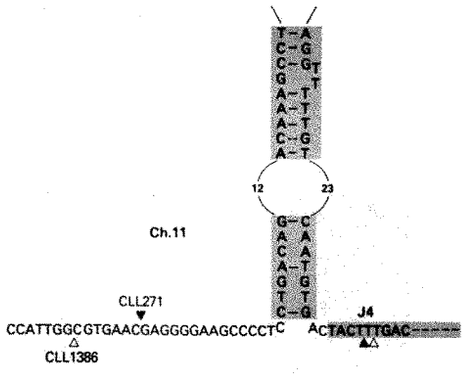


Fig. 5 A possible secondary structure between chromosome 11 and the *J*4 segment of the immunoglobulin gene.

ing the breakpoint was isolated from an LN87 genomic library as described above. The chromosomal breakpoint in LN87 cells was mapped very close to the *Sst*I[2] site in Fig. 1*B* by comparing the detailed restriction maps of normal chromosome 11 clones and a breakpoint-containing clone (data not shown). The chromosome 11 breakpoint in these cells is thus ~0.9 kb away from the site in the two CLLs. In addition, the breakpoint on chromosome 14 in LN87 cells seems to be quite different from that of CLL 271 and CLL 1386. It seems to be either 5′ to or within an immunoglobulin *V*-region gene, because a DNA probe, pLN104Bas (see Fig. 3), hybridized with multiple restriction fragments of human genomic DNA (data not shown). Such a pattern is characteristic of immunoglobulin heavy-chain *V*-region sequences[13].

Thus the chromosome breakpoints in the B-cell lymphoma LN87 are different, on both chromosomes 11 and 14, from those in the two CLLs we examined. It is possible that in the case of LN87, a different mechanism may have contributed to the chromosome translocation. Sequence analysis of the joining between chromosomes 11 and 14 on the $14q^+$ chromosome of LN87 cells should provide useful information concerning this issue.

These results also indicate that the breakpoints on chromosome 11 in three cases of B-cell neoplasia with the t(11;14) translocation, two CLLs and one B-cell lymphoma are all sufficiently close as to be detected by genomic Southern blot hybridization using a single restriction enzyme such as *Bcl*I and a single DNA probe such as *a* in Fig. 1*B*. This may make it possible to take practical advantage of specific DNA probes for the diagnosis of B-cell leukaemias and lymphomas carrying the t(11;14) translocation.

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In vivo somatic mutations in human lymphocytes frequently result from major gene alterations

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Somatic mutations, either spontaneous or produced by identifiable mutagens, are thought to be important in the aetiology of cancer and in the ageing process. The study of somatic mutations in human cells *in vivo* has recently been made possible by the development of techniques for enumeration and clonal expansion of lymphocytes mutated at the chromosome X-linked hypoxanthine phosphoribosyl transferase (HPRT) locus[1,2]. We have studied the molecular basis of *in vivo hprt* mutations in human lymphocytes and report here that a surprisngly high proportion (57%) involve substantial gene alterations which are not evident cytogenetically. These major gene alterations include deletions, exon amplifications and novel, sometimes amplified, bands on Southern analysis. Such changes emphasize the fluid nature of information in DNA and may be indicative of general mechanisms by which functional gene loss is involved in the aetiology of cancer and the homeostatic failure of ageing.

Mutant $HPRT^-$ cells can be selected for by the purine analogue 6-thioguanine (TG) as described previously[1,3]. Lymphocytes obtained from the blood of healthy adult males were cloned at limiting dilution either in the absence of TG (unselected, presumably $HPRT^+$ clones) or in the presence of 2.5 $\mu g\ ml^{-1}$ TG (selected, presumably $HPRT^-$ clones). Cloning efficiency in the absence of TG was 15–48% and the frequency of mutant cells ranged from 2.5×10^{-6} to 2.0×10^{-5}. TG-resistant clones were progressively expanded to $>5\times10^7$ cells by the intermittent addition of phytohaemagglutinin, irradiated allogeneic feeder lymphocytes from a female individual, and conditioned medium as a source of interleukin-2 (ref. 1). Genomic DNA was isolated from these monoclonal populations, digested with restriction endonucleases and electrophoresed on agarose gels; Southern analysis[4] was performed using a full-length complementary DNA probe for human *hprt*[5].

We have analysed 12 unselected clones and 21 selected clones from 7 individuals. In most cases, each genomic DNA sample was digested with three restriction enzymes (*Bam*HI, *Hin*dIII

Table 1 Major gene changes observed in 12 spontaneous $HPRT^-$ mutants on Southern analysis

		Change											
		*Eco*RI				*Hin*dIII				*Bam*HI			
Clone	Donor	D	N	A	AN	D	N	A	AN	D	N	A	AN
2419 TGA	R.R.	+		+		+		+		+		+	
2419 TG5	R.R.	+					+				+		
2419 TG6	R.R.			+	+			+	+	←ND→			
2409 TG1	J.D.			+	+			+	+			+	+
2409 TG2	J.D.			+	+			+	+			+	+
2409 TG3	J.D.			+	+			+	+			+	+
2412BTG1	J.W.		+	+				+		←ND→			
2412BTG2	J.W.		+		+	+	+		+		+		+
AM416TG6	R.C.	+				+				+			
BC49 TGR	P.H.				+				+				+
BC94 TGR	J.R.				+				+				+
84810F	L.P.			+				+		←ND→			

Clones are listed in arbitrary order. The indication of a change (+) implies a change with respect to the unselected clonal pattern for that donor. Changes indicates are: D, deletion; N, novel band; A, amplified exon; AN, amplified novel band. ND, not done.

and *Eco*RI) for which the coding and pseudogene fragments have been identified[6]. In 12 of 21 (57%) mutant clones, heterogeneous changes in Southern patterns were identified when compared with unselected clones from the same individual. The unselected clones of each individual showed no changes and generally agreed with the gene fragments described by Patel *et al.*[6]. The changes observed in mutant clones include a variety of deletions, and the appearance of novel bands and presumed amplifications of intragenic coding fragments and novel bands (Fig. 1; Table 1). As expected, pseudogenes residing on the autosomes showed no changes in any clone. Southern patterns obtained from one donor showed the same additional gene bands present in all of three selected and three unselected clones examined. We interpret these additional bands present in all six clones to represent a previously undescribed polymorphism and, as this individual subsequently gave a history of gout, family studies are in progress. However, the three selected clones from this individual also showed amplifications of both novel and previously identified coding fragments (Fig. 2). Note that, although these three clones showed similar abnormalities, they were independently derived.

Although major gene alterations of the *hprt* gene were readily detected at the molecular level, parallel cytogenetic studies using block staining and G-banding at the 600-band level showed no structural abnormalities of the X-chromosome in either selected or unselected clones. The messenger RNA products of the mutated genes are being investigated.

It is possible that the results were caused by an *in vitro* rather than *in vivo* phenomenon. However, the experiments involved addition of TG immediately after removal and separation of the lymphocytes and it is known that immediate TG selection results in the elimination of *hprt* gene mutations produced *in vitro*, as there is not time for such mutations to be expressed as a phenotypic change[7]. Therefore, the selected clones must have arisen from lymphocytes which were already mutated *in vivo*. It is formally possible that the initial mutation for each clone did not involve a major gene alteration and that the observed major gene alterations resulted from the emergence *in vitro* of secondarily altered subclones which had a proliferative advantage and which overgrew the original cells of the clone. To test this possibility directly, a subcloning experiment was performed in which lymphocytes were cultured in 96 wells containing TG, each well being subcultured into 12 wells on day 4, at which time any $HPRT^-$ lymphocytes would have undergone several divisions. After growth for 16 days in TG, two sets of subclones were chosen and expanded. Growth was present in 7 out of 12 wells in one set and 6 out of 12 wells in the other; the frequency of wells showing growth in each set indicated that the cells in most of the wells originated from a single cell and thus truly represented a subclone. The genomic DNA of each subclone was studied by Southern analysis and all subclones within each set were found to be identical; all of one set showed no change whereas all of the other (clone 84810F) showed the same *hprt* gene changes (amplification of exons). If major gene alterations develop during *in vitro* growth of lymphocytes, approximately half of each set of subclones should show abnormal Southern patterns and the abnormalities would be heterogeneous within a set. On the other hand, if major gene alterations are present *in vivo*, all subclones of a set would show either no abnormality or an abnormality which is identical for all members of a set. Our observation of the latter case provides direct evidence that the observed major gene alterations were present *in vivo* and had not arisen during clonal expansion *in vitro*.

The present experiments involved conditions of stringent TG selection and only mutations leading to a low or undetectable level of HPRT were likely to be recovered[1]. For this class of mutations, our observations indicate that *in vivo* somatic mutations at the *hprt* locus are at least as likely to result from major gene changes involving several kilobases (kb) of DNA as from more site-specific events such as point mutations or minor deletions. Indeed, it is likely that the observed frequency of gene alterations (57%) underestimates the true frequency, as

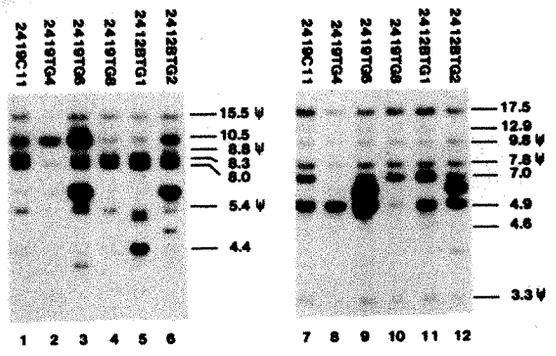


Fig. 1 Representative Southern transfer patterns of unselected ($HPRT^+$) and selected ($HPRT^-$) genomic DNA from two male donors (2419, 2412). Lanes 1–6, *Eco*RI digests; lanes 7–12, *Hin*dIII digests. Lane 1, unselected clone (2419 C11) showing normal enzyme digestion pattern with bands at 4.4 (not previously identified[6]), 5.4 (Ψ), 8.0, 8.3, 8.8 (Ψ) faint in all tracks, 10.5 and 15.5. (Ψ) kb. Lane 2, selected clone (2419 TG4) showing deletion of 8.3-kb band containing exons towards the 5′ end of the gene and a possible amplification of the 10.5-kb band. Lane 3, selected clone (2419 TG6) showing the appearance of a new amplified band of relative molecular mass 6.1 kb and amplification of the 10.5-kb band. Lane 4, selected clone (2419 TG8) which shows no evidence of Southern changes. Lane 5, selected clone (2412B TG1) showing amplification of a band at 4.4 kb and a new band at 5.2 kb. Lane 6, selected clone (2412B TG2) showing appearance of a new band at 6.1 kb, which may be amplified, and also a band at 4.8 kb, Lane 7, unselected clone (2419 C11) showing normal enzyme digestion pattern with bands at 3.3 (Ψ), 4.6 (not previously identified) 4.9, 7.0, 7.8 (Ψ), 9.8 (Ψ), 12.9 (not previously identified) and 17.5 kb. Lane 8, 2419 TG4 showing deletion of 7.0-kb band which hybridizes in unselected clones to the same cDNA fragment as that of the 8.3-kb band in the *Eco*RI digest (deleted in lane 2) and a possible amplification of the 4.9-kb band which correlates with the 10.5-kb band in the *Eco*RI digest of this clone. Lane 9, 2419 TG6 showing amplification of the 4.9-kb band and an amplified novel band at 6.4 kb. Lane 10, 2419 TG8 which shows no evidence of Southern changes. Lane 11, 2412B TG1 showing a possibly amplified band at 7.0 kb with this enzyme. Lane 12, 2412B TG2 showing appearance of a new band at 6.4 kb which may be amplified and a new band at 4.0 kb and a deletion of the 12.9-kb band.

Methods. For Southern transfers, 15 μg of genomic DNA extracted from cultured lymphocytes was digested with restriction enzymes, fractionated on 0.8% agarose gel and transferred to nitrocellulose by minor modifications of published procedures. Hybridization was carried out at 65 °C in 6×SSC 0.5% SDS, 5×Denhardt's, 0.01 M EDTA and 100 μg ml^{-1} sonicated denatured herring sperm DNA following prehybridization in the mixture which was the same except that it contained 250 μg ml^{-1} of the carrier DNA. The probe was a full-length human complementary DNA for *hprt* (insert cleaved from plasmid pHPT30 (ref. 6), *Pst*I/*Hha*I cut, yielding a fragment of 873 base pairs (bp) which has, in addition to the entire coding region, 18 bp of 5′-untranslated and 201 bp of 3′-untranslated sequence). The probe was nick translated to a specific activity exceeding 2.5×10^8 c.p.m. per μg and not less than 5×10^6 c.p.m. ml^{-1} hybridization mix was used. Hybridization was permitted for >16 h. Stringent washing was applied down to 0.5× SSC, 0.1% SDS, 1×Denhardt's at 65 °C. Autoradiographs were obtained on Ortho film using Lanex regular screens at −70 °C. Exposures were typically 48–72 h.

Southern analysis cannot resolve changes below a certain size unless a restriction site is involved. There is no reason to believe that events at the *hprt* locus are more or less frequent than at any other locus and we suggest that such changes in DNA are probably widespread throughout the genome.

It has been reported recently[8] that major changes in Southern patterns were present in 5 of 28 patients with Lesch-Nyhan syndrome. The changes were heterogeneous and included deletions, new bands and, in one family, amplifications. This observation, together with our own, suggests that loss or amplification of genetic material may be an important mechanism for variability in both germline and somatic cells.

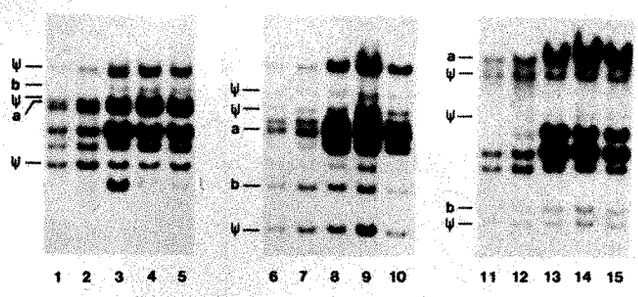


Fig. 2 Representative Southern transfer pattern of genomic DNA probed with cDNA for *hprt*. Two normal human male unselected (HPRT$^+$) clones are shown, 2409 Cl (lanes 1, 6, 11) and 2409 C2 (lanes 2, 7, 12), and three selected (HPRT$^-$) clones from the same individual, 2409 TG1 (lanes 3, 8, 13), 2409 TG2 (lanes 4, 9, 14) and 2409 TG3 (lanes 5, 10, 15). The enzymes used to digest DNA were *Eco*RI (lanes 1–5), *Hin*dIII (lanes 6–10) and *Bam*HI (lanes 11–15). The pseudogene allocations for *Eco*RI and *Hin*dIII digests are given in Fig. 1 legend. For *Bam*HI digests, the pseudogenes are shown at 3.4, 7.6, 14.5 and 25 kb (coincident with a coding region of the X-linked gene)[6]. Pseudogene fragments at 4.2 kb (*Eco*RI) and 2.0 kb (*Hin*dIII) do not show in this autoradiograph. It is clear that even allowing for somewhat greater loading of HPRT$^-$ DNA onto the gel, there has been a significant amplification of certain parts of the *hprt* gene in each of three mutants. In particular, bands labelled 'a' are amplified (*Eco*RI, 8.3 kb; *Hin*dIII, 7.0 kb; *Bam*HI, 22 kb) whereas bands labelled 'b' are not (*Eco*RI, 10.5 kb; *Hin*dIII, 4.9 kb; *Bam*HI, 3.7kb). The former are associated with exons 1 and 2 whereas the latter are associated with exons 3–6 (ref. 6). The simplest explanation of this is that the mutation has been occasioned by multiple reduplications of part of the gene containing exons 1 and 2. We also observe bands in these clones (HPRT$^+$ and HPRT$^-$) which are amplified in the mutants but which have not been previously assigned to the *hprt* gene. These results do not seem to be consistent with a restriction fragment length polymorphism and may therefore represent a non-pathological variant gene. We shall investigate the donor's family in more detail.

Direct evidence for the importance of somatic mutations in cancer comes from recent findings in familial and sporadic retinoblastoma[9] and in Wilms' tumour[10–13]. In both disorders, one autosomal gene at a critical site is believed to be abnormal in all cells as the result of a familial or germ-cell mutation and the other normal allele is believed to have become lost through a chromosomal mutation associated with deletion, inactivation or recombination. Of the 22 cases of Wilms' tumour studied by molecular techniques, chromosomal mutations have been detected in 12, that is 55%, similar to the percentage of *hprt* mutations that we have observed showing major gene changes on Southern analysis. We believe that our observations represent a more general case in that there was no selection or drift during clonal expansion, such as presumably occurs during the development of a tumour phenotype. If, as our findings suggest, somatic mutations generate gene changes in a widespread fashion throughout the genome, loss or amplification of genetic material might be a phenomenon of more general importance in cancer, acting either by causing expression of a recessive abnormal allele as already discussed, by leading to a deletion of regions separating promoters or enhancers from proto-oncogenes and thus producing oncogene activation, or by causing amplification of sequences containing oncogenes, resulting in increases in oncogene products, as has been observed in some neuroblastoma lines[14] and in the human promyelocytic HL60 cell line[15].

Somatic mutations have also been postulated to be involved in the aetiology of ageing. Mutations at the *hprt* locus have been observed to accumulate with age[16–18] and one can readily envisage that accumulating losses or amplifications of genetic material could lead to progressive deterioration of cellular function with age.

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Amplification and enhanced expression of the c-*myc* oncogene in mouse SEWA tumour cells

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SEWA tumour cells are derived from an osteosarcoma induced in an A.SW mouse by infection with polyoma virus[1]. Cytogenetic analyses have revealed three different characteristic chromosomal abnormalities diagnostic for the presence of amplified genes: 'double minutes' (DMs), homogeneously staining chromosomal regions (HSRs) and C-bandless chromosomes (CMs; for review see ref. 2). DMs may undergo fluctuation in number depending on the conditions in which the cells grow. Their number usually increases after injection of cells into a mouse and often is reduced to undetectable levels when the cells are explanted back into tissue culture; when the cells are re-introduced into the mouse, they again acquire multiple DMs[3]. We show here that cells of SEWA lines carrying DMs, HSRs or CMs contain amplified copies of the proto-oncogene c-*myc* and enhanced levels of c-*myc* messenger RNA and c-*myc* protein. DMs or CMs are the sites of c-*myc* amplification in two different SEWA lines.

We used the SEWA cell lines 4031Tc3 (DMs), StamA (HSR) and Rec4Tc2 (CMs) to screen for amplification of oncogenes (for a genealogy of SEWA cells see ref. 2). Preliminary tests, in which we hybridized a ^{32}P-labelled complementary DNA synthesized from poly(A)$^+$ RNA to nitrocellulose filters containing different oncogene DNAs[4], had provided evidence for abundant c-*myc* mRNA in SEWA cells (data not shown). We therefore investigated whether c-*myc* is amplified in SEWA cells. Total genomic DNA was cleaved with restriction endonucleases, fractionated on agarose gels and blotted to nitrocellulose filters to which ^{32}P-labelled c-*myc* probe was hybridized. For comparison, we tested DNA from testes of the A.SW mouse and DNA from human cells of the COLO 320-DM line, in which an apparently normal c-*myc* allele and a mutant allele rearranged at the 5′ end are each amplified ~25-fold (ref. 5 and M.S. *et al.*, manuscript in preparation). The autoradiographic signal

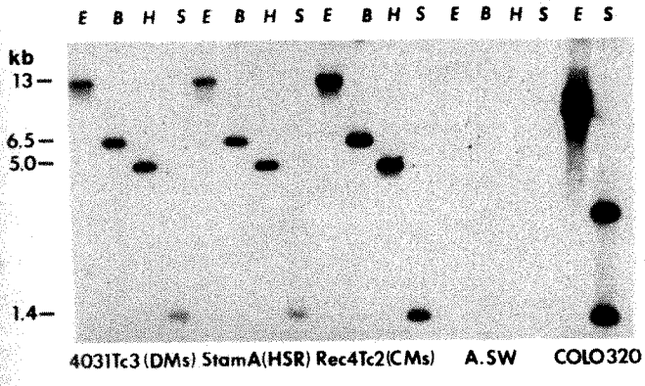


Fig. 1 Analysis of c-*myc* in the DNA of SEWA cell lines. NIH 3T3 and COLO 320-HSR and -DM cells served as controls. Approximately 10 μg of DNA was digested with restriction endonucleases (E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sac*I), fractionated on agarose gel and transferred to nitrocellulose filter, to which ^{32}P-labelled *Pst*I fragment of exon II of human c-*myc* was hybridized. COLO 320 DNA digested with *Eco*RI was from the DM subline, DNA digested with *Sac*I from the HSR subline. DNAs were analysed with a single-copy gene probe (Ha-*ras*) to show that approximately equal amounts of DNA of each line were being used (result not shown).

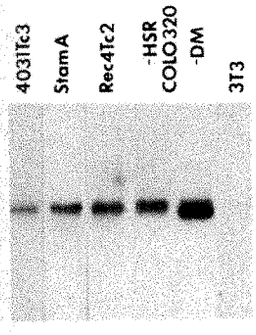


Fig. 2 Characterization of RNA transcribed from c-*myc* in SEWA cells. NIH 3T3 cells and COLO 320-HSR and -DM cells were used for comparison. Poly(A)$^+$ RNA (5 μg per lane) was fractionated on agarose gel in the presence of 2.2 M formaldehyde, transferred to nitrocellulose filters and hybridized to c-*myc* probe.

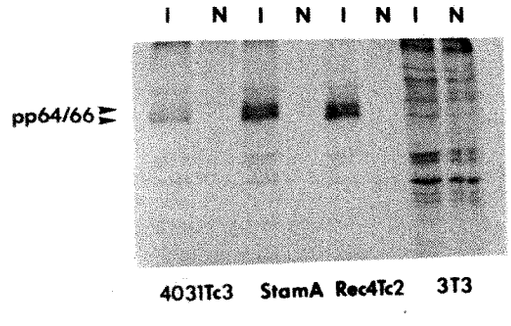


Fig. 3 Immunoprecipitation of pp64/pp66$^{c\text{-}myc}$ from SEWA cells. NIH 3T3 cells were used for comparison. I, immune serum from rabbits immunized with a peptide from the C-terminus of human c-*myc*[6]; N, non-immune serum collected before immunization.

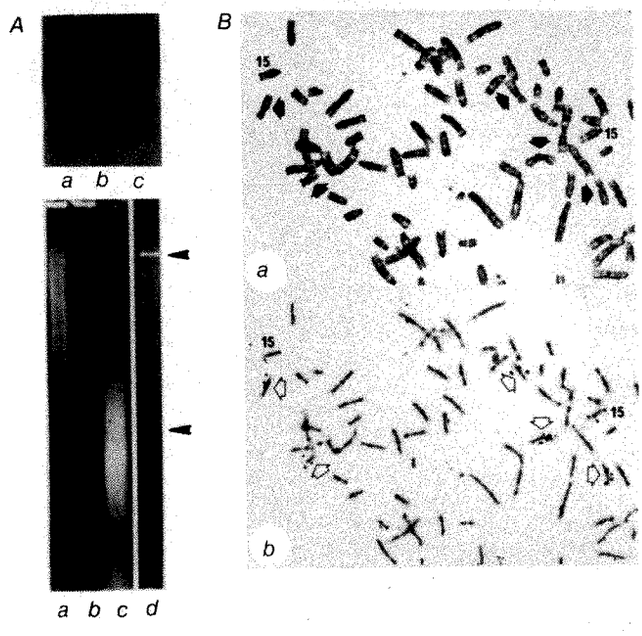


Fig. 4 Presence of amplified c-*myc* in DMs and CMs. *A*, Localization in DMs. Approximately 10 μg of DNA from intact nuclei and undisrupted cells (lane *a*), 5 μg of the fraction containing the macrochromosomes (lane *b*) and an estimated 0.5 μg of the DM fraction (lane *d*) were fractionated on an agarose gel and blotted onto nitrocellulose filter. The upper part of the figure shows the result obtained by hydridizing *myc* probe to the filter, the lower part shows the same gel stained with ethidium bromide. The ethidium bromide stained gel displays the enrichment for specific DNA sequences, presumably repetitive DNA (lane *d*, arrowheads indicate bands due to DNA fragments ~16 and 0.8 kb in size), which do not hybridize with c-*myc* probe, however. The intense fluorescence in lane *c* is probably due to the presence of RNA contained in ribosomes which co-sediment with DMs; the signal disappears following treatment of the gel with pancreatic ribonuclease (lane *e*). *B*, Localization in CMs. SEWAC-3 (a derivative of Rec4) containing CMs was used. Arrows indicate CMs. *a*, Metaphase spread after trypsin/Giemsa treatment. *b*, The same spread after destaining in methanol and *in situ* hybridization to ^{3}H-labelled c-*myc* probe. Note that four of the silver grains in the figure which are not situated over CMs are localized to chromosome 15 (indicated), which contains the c-*myc* gene.

Methods. Approximately 10^8 exponentially growing cells were arrested in metaphase by colchicine (0.05 μg ml^{-1} for 6 h) and collected. Chromosomes were released by gently drawing the cell suspension up and down three times through a 25-gauge needle in chromosome isolation buffer (15 mM Tris-HCl, 0.2 mM spermine, 0.5 mM spermidine, 2 mM EDTA, 0.5 mM EGTA, 80 mM KCl, 20 mM NaCl, 14 mM mercaptoethanol, *p*H 7.2)[9] containing 0.34 M sucrose, 0.1% digitonin and 0.5 M 2-methyl-2,4-pentanediol (Kodak). Nuclei and undisrupted cells were pelleted for 10 min at 500 r.p.m. in a JS 4.2 rotor (Beckmann), and subsequently the macrochromosomes were removed by repeated centrifugation at 4,000 r.p.m. The final supernatant, which on the basis of inspection by light microscopy was virtually free of macrochromosomes, was centrifuged at 25,000 r.p.m. (SW 50.1, Beckmann) for 45 min to pellet the DMs. DNA was prepared from the intact nuclei and undisrupted cells, from the pellets containing the macrochromosomes and from the pellets containing the DMs, and was digested with *Eco*RI.

obtained for the DNA of all three SEWA lines tested was much stronger than that obtained for the DNA from the A.SW mouse. SEWA cell lines 4031Tc3 and StamA contained ~15–20-fold and line Rec4Tc2 ~20–30-fold amplification of c-*myc* (Fig. 1). The size of the DNA fragments detected with the c-*myc* probe was the same in all three SEWA lines and in the normal mouse DNA fragment, indicating that the amplified c-*myc* gene in the SEWA calls has not undergone major rearrangement.

We next used RNA blotting procedures to test whether c-*myc* amplification is associated with a proportional enhancement of the level of c-*myc* mRNA. For comparison, we tested polyadenylated RNA from NIH 3T3 cells and from cells of the COLO 320-HSR and -DM lines containing ~25-fold enhanced levels of mRNA[5]. COLO 320-HSR cells have an mRNA of normal size, ~2.5 kilobases (kb), and COLO 320-DM cells carry predominantly a 2.3-kb c-*myc* mRNA as the result of c-*myc* rearrangement. The size of the c-*myc* mRNA in the SEWA cells and the COLO 320-HSR cells was indistinguishable (Fig. 2). Levels of c-*myc* mRNA in lines 4031Tc3, StamA and Rec4Tc2 were elevated 5–10-fold, 15-fold and 25-fold, respectively, com-

pared with NIH 3T3 cells; the level in Rec4Tc2 cells approached that found in the COLO 320 cells. The mouse c-*myc* gene encodes two forms of the c-*myc* protein, with relative molecular masses of 64,000 and 66,000 (pp64/66)[6]. Immunoprecipitation with anti-*myc* serum[6] showed that the level of c-*myc* protein was greatly increased and that the protein itself was unaltered (Fig. 3).

We have shown recently that DMs and HSRs in several human and murine tumour cells contain amplified cellular oncogenes (for a review see ref. 7). To test whether amplified c-*myc* is localized in DMs of cells of SEWA line 4031Tc3, a chromosomal fraction enriched for DMs was prepared according to the procedure previously used to localize c-Ki-*ras* in DMs[4]. DNA was isolated from intact nuclei and undisrupted cells, from pellets containing the macrochromosomes and from pellets containing the DMs. It was digested with restriction endonuclease *Eco*RI, fractionated on agarose gels and transferred to nitrocellulose paper to which ^{32}P-labelled c-*myc* probe was then hybridized. The autoradiographic signal obtained using ~0.5 μg of DNA from the DM fraction (Fig. 4*A*, upper part, lane *c*) was much more intense than that obtained for 5.0 μg DNA from the macrochromosomes (lane *b*), and only slightly less intense than that seen for 10 μg DNA isolated from total nuclei and undisrupted cells (lane *a*). These results indicate that the DM fraction is enriched for c-*myc* and that DMs are the site of c-*myc* amplification. In another experiment, when SEWAC-3 cells (a derivative of SEWARec4 containing CMs) were hybridized *in situ* to ^{3}H-labelled c-*myc* probe, the average number of grains found over CMs was 10 times greater than that over other chromosomes (Fig. 4*B*, lane *b*), indicating that CMs are the site of amplified c-*myc*.

The generation of DMs following transfer of SEWA cells from *in vitro* culture into the mouse, and the loss of DMs following re-transfer of the cells into tissue culture has long been thought to reflect changes in the copy number of genes involved in malignancy[8]. The present results show that c-*myc* is amplified and overexpressed in SEWA cells and, at least in cells of lines 4031Tc3 and C-3, is probably localized in DMs and CMs, respectively. Although the amplified unit of DNA probably contains considerable genetic information in addition to the c-*myc* gene, it is possible that enhancement of expression of c-*myc* after amplification confers on the host cells a selective advantage for tumour formation in the natural host. This idea is supported by our finding that the degree of amplification of c-*myc* observed in different SEWA lines is correlated with the rate at which tumours develop when the cells are injected into a mouse (T.M. *et al.*, manuscript in preparation). Southern blotting procedures revealed that SEWA cells, which are derived from a polyoma virus-induced tumour[1], contain three to five copies of polyoma virus (data not shown). It is unclear which function, if any, these polyoma sequences contribute to the malignant phenotype in the presence of amplified c-*myc* gene. We are now analysing the possible significance of c-*myc* amplification in SEWA cells in more detail.

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Size variation in chromosomes from independent cultured isolates of *Plasmodium falciparum*

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The complexity of the life cycle of the protozoan malaria parasite *Plasmodium falciparum* has hindered genetic analysis[1]; even the number of chromosomes in *P. falciparum* is uncertain[2]. The blood stages of rodent malaria parasites are haploid[1,3] and hybridization with cloned complementary DNAs similarly suggests a haploid genome in *P. falciparum* blood stages (ref. 4 and our unpublished results). A novel approach to karyoptic and linkage analysis in *P. falciparum* has been provided recently by the technique of pulsed-field gradient (PFG) gel electrophoresis[5], which allows the fractionation of DNA molecules of 30–3,000 kilobases (kb), a range including the sizes of intact chromosomal DNA molecules from eukaryotes such as yeast[5] and trypanosomatids[6]. We describe here the fractionation by PFG electrophoresis of chromosomal DNA molecules from *P. falciparum* into at least seven discrete species which vary in size by up to 20% between different isolates. Several genes for *P. faciparum* antigens which contain repetitive sequences are located on different chromosomes. Surprisingly, two of the chromosomes seem to contain the same sequences.

Intact chromosomal DNA[5,6] was prepared by immobilizing the organisms in low-gelling point agarose and then deproteinizing *in situ* (see Fig. 1 legend). The chromosomal DNA molecules were subsequently separated by PFG electrophoresis in a 1.5% agarose gel. In this procedure, an electric field is applied in alternate pulses first in one direction, then in a direction oriented 90° to the first. Because the DNA molecules are considerably longer than the pore size of the gel, they must align with the field in order to migrate 'end-on' through the gel. As the time to re-orient is a function of size, the molecules can be resolved if the pulse time is tuned to an appropriate frequency[5]. We found that the optimal pulse time for *P. falciparum* chromosomes was ~80 s. The separated chromosomal DNA molecules can then be stained with ethidium bromide (EtBr) and blotted to nitrocellulose for gene localization by hybridization[5].

Fractionation of the chromosomes of cloned *P. falciparum* line E12 (ref. 7), derived from the Papua New Guinea isolate FCQ27/PNG (FC27), is shown in Fig. 1*a*; E12 and FC27 were identical (data not shown). Migration is nonlinear because of the focusing effect of the asymmetrical electrode array[5]. Hence, the DNA molecules in each track lie on a curve, and equivalent bands in successive tracks form a curve across the gel. In the region of highest mobility the DNA formed a smear (S in Fig. 1). Hybridization data (see below) demonstrated that the smear contains fragments of the larger chromosomes, but this does not exclude the presence of other components, for example, mitochondrial DNA or 'minichromosomes'.

In the central region, four sharp bands, designated chromosomes 1–4, were clearly resolved (Fig. 1*a*). To estimate their sizes, yeast chromosomes were used as markers (Fig. 1*c*). The 17 yeast chromosomes were resolved into about 11 bands (Fig. 1*c*) plus a lower band of mitochondrial (YM) DNA. Chromosomes 1–4 of *P. falciparum* clone E12 migrated in the same range as the larger yeast chromosomes. Taking the sizes of the three smallest yeast chromosomes to be about 0.3, 0.5 and 0.7 megabases (Mb) and the largest to be about 2.0 Mb (ref. 5), we estimated that E12 chromosomes 1–4 are roughly 0.8, 1.2, 1.6 and 1.8 Mb in size, respectively. From their discrete sizes and from hybridization data (see below) showing that they contain different sequences, we conclude that they are intact chromosomes.

Nearer the top of the gel, bands were less clearly resolved (see Figs 1–3). Hybridization data (see below) indicate that there are at least three chromosomes in this region. Chromosomes 5 and 6 were usually evident as discrete bands while chromosome 7 smeared from the well, partially overlapping chromosome 6. By extrapolation, we have assigned chromosomes 5–7 nominal sizes of 2.2, 2.6 and 3.0 Mb respectively (Fig. 4), but there are no accurately known size markers in this range. Some EtBr-staining material consistently remained in the well. Hybridization suggested that it contains a poorly reproducible proportion of all the chromosomes, presumably trapped in debris. The possibility of an additional chromosome(s) in this region cannot be excluded.

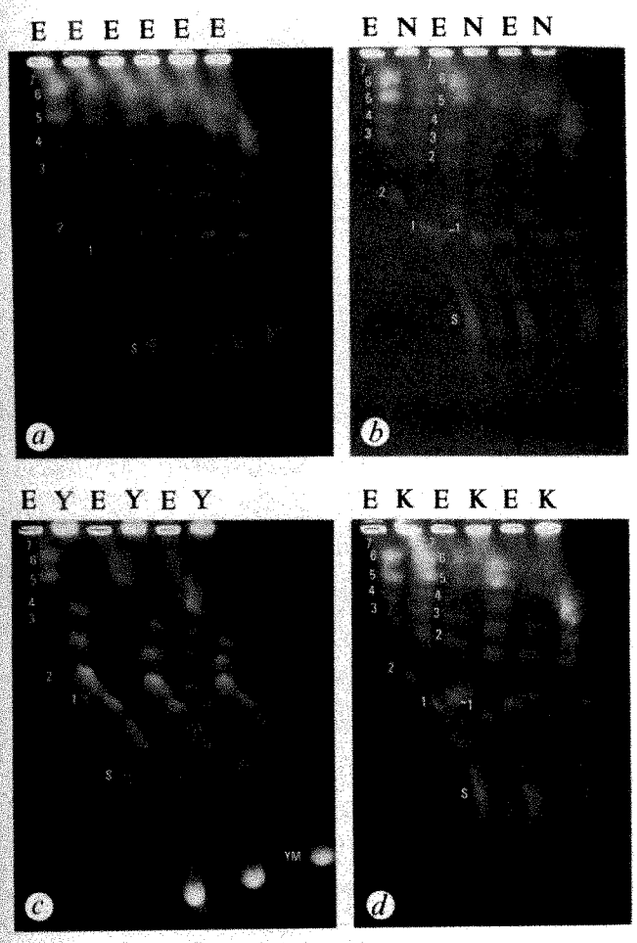


Fig. 1 Fractionation of *P. falciparum* chromosomes by pulsed field gradient gel electrophoresis. In *a–d* the chromosomes were from clone E12 (E) derived from *P. falciparum* isolate FC27, isolate NF7 (N) or K1 (K). The marker chromosomes (Y in panel *c*) were from yeast (*Saccharomyces cerevisiae*, strain J69b1). The samples were run in for 2 h at 200 V in the north–south direction, then electrophoresed for 22 h with 80-s pulses, 250 V in the north–south direction (top–bottom) and 100 V in the east–west direction (left–right) at 18 °C, and stained with EtBr.

Methods. *P. falciparum* isolates were maintained *in vitro*[16] on human erythrocytes. Cultures were collected at 5–10% parasitaemia and the parasites were concentrated by lysing the red cells with saponin[17], followed by two washes. The parasites were resuspended in phosphate-buffered saline at $\sim 6 \times 10^9$ organisms per ml, and added to an equal volume of molten 2% low-gelling agarose in saline at 37 °C, and cast into 1.6 × 5 × 5 mm blocks[5]. The blocks were treated with 0.5 M EDTA/0.01 M Tris/1% Sarkosyl/2 mg ml^{-1} proteinase K for 48 h at 42 °C[5] and stored at 4 °C. Before electrophoresis the blocks were cut into strips ~1.6 × 1.0 × 5 mm, soaked in 0.9 × TBE[5] for 1 h and then inserted into the slots of a 1.5% agarose (Marine Colloids, type ME) gel in 0.9 × TBE and sealed into the slots with 1% low-gelling agarose. Yeast chromosomes were prepared as described previously[5]. The PFG apparatus was constructed essentially as described elsewhere[5], with 15 diode-isolated electrodes in the west, north and east sides and a single electrode in the south-west corner. The gel (225 × 215 × 5 mm) was supported on 2-mm-thick Perspex blocks, to allow cooling by flow of the electrolyte under as well as over the gel. Cooling was achieved by a tap-water heat exchanger and the electrolyte (0.9 × TBE) was recirculated between buffer reservoirs at the north and south ends at 600 ml min^{-1} with a solution metering pump.

Comparison of chromosomes from *P. falciparum* clone E12 with those of isolates NF7 from Ghana and K1 from Thailand revealed striking size polymorphisms (Fig. 1*b*, *d*). Chromosome 1, the smallest chromosome of each isolate, differed in size between the three isolates, as can be seen by comparing the alternate tracks of E12 and NF7 (Fig. 1*b*) and of E12 and K1 (Fig. 1*d*). The sizes of chromosome 1 were estimated to be ~0.8 Mb (E12), 0.9 Mb (NF7) and 1.0 Mb (K1).

No chromosome of a size similar to E12 chromosome 2 was present in NF7 or K1, whereas both NF7 and K1 possesses a chromosome intermediate in size between E12 chromosomes 4 and 5. All three isolates have chromosomes similar in size to E12 chromosomes 3 and 4, but hybridization indicated that this apparent similarity is misleading (see below). All three isolates have chromosomes apparently equivalent in size to E12 chromosomes 5–7, but the resolution is poor in this region.

To clarify the relationships between individual chromosomes in the three isolates, we preparatively isolated FC27 chromosomes by PFG electrophoresis, digested each with *Eco*RI, subcloned the fragments into pUC9 (ref. 8) and hybridized individual chromosome-specific plasmids to blots of E12, NF7 and K1 chromosomes separated by PFG electrophoresis (Fig. 2). Two independent probes that hybridized only to chromosome 1 in E12 also hybridized only to the smallest chromosome in NF7 and K1 (data not shown), as did a cloned cDNA (see below). Hence, the smallest chromosomes of each isolate are counterparts, designated chromosome 1. Hybridization with a probe (pF2.1) specific for E12 chromosome 2 (Fig. 2*b*) revealed that the corresponding chromosome in K1 was much larger (Fig. 2*b*). The same results were obtained with this probe when E12 and NF7 were compared (data not shown). Hence, the second smallest chromosome in each isolate is a counterpart of E12

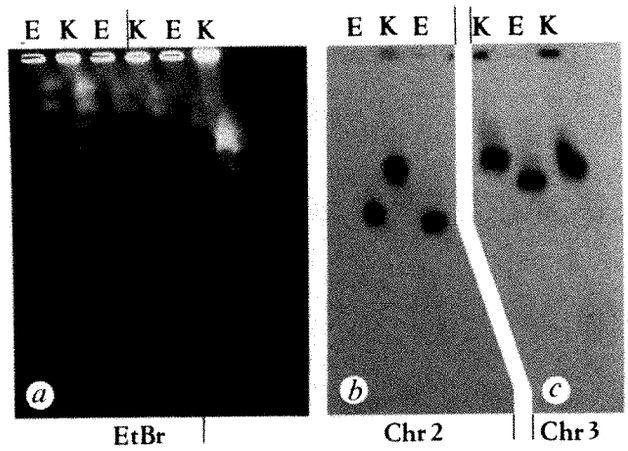


Fig. 2 Relationships between the chromosomes of *P. falciparum* cloned line E12 (E) and isolate K1 (K). Chromosomes were fractionated as in Fig. 1 and stained with EtBr (*a*). The DNA was then blotted to a nitrocellulose filter which was cut into two parts. The left and right parts were hybridized with a cloned FC27 chromosome 2 (*b*) and chromosome 3 (*c*) probe.

Methods. FC27 chromosomes were fractionated as in Fig. 1*a*. Bands corresponding to each chromosome were sliced from the preparative gel, electrophoresed into and then extracted from low-gelling agarose. Chromosome-specific hybridization probes were generated using *Eco*RI digestion of DNA from these individual chromosome bands and ligation of fragments into the plasmid vector pUC9 (ref. 8). Fragments released from the resulting clones pF2.1 and pF3.3 by *Eco*RI digestion were gel-purified, nick-translated and used directly as hybridization probes as described previously[18].

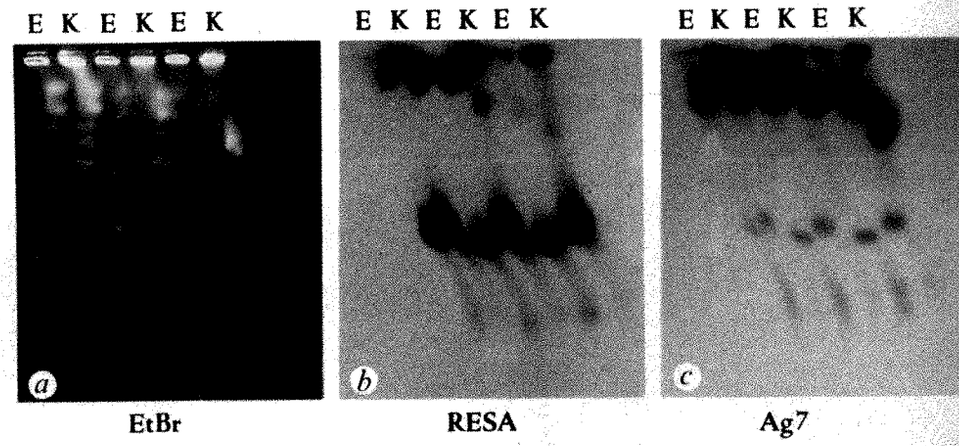


Fig. 3 Chromosomal locations of genes for repetitive antigens. Chromosomes from E12 and K1 were fractionated as in Fig. 1 and stained with EtBr (*a*). The DNA was then blotted to nitrocellulose filters and hybridized with a RESA probe (clone Ag13, from ref. 12) (*b*). After autoradiography, the filter from *b* was rehybridized with a fivefold greater concentration of an Ag7 (ref. 18) probe and autoradiographed again (*c*). Hybridization of both these probes to the origin for K1 is probably an artefact resulting from trapped DNA (see also Fig. 2*c*).

chromosome 2, even though it differs in size by about 0.3 Mb (that is, ~20%) between isolates. Similarly, three independent probes (pF3.3, pF3.9 and pF3.1S) specific for E12 chromosome 3 (Fig. 2*c* and data not shown) revealed that the corresponding chromosome in K1 was considerably larger. Thus, the third smallest chromosome in each isolate is a counterpart of E12 chromosome 3, even though E12 chromosome 3 is considerably smaller (by ~0.2 Mb) than that of K1 or NF7.

K1 and NF7 each contain a chromosome that is intermediate in size between E12 chromosomes 4 and 5. We presume that this chromosome 4 corresponds to chromosome 4 of E12, which is about 0.2 Mb smaller, because we have accounted above for chromosomes 1–3 in each isolate, and hybridization with other DNA probes (see below) shows that chromosomes 5–7 are equivalent (Fig. 4). Remarkably, all four chromosomes that can be clearly resolved are considerably larger in NF7 and K1 than are their counterparts in E12 and FC27.

Several antigens of *P. falciparum* are unusual proteins containing multiple repeats of oligopeptide sequences[4,9-14]. Assignment of these genes by hybridization to separated chromosomes would provide an approach to analysis of the genetic organization of genes coding for these repetitive antigens. Hybridization of a cloned sequence encoding RESA, a repetitive *P. falciparum* antigen located at the membrane of the newly infected erythrocyte[12], indicates that the RESA gene is located on the polymorphic chromosome 1 in both E12 and K1 (Fig. 3*b*). The RESA gene probe also hybridized to chromosome 7 but to a much lower extent, in agreement with our observation that several restriction fragments cross-hybridized to RESA[12]. The gene for a second repetitive antigen, an isolate-specific secreted protein designated the S-antigen[9], was also located on chromosome 7 (data not shown), providing further compelling evidence that chromosome 7 is indeed distinct from chromosomes 5 and 6.

Figure 3*c* shows hybridization of a cDNA probe encoding a third repetitive antigen designated Ag7, which apparently becomes associated with the schizont-infected erythrocyte and contains hexapeptide repeats (our unpublished observations). The Ag7 probe hybridized to both chromosomes 5 and 6 in E12 and K1 (Fig. 3*c*) and in NF7 (data not shown). Its localization on two chromosomes was surprising, as the same Ag7 probe hybridized to a single polymorphic restriction fragment after digestion with *Eco*R1, *Aha*III or *Hin*dIII.

A cDNA probe encoding a fourth repetitive antigen, designated FIRA (for *Falciparum* interspersed repeat antigen, ref. 4) also hybridized to both chromosomes 5 and 6 in E12, K1 and NF7 (data not shown). Again, conventional Southern hybridization had suggested a single gene in each isolate[4], with polymorphisms between isolates. Three other cloned sequences derived from isolated chromosomes (pF5.9, pF5.14 and pF5.17, data not shown) all hybridized to chromosome 5 as well as chromosome 6. Hence, all five sequences that hybridized to chromosome 5 also hybridized to chromosome 6, in each of the isolates including clone E12. This is a paradoxical observation because Ag7 and FIRA are both highly polymorphic (ref. 4 and

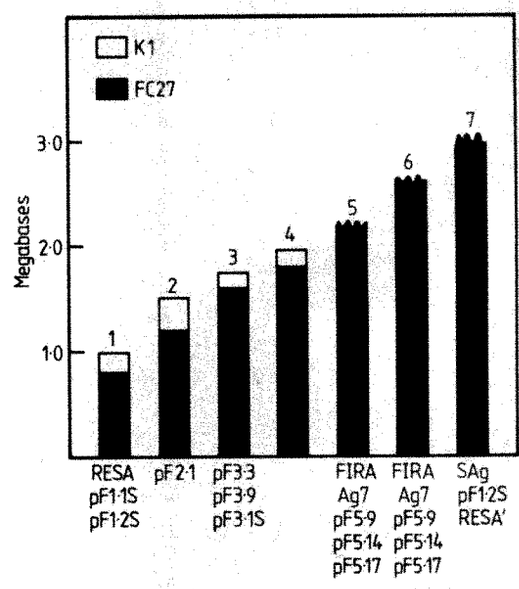


Fig. 4 Karyotypes of FC27 and K1. The heights of the bars represent the approximate sizes of chromosomes 1–7; the wavy lines depicting chromosomes 5–7 imply considerably less accuracy in these sizes. The scale (in Mb) is indicated at the left. Hybridization results are summarized below each chromosome. NF7 was indistinguishable from K1, except that chromosome 1 was ~0.9 Mb. Probes designated pF were generated from isolated chromosomes. RESA' indicates cross-hybridization (Fig. 3*b*).

our unpublished observations). In seven different isolates, including those used here, single restriction fragments were detected by each probe, although the size of these fragments differed between isolates. Hence, each isolate was apparently homozygous at each of these two highly polymorphic loci, so we concluded that the blood stages of *P. falciparum* are haploid, in agreement with genetic studies[1]. However, the single alleles of Ag7 and FIRA present in each of these isolates seem to each reside on both chromosomes 5 and 6. On the basis of EtBr binding, chromosomes 5 and 6 appear to be equimolar and present at the same copy number (presumably 1 per cell) as chromosomes 1–4. A possible resolution of this paradox could be that chromosome 5 is an exact but incomplete copy of chromosome 6. Other variations of this model are obviously possible, the crucial point being that *P. falciparum* appears to be haploid for chromosomes 1–4 and diploid for a chromosome detected as 5–6 because the two copies are of different sizes. At present, we have no independent method to confirm this unexpected observation. It may be an artefact of this new technique, perhaps resulting from chromosome breakage at a specific site.

The data described here illustrate the value of PFG electrophoresis in cytogenetic analysis of a protozoan parasite that

otherwise is genetically intractable. These results show clearly that genes for repetitive antigens are distributed among the chromosomes of *P. falciparum* rather than clustered on one chromosome. The karyotpye of FC27 was remarkably different from those of NF7 and K1, in that chromosomes 1-4 were all considerably smaller, and karyotyping by PFG electrophoresis has enabled us to distinguish these three and another two isolates (7G8 and V1) examined so far.

The phenomenon of uniformly larger chromosomes in one isolate compared with another is reminiscent of differences in the chromosomes of closely related grasshoppers which differ in their content of repetitive heterochromatic sequences[15]. However, deletions occurring *in vitro* could confer a growth advantage and so cultured lines may be rapidly overtaken by such variants. The genetic differences underlying these karyotype differences may be of considerable importance, both clinically and in the efficacy of any future vaccines.

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Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate

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Post-meiotic segregation of alleles, which is seen, for example, in the 5:3 distribution of alleles in the products of a single meiosis in fungi, has been thought to be due to the non-repair of heteroduplex regions formed during genetic recombination. In current models of genetic recombination, heteroduplex DNA is formed either as the primary intermediate generated by two interacting non-sister chromatids[1] or as a short region flanking a double-stranded gap[2]. The frequency of post-meiotic segregation differs for different alleles, and this is presumed to reflect the varying efficiencies with which different types of mismatches in the heteroduplex are repaired. To gain some insight into this process, we have now determined the nucleotide sequences of various yeast alleles with different post-meiotic segregation frequencies and compared the mismatches predicted to occur in heteroduplexes of these alleles with wild-type DNA with those repaired with varying efficiency in bacterial systems. A striking correlation is observed, with the mismatches predicted for high post-meiotic segregation frequency alleles being similar to mismatches repaired with low efficiency in bacteria. These results support the view that post-meiotic segregation frequency reflects heteroduplex repair efficiency and the contention that meiotic gene conversion is the result of the successful repair of heteroduplex mismatches.

The cloning of the *ADE8* gene has been reported previously[3]. Two methods were used to clone the *ade8-18* allele[4], both in an *ade8-18* host; these were mitotic gap repair[5] and meiotic gene conversion of the *ADE8* plasmid. Figure 1*a* shows a detailed restriction map defining the fragments subcloned for dideoxy sequencing[6] while Fig. 1*b* displays the nucleotide sequence of the *ADE8* gene. The *ade8-18* mutation is a 38-base-pair (bp) deletion removing the third position of codon 88 to the first position of codon 101, inclusively. This accounts for the observed non-revertibility of the allele[7]. The *ADE8* sequence codes for a 214-amino-acid protein, in close agreement with the predicted 232-amino-acid length based on meiotic and X-ray mapping data[7]. The deletion removes a unique *Xho*I restriction site in the coding sequence; this site is absent in both independently isolated *ade8-18* plasmids.

Table 1 Shared sequences in *ADE8* and *ARG4*

Sequence	Location in *ADE8*	Location in *ARG4*
AAGATCC	619	221
AAGGCAA	614	29
AAGGTTT	551, 1,001, 1,134	231
ACAAAGG	548, 575	526
AGATCCC	620	371
ATGTCAG	817	38
CAAAGGG	576, 953	527
CGATATT	741	343
GATCCTT	700	89
GCGGGCT	686	176
AAGGTTTG	551	231
TTTGAGAAA	629	400
TACAAAGGG	574	525

Numbering for *ADE8* begins with the first nucleotide in Fig. 1*b*. For reference, nucleotides 646-683 are deleted in the *ade8-18* mutation. Numbering for *ARG4* begins with the first nucleotide in Fig. 2. For reference, *arg4-17* is at nucleotide 164 and *arg4-16* is at nucleotide 378.

Figure 2 shows the nucleotide sequence of the 566-bp *ARG4* *Sst*I fragment. The ochre mutation *arg4-17*, with low post-meiotic segregation, is an A to T transversion in the first position of codon 43. The plasmids pSZ524 and pSZ524-17 (provided by T. Orr-Weaver and J. Szostak) were the sources of *ARG4* and *arg4-17* DNA. The *arg4-16* mutation, showing high post-meiotic segregation, is a G to C transversion in the second position of codon 114, substituting proline for arginine. The *arg4-16* allele was cloned by mitotic gap repair[5]. The *ARG4* sequence in Fig. 2 agrees with that recently published[8] except at position 346, where we read T instead of G, resulting in aspartate rather than glutamate.

The finding that heterozygosity for the *ade8-18* 38-bp deletion displays high post-meiotic segregation (54% among aberrant tetrads) is unexpected. Previous studies of *HIS4* deletions showed that a 400-bp deletion (*his4-Δ15*) displays gene conversion but no post-meiotic segregation, although a presumed point mutation (*his4-4°*) in the deleted region displays similar non-mendelian segregation levels, but 10.3% post-meiotic segregation[9]. Single-base insertions also seem not to result in high post-meiotic segregation (Table 2). The relationship between deletion size and the frequency of post-meiotic segregation warrants further investigation.

The high frequency of post-meiotic segregation which is characteristic of the *ade8-18* allele does not seem to be a simple

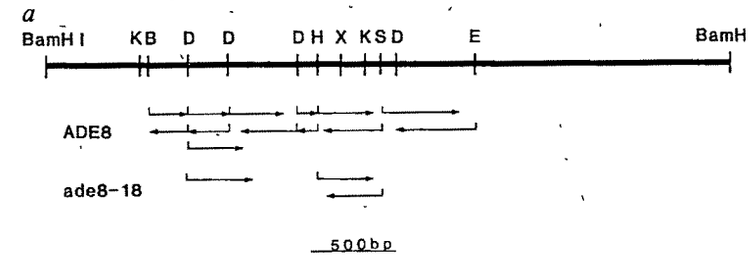


Fig. 1 *a*, Restriction map of *ADE8* DNA and sequencing strategy. The indicated fragments were subcloned into M13mp8 and M13mp9 for nucleotide sequencing by the dideoxy method[6]. B, *Bgl*II; D, *Dde*I; E, *Eco*RI; H, *Hpa*I; K, *Kpn*I; S, *Sal*I; X, *Xho*I. *b*, Nucleotide sequence of the *ADE8* gene. The sequence presented begins with the 412-bp *Dde*I fragment in *a*, and reads from left to right, ending at the consensus poly(A) addition signal[21] in the *Sal*I/*Eco*RI fragment. Putative control regions 5′ to the open reading frame are underlined. The role of the 'amino-acid general control' sequence TGACTC[22] is unknown with regard to *ADE8* transcription. The 38-bp deletion comprising the *ade8-18* mutation is also underlined.

b

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TCAGGTGACATCTAGGGGC GTTTACTGTAAGAATATAAAGTACAATCCAAAAAGGAAAATTATACTTGTTTAAGAAAAGAGGAACCTTCTGCTCGCTCTAAAATGGTAGTTCTAGTGA  119

TAGTTTAAAAATGCATTTTCCTTCCGACTTCGAAGATAGATTTCAGCATAAAGACTAAAAGCGTCAGAAGGATTCGAATGAGTCGACTCGTTGCTAGTGACTCTGACCTGTTTTTTATTC  238

CAGCAAGAGGAAAGTTATATAGAGTAGTTTATGAAGAGAAGACACTTCTAAAGTTGAATAACGCTCCTAGCCTCAAGGTTGATGATTTAACTTCCAGCAAGCGCAGGTGAGAGCAACA  357

                                                                1                                                  10                                                  20
                                                                met ala arg ile val val leu ile ser gly ser asn leu gln ala leu ile asp ala gln lys
CACATCAATAATGTTTCCAAAAGCT                                       ATG GCC AGA ATT GTC GTA TTA ATT TCG GGC TCA CGT TCC AAC TTG CAA GCC TTG ATC GAT GCC CAA AAG  451

                                                                30                                                  40                                                  50
gln gly gln leu gly glu asp ala his ile val ser val ile ser ser ser lys lys ala tyr gly leu thr arg ala ala asp asn asn
CAG GGC CAA TTA GGC GAG GAT GCT CAT ATT GTC TCT GTC ATA TCT TCC AGC AAG AAG GCA TAC GGC TTA ACT AGG GCA GCG GAC AAT AAT  541

                                                                60                                                  70                                                  80
ile pro thr lys val cys ser leu tyr pro tyr thr lys gly ile ala lys glu asp lys ala ala arg ala lys ala arg ser gln phe
ATC CCC ACA AAG GTT TGT TCG CTA TAC CCA TAT ACA AAG GGT ATT GCG AAG GAG GAC AAA GCC GCA AGG GCC AAG GCA AGA TCC CAG TTT  631

                                                                90                                                  100                                                 110
glu asn asp leu ala lys leu val leu glu glu lys pro asp val ile ile cys ala gly trp leu leu ile leu gly ser thr phe leu
GAG AAC GAC CTC GCA AAA CTA GTG CTC GAG GAA AAG CCT GAC GTT ATC ATC TGC GCG GGC TGG TTA TTG ATC CTT GGA TCT ACT TTC TTG  721
                                                                ade8-18 deletion
                                                                120                                                 130                                                 140
ser gln leu gln ser val pro ile leu asn leu his pro ala leu pro gly cys phe asp gly thr thr his ala ile glu met ala trp
AGC CAA CTG CAA TCT GTC CCG ATA TTA AAT CTG CAT CCA GCG CTA CCA GGT TGT TTC GAT GGT ACC ACA CAC GCA ATT GAA ATG GCA TGG  811

                                                                150                                                 160                                                 170
arg lys cys gln asp glu asn lys pro leu thr ala gly cys met val his tyr val ile glu glu val asp lys gly glu pro leu val
CGC AAA TGT CAG GAC GAG AAC AAG CCT CTG ACG GCC GGA TGC ATG GTG CAC TAT GTC ATC GAG GAG GTC GAC AAG GGT GAG CCA TTG GTC  901

                                                                180                                                 190                                                 200
val lys lys leu glu ile ile pro gly glu glu thr leu glu gln tyr glu gln arg val his asp ala glu his ile ala ile val glu
GTA AAA AAG CTA GAA ATC ATA CCT GGC GAA GAA ACA CTA GAG CAG TAC GAG CAA ACG GTA CAC GAT GCT GAG CAC ATC GCG ATT GTA GAA  991

                                                                210                 214
ala thr tyr lys val leu gln gln leu his lys OC
GCT ACA TAT AAG GTT TTA CAG CAG CTT CAC AAA TAA GAATAGTTCCCATATTTATACTTTTATATTTGGCATTCTTCAAAGTGAGGCGCGAAACATCTCGCGTCTCGTAA 1099

ATCATGATCAATGGATTGTGACAAAACGATCTTAAAGGTTTCGAATAAAATGT
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```
                                                                1                                                  10                                                  20
                                                                met ser asp gly thr gln lys leu trp gly gly arg phe thr gly glu thr asp pro leu
1   CAAAAGCAGGTAACTATATAACAAGACTAAGGCAAAC                       ATG TCA GAC GGC ACT CAA AAA CTA TGG GGT GGG AGA TTC ACT GGT GAA ACC GAT CCT TTG

                                                                30                                                  40                                                  50
    met his leu tyr asn ala ser leu pro tyr asp tyr lys met tyr lys ala asp leu glu gly thr lys val tyr thr ala gly leu gln
98  ATG CAC CTT TAC AAT GCG TCT CTT CCG TAT GAT TAT AAG ATG TAT AAG GCA GAT TTA GAA GGA ACT AAA GTA TAC ACA GCG GGC TTG CAG
                                                                                                                    T (arg4-17)
                                                                60                                                  70                                                  80
    lys leu gly leu leu thr glu thr glu leu ala lys ile his glu gly leu ala glu ile lys lys glu trp asp ala asp lys phe val
188 AAG TTG GGT CTT CTA ACG GAG ACA GAA TTG GCA AAG ATC CAT GAA GGT TTG GCT GAA ATC AAA AAA GAA TGG GAC GCT GAC AAA TTT GTC

                                                                90                                                  100                                                 110
    arg his pro asn asp glu asp ile his thr ala asn glu arg arg leu gly glu leu ile gly arg asp ile ala gly lys val his thr
278 CGT CAT CCA AAC GAC GAG GAT ATC CAT ACT GCG AAT GAA AGA CGT CTT GGT GAA CTA ATT GGC CGC GAT ATT GCT GGT AAA GTC CAC ACC

                                                                120                                                 130                                                 140
    gly arg ser arg asn asp glu val val thr asp leu arg ile tyr cys arg asp ile val asn asp thr leu phe pro ala leu lys gly
368 GGT AGA TCC CGT AAT GAT GAA GTT GTT ACC GAT TTG AGA ATA TAC TGT CGT GAC ATT GTC AAT GAC ACC CTC TTT CCA GCT TTA AAG GGC
          C (arg4-16)
                                                                150                                                 160                                                 170
    leu val glu val leu ile lys arg ala glu gly glu ile asp val leu met pro gly tyr thr his leu gln arg ala gln pro ile arg
458 TTG GTT GAA GTT CTA ATT AAG AGG GCC GAA GGT GAG ATA GAT GTC TTA ATG CCA GGC TAC ACA CAT TTA CAA AGG GCA CAA CCT ATT AGA

    trp ser his trp leu ser
548 TGG TCT CAT TGG TTG AGC T
```

Fig. 2 Nucleotide sequence of the *ARG4 Sst*I fragment. The *ARG4* and *arg4-17°* sequences were obtained from the plasmids pSZ524 and pSZ524-17 provided by T. Orr-Weaver and J. Szostak. The *arg4-16* allele was cloned by mitotic gap repair[5] of *Sst*I-digested pSZ524 in an *arg4-16* host. The three *Sst*I fragments were subcloned into M13mp8 in both orientations for dideoxy sequencing[6]. The *arg4-16* and *arg4-17°* mutations are given below the wild-type sequence.

function of the location of the *ADE8* gene in the genome. When the *ade8-18* heterozygosity was transferred to the *TRP1* locus, the non-mendelian segregation frequency fell from ~10% to the ~1% level observed for *trp1* alleles, yet ~50% of the non-mendelian segregations were post-meiotic segregation events[4]. Similarly, the *ade8-18* post-meiotic segregation frequency does not seem to depend wholly on intragenic location as the *ade8-14* allele is meiotically inseparable yet displays only 11% post-meiotic segregation (S.F. and K.L., unpublished results)[7,10].

The two main recombination models now under consideration provide contrasting predictions for the features determining post-meiotic segregation. Both models feature post-meiotic segregation arising from heteroduplex DNA but differ in the mechanisms which generate this hDNA.

The Aviemore or Meselson–Radding model[1] features asymmetrical DNA strand transfer between interacting homologues, resulting in an extensive hDNA region. DNA mismatches arising in the hDNA are ordinarily recognized and corrected, resulting in gene conversion or (cryptic) restoration. Post-meiotic segregation occurs when such mismatches escape correction during meiosis, and are resolved via subsequent replication. Therefore, specific alleles displaying high frequencies of post-meiotic segregation would be expected to generate hDNA mismatches which are inefficiently corrected.

The double-strand break repair model[2] accounts for recombination through the repair of double-stranded DNA gaps. The process uses a homologous chromatid as the repair template. Short hDNA regions are formed flanking the repaired double-stranded gap. Mismatch repair presumably does not occur in this model, and most mismatches will generate post-meiotic segregation. Rare alleles displaying high frequencies of post-meiotic segregation must occur in hDNA more often and are postulated to create or abut on 'pause sites' for the nuclease activity which enlarges the initial double-stranded break[2]. Such a pause site could halt or retard enlargement of the gap on one or both strands.

The *ADE8* and *ARG4* DNA sequences were searched for shared sequences which could represent pause sites. Thirteen separate sequences of 7, 8 or 9 bp are shared in the regions of

Table 2 Mismatch specificity of post-meiotic segregation in yeast and transformation in *Streptococcus*

S. cerevisiae			*S. pneumoniae*		
Diploid	Mismatch	Post-meiotic segregation frequency	Efficiency class	Mismatch	Relative efficiency
ADE8/ade8-18	38-bp deletion	54.1%	VHE	33-bp deletion	0.86–1.06
ARG4/arg4-16	C/C or G/G	32.5%	HE	C/C or G/G A/G or C/T	0.49–0.64
ARG4/arg4-17°	A/A or T/T	5.3%	IE	A/A or T/T	0.17–0.26
arg4-17°/arg4-17°	A/C or G/T	4.2%	LE	A/C or G/T	0.0026–0.085
HIS4/his4-519	G insertion	0			
HIS4/his4-518	C insertion	0			

The frequencies of post-meiotic segregation are from ref. 9, except *his4-519* (197 tetrads)[18] and *his4-518* (712 tetrads, S.F. and K.L., unpublished results). The *his4-518* sequence is from M. Culbertson (personal communication): ACC→ACCC and the *his4-519* sequence is from ref. 19. The nomenclature for *S. pneumoniae* efficiency classes is from ref. 16, where one to four sequenced examples of each class were reported. The relative transformation efficiencies are from Lacks[15]. Large deletions[15] and presumed frameshifts[20] are usually LE markers in *Streptococcus*. VHE, very high efficiency; HE, high efficiency; IE, intermediate efficiency; LE, low efficiency.

interest (*ADE8 Hpa*I/*Sal*I fragment and *ARG4 Sst*I fragment) (see Table 1). Although one of the sequences abuts on the *arg4-16* site and is also represented only 19 bases away from the *ade8-18* breakpoint, another sequence is equally close to *ade8-18* and the low post-meiotic segregation *arg4-17* site. No new homology is generated by any of the mutations studied in these regions. Therefore, our data neither support nor rule out the presence of a pause site near the two high post-meiotic segregation alleles.

Table 2 lists the *Saccharomyces cerevisiae* hDNA mismatches generated by the alleles whose sequences were determined in the present study. No other data on mismatch repair efficiencies for specific mismatches in *S. cerevisiae* exist. However, post-meiotic segregation is observed for specific G to C transversions creating C/C or G/G mismatches during meiotic conversion of transfer RNA genes in *Schizosaccharomyces pombe*[11]. Analysis of two bacteriophage T4 frameshift mutations which could have arisen through hDNA repair on a quasipalindromic sequence containing a two-base bulge (that is, an insertion relative to the other 'strand') and three sets of base-pair mismatches, G/G, T/G and C/C, yielded one mutant in which all three base pair mismatches had been corrected along with the bulge, while in the other mutant only the C/C mismatch survived the correction process[12].

There is a striking correlation between the *S. cerevisiae* data shown in Table 2 and the relative transformation efficiencies of various markers in *Streptococcus pneumoniae*. Exogenous DNA is converted to single-stranded DNA upon or concomitant with entry into the *S. pneumoniae* cell[13]. The DNA single strands are then taken up by host DNA[13], presumably resulting in hDNA and D-loops in homologous regions. Marker transformation efficiency is interpreted as being a function of the probability of hDNA mismatch recognition[14]. Once recognition occurs, incoming information is excised. Thus, transformation efficiency is inversely related to hDNA mismatch recognition[14]. The right half of Table 2 presents the four identified transformation efficiency classes[15] and the potential hDNA mismatches arising from sequenced examples[16]. Comparisons within Table 2 reveal that the frequency of post-meiotic segregation in yeast and transformation efficiency in *Streptococcus* seem to be based on similarly specific hDNA mismatch correction. Correction of individual base pair mismatches by the methylation-directed system in *Escherichia coli* is also similar. For example, C/C, A/G and C/T are inefficiently corrected, A/A is fairly efficiently corrected and A/C and G/T are well corrected[17].

Based on the above correlations, we are constructing diploid strains harbouring all six possible pairs of mismatches at a single site. These will provide critical information concerning the nature of the meiotic recombination intermediate. If the intermediate is primarily hDNA, the frequency of post-meiotic segregation should vary with the mismatch generated. However, if the intermediate is primarily a double-stranded gap, post-meiotic segregation frequency will depend only on the sequence present on the gapped or recipient chromatid. In this instance, similar post-meiotic segregation frequencies would be expected for all six diploids unless a particular mutant allele creates a pause site not present in the wild-type strain.

In conclusion, we have presented data which comprise a framework for a molecular analysis of the meiotic recombination intermediate. If the intermediate is a double-stranded gap, 54% of the gaps formed near the *ade8-18* site must have a hDNA segment of at least 38 bp, perhaps due to a pause site. On the other hand, we find that the frequency of post-meiotic segregation is correlated with the nature of specific hDNA mismatches analysed and our data therefore suggest a hDNA intermediate. In fact, data for two point crosses at *ARG4* suggest that the A/A or T/T mismatch generated by *arg4-17°* can promote correction of the *arg4-16* C/C or G/G mismatch[9]. Thus, sites separated by 213 bp can be involved in the same hDNA region. If further experiments confirm that segregation frequency is controlled by the structure of the mismatch itself, rather than the sequence environment, it will be possible to estimate more accurately the minimum contribution of hDNA to the meiotic recombination intermediate.

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"The GLC has taken an essentially negative attitude on most of the major planning issues in London... On several such issues its contribution has been incompetent, irrelevant, or irresponsible."

Kenneth Baker, in Committee debate on Local Government Bill, 15 January, 1985.

In the alterations it has submitted, to the Greater London Development Plan (GLDP) the GLC sets policies and programmes for co-ordinated action to improve London's environment. Among the GLC's own initiatives are:-

Protecting 88,000 acres of Green Belt from major building.
Funding for new country parks, such as Colne Valley and Lee Valley.
Maintaining 20,000 acres of urban parks and open spaces.
Creating large urban parks at Mile End (Tower Hamlets) and Burgess Park (Southwark).
A specialist ecology team to advise on and grant aid work to protect wild life and natural environments.
Redevelopment of obsolete urban environments for community use — Coin Street and Courages.

- Reclaiming polluted lands at Woolwich Arsenal, Battlebridge Basin, and Wandsworth Gas works for housing, recreational and industrial use.
- Reclaiming land spoilt by minerals working for recreational uses.
- Protecting London from flooding and encouraging access to and greater use of the Thames.
- Tackling London's high levels of noise, vibration, air, land and water pollution.
- Protection of and repairs to London's historic buildings and areas, such as Covent Garden.
- The Secretary of State refused to consider alterations to the GLDP; how will their initiatives be continued without a co-ordinated plan and the resources of an elected London-wide planning authority?

GLC
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Reader Service No.03

Heresy of immanence

John Maddox

The Probability of God. By Hugh Montefiore.
SCM Press, 26-30 Tottenham Road, London: 1985. Pbk £6.95.

ON MATTERS of dogma the Anglican Church has an almost Unitarian god-of-your-choice flexibility, typified by the vicar in the 1960s revue "Beyond the Fringe" who was indistinguishable from a social worker and whose slogan was "Call me Bill; that's the kind of vicar I am". Hugh Montefiore, Anglican Bishop of Birmingham since 1978, is that kind of bishop.

The Probability of God is only marginally a theological work which, in that respect, has much in common with the opinions of the turbulent northern bishops such as the new incumbent at Durham, who is forever shocking the popular newspapers by saying that he doubts the doctrine of trans-substantiation. The real purpose of the book is to demonstrate the immanence of God (or, more ecumenically, I suppose, some God) from the explanatory incompleteness of scientific theories, from cosmology to evolution.

To be frank (and fair), the book is a good and even entertaining read. Over the past few years, Montefiore has taken a serious and continuing public interest in matters such as the environment and arms control. He is both reasonable and earnest, willing to acknowledge that he does not know science at first hand, insistent nevertheless that it must be possible for an honest enquirer to get to the bottom of it (in which he is right) and, then, willing to take up the cudgels in the defence of what seems sanity. If there have to be bishops, nobody could ask for better.

The book is as reasonable as the man. On cosmology, for example, Montefiore does not seize on the big bang as proof that there is (or at least was) a Creator; that, he says, would be "to confuse secondary with primary causes". Instead, he follows the recent writings of Professor Paul C. Davies in emphasizing the numerical coincidences without which life as we know it would not have been possible — the small but not-too-small degree of anisotropy at the beginning that made it possible for galaxies to form; the particular ratio of gravitational and electromagnetic forces which ensures that all stars are not red dwarfs; the particular value of the coupling constant of the strong nuclear force which means that heavy elements (which we need in our enzymes) are stable but which is not so great that the post-big-bang ratio of hydrogen to helium would have been negligible.

Montefiore's refrain is typified by the declaration that "It seems remarkable that the possibility of a planet where life as we know it could evolve should depend upon the precise value of this, the weak nuclear coupling constant". The anthropic principle, which has it that people can observe only a universe conducive to their own existence, is dismissed, but unconvincingly. Lovelock's Gaia hypothesis is a natural next: if the temperature on the surface of the Earth has been that which has been suitable for the survival of living things since the Precambrian, the ups and downs of the solar constant, or the variations of the chemical composition of the atmosphere notwithstanding, does it not follow that there must have been some intervention on behalf of us and our kind?

The texture of the two evolutionary chapters is different, even though Montefiore again begins by not taking the easy way out by letting everything hang on the belief that there has been too little time since the beginning of the Solar System for such a complicated set of living species as are now extant to have evolved. Montefiore argues instead from particular examples, but first asserts that science has made a dogma of natural selection. The rest is a collection of gee-whizz stories, "puzzle after puzzle". Why are our viscera the way they are? Why should *Drosophila* larvae be interposed between egg and adult? Why several independent and apparently successful attempts to evolve an eye? Popper's description of Darwinism as metaphysical (because it cannot be falsified) is mentioned, Popper's recantation is not.

In passing, Montefiore roundly castigates the Creationists, condemns Lysenko, concludes regretfully that there is no hope for Christians in Lamarck but plainly has a soft spot for Dr Ted Steele, the immunologist who caused a minor sensation a few years ago by claiming that immunological genetic changes may be inherited in the germ line. Mystifyingly, he also pats Rupert Sheldrake on the back, noting that the tendency for living cells to assemble in ordered ways can be explained if, indeed, there are "morphogenetic fields" which, for example, guide neurones to the connections with others that make them function properly.

Montefiore's God, therefore, is not a once and for all God, one who drew up the laws of physics and then left us to it, nor a "God of the gaps", one who may have intervened from time to time, but is instead an immanent God, embedded in the Universe and helping to make it tick from one instant to the next. By means of an argument reminiscent of Arthur Koestler's *Roots of Coincidence*, a tract in favour of the paranormal, Montefiore concludes that the nature of the Universe makes the hypothesis of God probable, whereupon more familiar religious arguments lead to Christianity and all that. In passing (and at the outset), he rejects the notion that religion is a code by which people may distinguish ethical and moral behaviour from other kinds of behaviour, and which is therefore orthogonal to rational consideration of the Universe.

Although the cosmological part of Montefiore's argument would fall apart if the ambitions of particle physicists to unify the four forces of nature were to succeed, and demonstrate that the cosmic coincidences can be derived from the laws of physics, the flaw in the whole argument is not in the detail but in the grand design. Montefiore writes as if present scientific understanding of the Universe were complete, as if we shall always be stuck, for example, with the model of the big bang (in its contemporary form, no older than the discovery of the microwave background radiation). It would be an interesting exercise if somebody were to rewrite Montefiore's book at intervals of, say, five years; his natural theology (*pace* David Hume) would repeatedly be found to derive from different sets of circumstances.

Indeed, Montefiore's whole enterprise is more hazardous, and dangerous, than his reasonable tone implies. His immanent God, working in everything, qualifies him as a pantheist. One does not have to go quite as far as Hermann Bondi, whom Montefiore quotes as having described religion as a "dangerous and habit-forming evil", to be deeply suspicious of how pantheism could corrupt the process of scientific enquiry. At what point in the investigation of the evolution of the human eye, for example, will investigators feel free to chicken out, ascribing some parts of the process to natural selection and others to God? Montefiore says his is not the "God of the gaps", but it sounds a bit like the God of the unsolved problems.

Religious belief is common, even among scientists, and, in moderation, is at worst harmless. Many believers are content with what seems to them the sensible position that religious belief is neither dependent on nor even connected with the character of the Universe in which they live and carry out experiments. Others take the view that physical divine intervention ended with the big bang. Explanations of the real world that suppose there is an invisible hand somewhere, making sure that hidden purposes are fulfilled even in the face of the laws of physics are less common but, in their nature, more challenging. Montefiore's book, although honest to the point of saying that "the possibility that there is no God remains open" (but is "wildly improbable"), asks of its readers a concession to irrationality that most will find impossible to make. □

John Maddox is Editor of Nature

Ringing the changes in chemistry

Robert Ramage

Comprehensive Heterocyclic Chemistry: The Structure, Reactions, Synthesis and Uses of Heterocyclic Compounds.
Chairmen of the editorial board Alan R. Katritzky and Charles W. Rees.
Pergamon: 1984. Eight volumes. £1375, $2200.

HETEROCYCLIC chemistry is a crucially important area of organic chemistry; it embraces a wide range of structural types and applications; and the pharmaceutical industry is, to a large extent, sustained by developments in the structure, reactivity and synthesis of heterocyclic systems. Pergamon's eight-volume series has these three themes as the unifying factor, which has enabled the principal editors and the volume editors to achieve an internally consistent presentation embodying coverage of a remarkable range of topics. The necessary editorial control exercised in the compilation of 154 chapters has not stifled the independent scholarship of the many contributing authors — their collective efforts have resulted in a work which can truly be considered to be "comprehensive".

The series is subdivided into six parts, and the approach adopted by the editors can best be illustrated by the general individual chapters on structure, reactivity and synthesis in Vols 2, 4, 5 and 7. These contributions are not only interesting in themselves but are excellent introductions to the subsequent, more specialized material in each section.

The editors have used Vol. 1 (which constitutes Part 1) to deal with general matters such as nomenclature, review literature, biosynthesis and applications of heterocyclic compounds, thus allowing the following sections to deal more with fundamental principles. In addition, the first volume treats an assortment of lesser known heterocyclic systems which could not easily be assimilated into the accounts of the main heterocyclic systems. Chapter 1.03 on the review literature of heterocycles includes an impressive yet accessible collection of a wide range of references, subdivided into synthesis, reactions and properties, while biological applications are succinctly discussed in chapters dealing with biosynthesis, toxicity, and pharmaceutical, agrochemical and veterinary products. Chapter 1.17 by K. Dimroth, on heterocyclic rings containing phosphorus, is especially impressive in content and organization and will allow chemists to appreciate more fully this less-traditional area of the subject.

Part 2 covers six-membered ring systems incorporating one nitrogen atom (Vol. 2) and then oxygen, sulphur, or two or more nitrogen atoms (Vol. 3). The treatment of these topics is consistent with the general editorial policy of logical development from structure through reactivity to synthesis. Highlights here are Chapter 2.03, a strategic discussion of synthesis of six-membered rings with varying degrees of unsaturation, and Chapters 2.05 and 2.06 which illustrate many facets of pyridine reactivity in monocyclic and bicyclic systems. Volume 2 will certainly provide good background reading in this general area, while the more specialized topics of Vol. 3 are treated in a clear, interesting manner. Together, these two volumes contain the fundamental chemistry required for understanding of many of the biologically important heterocyclic systems.

In Vol. 4 (Part 3 of the series), the approach to five-membered rings containing one hetero-atom is in accord with the pattern set out in the previous volumes. The introductory section (Chapters 3.01, 3.02 and 3.03) has been authoritatively written by C.W. Bird and G.W.H. Cheeseman, and there are useful accounts of the application of spectroscopic methods to structural problems of five-membered heterocycles, resonance and tautomerism. Pyrroles and their benzo derivatives are dealt with in some detail, and are followed by an excellent chapter by K.M. Smith on porphyrins and related compounds which examines in depth the synthesis of simple corrinoids and vitamin B_{12}. The treatment of furan and thiophene chemistry is both a critical and an extensive contribution.

Both Vols 5 and 6 (Part 4) include accounts of a remarkable range of heterocycles containing two or more hetero-atoms. Each class of ring system is treated consistently, and here are to be found heterocyclic types ranging from the biologically important imidazoles and purines to the more specifically chemically interesting systems of importance in organic synthesis.

Volume 7 (Part 5) is used to collate the less voluminous chemistry of heterocyclic systems having small and large rings. The treatment of small-ring chemistry is instructive and well written, although the coverage of β-lactam antibiotics is of necessity rather too brief to be of more than general interest. The chemistry of seven-membered and larger rings is dealt with clearly and concisely, and crown ethers and cryptands are thoroughly reviewed by A.D. Hamilton who takes the subject from consideration of crystal structures to biological and synthetic applications of coordination of these macrocycles containing several hetero-atoms.

The final volume (and part) contains the index, helpfully divided into physical data, author, subject and ring. In addition, the work as a whole benefits from an ingenious reference code system which gives the reader easy access to original papers and source material such as books and reviews.

Comprehensive Heterocyclic Chemistry makes a unique contribution to the literature of organic chemistry. The references quoted are mainly post-1965, and together the eight volumes provide chemists, and biologists working at the chemical interface, with an impressive body of collected knowledge which is not available from any other source. Expensive though it is, chemistry libraries cannot afford to be without this series. □

Robert Ramage is Forbes Professor of Organic Chemistry at the University of Edinburgh.

Bacterial diplomacy

Howard C. Berg

Motility and Taxis in Prokaryotes.
By A.N. Glagolev.
Harwood: 1984. Pp.279. $126.

MODERN work on bacterial motility and taxis dates from the demonstration by Julius Adler (*Science* **166**, 1588–1597; 1969) that the bacterium *Escherichia coli* has specific chemoreceptors: proteins near its outer surface that bind particular amino acids or sugars, causing the cell to respond favourably to changes in its environment, even in the absence of transport or metabolism. This meant that one could study behaviour without having to worry much about energetics, using genetic tricks to unravel steps in the sensory-transductive pathway.

In the Soviet Union, interest in this field was sparked by Vladimir Skulachev, well known for his work on chemiosmotic proton circuits, following the discovery by Larsen, Adler, Gargus and Hogg (*Proc. natn. Acad. Sci. U.S.A.* **71**, 1239–1243; 1974) that bacterial flagella are powered, not by ATP, but rather by the high-energy intermediate of oxidative phosphorylation. Work in the Soviet Union, the United States and Japan has since shown that the immediate source of energy for flagellar rotation is, indeed, the protonmotive force. Alexei Glagolev, a protégé of Skulachev, now raises the Mitchellian banner and argues that $\Delta\tilde{\mu}H^+$ plays an important role in tactic sensing and signalling.

This is a research monograph, not a book for the casual reader. It begins with a compact survey of bioenergetics, summarizes work on the motile mechanics of flagellated cells and gliders, and then presents an extensive discussion of various forms of directed movement, including chemo-, photo-, thermo-, visco- and magneto-taxis. The book brings together the results of a number of experiments described previously by the author and his colleagues in a series of short papers appearing primarily in *FEBS Letters* and *FEMS Microbiology Letters*.

While many of the phenomena described in these papers and reviewed here are striking and warrant serious attention — experiments on the behaviour of long trichomes

of the gliding cyanobacterium *Phormidium uncinatum* are particularly elegant — I find myself sceptical of the work as a whole. A modern-day bioenergeticist comes armed with a number of chemical weapons (ionophores, uncouplers and inhibitors) with which I have had limited experience. How secure are the conclusions drawn from experiments in which such weaponry is applied? These reagents are not all highly specific. For example, the calcium ionophore A23187 has poor selectivity for Ca^{2+} over Mg^{2+} and can even exchange Na^+ for H^+. The ATPase inhibitor DCCD reacts with alcohols and amines, not only with carboxyl groups in hydrophobic pockets. Furthermore, not all such reagents readily penetrate the outer membrane of gram-negative bacteria. The permeability properties of the outer membrane of *E. coli* are reasonably well understood, but what about *Ph. uncinatum, Rhodospirillum rubrum* or *Vibrio harveyi*, to name but a few of the organisms discussed here, or the archaebacterium *Halobacterium halobium*? These systems are complex. More than one function can be perturbed by chemical modification. How is the novice to judge the reliability of this approach when discussions are so brief?

My concern is reinforced by recent results obtained by the genetic approach. For example, by comparing the effects of the inhibitors DCCD and KCN on both phototaxis and motility in *H. halobium*, Glagolev argues that phototaxis in this organism is mediated by changes in $\Delta\bar{\mu}H^+$. This bolsters his argument that sensory systems in bacteria might have evolved from a primitive protometer. However, as he readily acknowledges in his addendum (August 1983), the discovery that phototaxis in *H. halobium* is mediated by a slow-cycling rhodopsin distinct from either bacteriorhodopsin or halorhodopsin — see Spudich and Bogomolni *Nature* **312**, 509-513 (1984) — makes this interpretation highly unlikely. Or take the molecular model for chemotaxis (Fig.38), which includes (i) activation of cGMP by an attractant-receptor-MCP (methyl-accepting chemotaxis protein) complex, (ii) blockage by cGMP of the chemotaxis signal, comprised of a repellent-receptor-CheB (methylesterase) complex, which (iii) affects the flagella by binding to CheZ (a cytoplasmic protein), thus liberating CheC (the flagellar switch) and allowing Ca^{2+}-dependent (possibly membrane potential-dependent) flagellar reversals. As far as I am aware, evidence for the involvement of either cGMP or Ca^{2+} in this process is equivocal, while mutants deleted for *che*B and/or *che*R (the methyltransferase) have normal excitation kinetics. So I find this model speculative in the extreme.

I therefore do not agree with the writer of the book's promotional blurb, who claims that this is "the first rigorous survey of the major new discoveries pertaining to bacterial behaviour". But I do find the book a lively, honest, even exuberant work, full of interesting facts and figures, that carries the discussion of bacterial behaviour well beyond the narrow confines of the *E. coli/S. typhimurium* school.

One of the problems, regrettably, is that our Soviet colleagues have not had the benefit of much feedback from the West — one learns a great deal from peer review and the grapevine. Nor have we absorbed as much as we should of their bioenergetic expertise. The channels of communication should be strengthened. We need more bacterial diplomacy. □

Howard C. Berg is Professor of Biology at the California Institute of Technology, Pasadena.

Only natural

L. Crombie

The Chemistry of Natural Products.
Edited by R.H. Thomson.
Blackie/Methuen: 1985. Pp.467.
£46, $75.

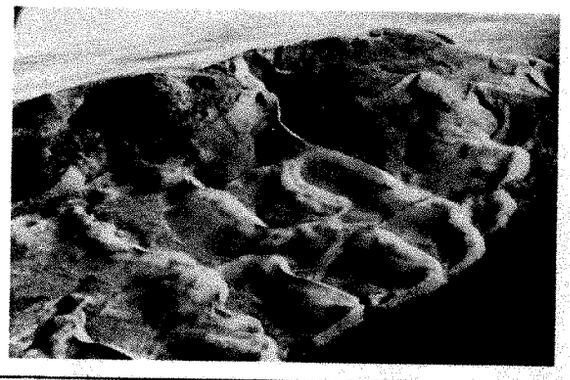
ONE of the great barriers to a general appreciation of organic chemistry is the system of structural formulae employed by organic chemists. This is a pity, but understandable. The structural formula, with its dense information content, has evolved to its present form over a century and the practised organic chemist can take in at a glance an enormous amount of precise and detailed scientific information. Like any other language this pictogram system has to be learnt and practised, but once fluency is attained it is easy to use and, mathematical notation apart, is more universally accepted in science that any other language.

The Chemistry of Natural Products is full of such formulae, beautifully drawn and presented, and very helpful they are too. However, despite their plentiful use, the field of natural products is so large that a writer or editor must be very selective in the choice of topics to be covered in a single book. Professor Thomson has assembled a group of ten excellent authors to write about some of the principal sectors of the subject — carbohydrates, aliphatic and aromatic compounds, terpenes, steroids, alkaloids, nucleosides, nucleotides and nucleic acids, amino acids, peptides and proteins, and porphyrins and related compounds. Even so, limitations have had to be imposed. Authors were asked to emphasize stucture, chemistry and synthesis, and make only passing reference to biosynthesis. Additionally, their brief was to describe what has been happening in each of these areas during the past ten years. This of course places some restrictions on the readership which has to have an appreciable basic knowledge of the subject: for such people, however, the book is stimulating and valuable for keeping abreast of developments.

Undergraduates will probably find the chapters too terse and lacking in detailed explanation for direct use, but this should prove to be a useful book to their teachers for updating lecture notes. Postgraduates, especially those undertaking research in this area, will find it invaluable for acquiring a broader, up-to-date view of their subject. However, a price has had to be paid for this wide coverage in that, because of constraints on space, some authors have adopted a rather telegraphic style reminiscent of *Annual Reports of the Chemical Society* or certain of the *Specialised Periodical Reports*. For the purposes of this book, I would have preferred to have seen some reduction in the number of subjects covered and rather more relaxed and extended treatments.

Looking over these various topics, I sense that the contributors were necessarily assigned tasks of unequal difficulty. It is distinctly harder for authors of general sections such as "Aliphatic Compounds" or "Aromatic Compounds" to produce an exciting account because of the diversity of their material. On the other hand, more homogeneous topics — "Porphyrins and Related Compounds" or "Amino Acids, Peptides and Proteins" for example — make attractive reading and were doubtless more pleasurable to write. The book itself is a good example of a stout, well-produced product at an acceptable price. □

L. Crombie is the Sir Jesse Boot Professor of Organic Chemistry at the University of Nottingham.



Making waves — climbing parabolic sand dunes approaching Juniper Buttes, Snake River Plain, Idaho; wind direction is from lower left to upper right. The picture is reproduced from Wind as a Geological Process (on Earth, Mars, Venus and Titan), *by Ronald Greeley and James D. Iversen. Publisher is Cambridge University Press, price is £35, $59.50.*

June meetings

5 June 1985, **Seminar on Laboratory Automation**, London, UK (Marketing Director, V.A. Howe & Co., 12-14 St Anne's Crescent, London SW18 2LS, UK)
10–15 June 1985, **Bari International Conference on F.VIII/von Willebrand Factor-Biological and Clinical Advances**, Pizzomunno Vieste del Gargano, Italy (Dr N. Ciavarella, Hemophilia Centre, Policlinico, University, 70124 Bari, Italy)
25–28 June 1985, **International Symposium on Forest Utilization of Municipal and Industrial Wastewater and Sludge**, Seattle, Washington, USA (K. Grier, College of Forest Resources AR-10, University of Washington, Seattle, WA 98195)

August meetings

24–27 August 1985, **International Conference on the Immonogenetics of Type I Diabetics and Autoimmune Thyroid Disease**, St. John's, Newfoundland, Canada (N.R. Farid, Prof. of Medicine, Chief-Division of Endocrinology and Metabolism, Memorial University of Newfoundland, St John's, Newfoundland, Canada)

September meetings

2–6 September 1985, **International Conference on Atmospheric Carbon Dioxide; Its Sources, Sinks and Global Transport**, Kandersteg, Switzerland (Ulrich Siegenthaler, Physics Institute, Sidlerstrasse 5, CH-3012 Bern, Switzerland)
16–19 September 1985, **European Congress for Artificial Organs**, Athens, Greece (Prof. S. Raptis, M.D. P.O. Box 14127, 11510 Athens, Greece)
16–20 September 1985, **International Meeting on Quantum Chemistry**, Strasbourg, France (Dr A. Veillard, Université Louis Pasteur, Institut Le Bel, 4 Rue Blaise Pascal, 67000 Strasbourg, France).
17–19 September 1985, **International Symposium on Lyme Disease and Related Disorders**, Vienna, Austria (Dr G. Stanek, M.D. Hygiene Institute of the University, A-1095 Vienna, Austria).
30 September–3 October 1985, **European Symposium on Hormones and Cell Regulation**, Strasbourg, France (Dr R.J.B. King, Imperial Cancer Research Fund Laboratories, Hormone Biochemistry Department, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK).

October meetings

3–4 October 1985, **International Meeting on Haemodynamics and Atherosclerosis**, Wellington, New Zealand (Dr W.E. Stehbens, Department of Pathology, Wellington Clinical School of Medicine, Wellington, 2, New Zealand).
3–5 October 1985, **2nd Congress of the European Society of Magnetic Resonance in Medicine and Biology** (with Exhibition), Montreux, Switzerland (Dr M.A. Hopf, 1 route de Florissant CH-1206 Geneva, Switzerland)
8–10 October 1985, **International Congress and Exhibition for Biotechnology**, "Biotechnica", Hannover, West Germany (Deutsche Messe — und Ausstellungs — AG, Messegelande, D-3000, Hannover 82, FRG).
20–25 October 1985, **International Conference on Leukotrienes and Prostanoids in Health and Disease**, Jerusalem, Israel (Prof. U. Zor, Dept. of Hormone Research, Weizmann Institute of Science, Rehovot, Israel 76100)
21–24 October 1985, **International Conference on Pesticides Toxicity, Safety and Risk Assessment**, Lucknow, India (Dr T.S.S. Dikshith, General Secretary of the Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Post Box No. 80, Lucknow - 226001, India).
23–25 October 1985, **Congress of the International Society for Research on Civilization Diseases and Environment**, Budapest, Hungary (Congress Bureau MOTESZ, Budapest PO Box 32, H-i1361, Hungary).
28 October–1 November 1985, **International Symposium on Source Term Evaluation for Accident Conditions**, Columbus Ohio, USA (M. Jankowski, Div. of Nuclear Safety, c/o Int. Atomic Energy Agency, Vienna International Centre, PO Box 100, A-1400 Vienna, Austria).

November meetings

18–21 November 1985, **Scientific Consensus Conference on methods for assessment of the cariogenic potential of foods**, Texas, USA (Dr Dominick P. DePaola, Chairman Consensus Conference Planning Committee, Dean, U.T.H.S.C.S.A., DS 7703 Floyd Curl Drive Antonio, Texas 78284, USA)
25 November–6 December 1985, **International Symposium on Optical and Electro-Optical Applied Science and Engineering**, Cannes, France (J. Prado, Cannes Conference Coordinator, ANRT–Association Nationale de la recherche Technique 101, Avenue Raymond Poincaré, 75116 Paris, France)

Appointments

President Reagan has announced his intention to nominate **Dr John H. Moore** as Deputy Director of the National Science Foundation (NSF). Dr. Moore has been Associate Director and Senior Fellow of the National Science Board, the policy making body of NSF since 1982. Dr Moore would succeed Donald Newton Langenberg.

The Minister of Agriculture, Fisheries and Food has appointed **Dr Robert N. Crossett**, Chief Scientist (Fisheries and Food) of the Ministry, to be a member of the Agricultural and Food Research Council.

Please send 'Announcements' entries to: The Editor, Nature, 4, Little Essex Street, London WC2R 3LF, UK

The KROC Foundation has endowed a $1 million chair at UC-San Francisco to support biomedical research on rheumatic and other immunological diseases. The first holder of the Robert L. Kroc Chair in Rheumatic and Connective Tissue Diseases is **Edward J. Goetzl**, professor of medicine and director of the Division of Allergy and Immunology at UCSF.

Weizmann Institute chemist **Professor Zippora Shakked** has been named first incumbent of the Helena Rubinstein Chair in Structural Biology. Prof Shakked is a member of the Institute's Department of Structural Chemistry, applying X-ray crystallography methods to investigate compounds responsible for the expression of inherited traits.

Professor J. MacMillan, Professor of Organic Chemistry in the University of Bristol, has been appointed as Alfred Capper Pass Professor of Organic Chemistry in succession to Professor Whiting, who is retiring. Professor Whiting will be re-engaged part-time, as Professor of Organic Chemistry.

Dr J.D. Baum has been appointed to Bristol University's Chair in Child Health upon the retirement of Professor N.R. Butler at the end of July 1985.

Dr J.P. McGeehan, Senior Lecturer in Communications Engineering at the University of Bath, has been appointed to Bristol University's Chair in Communications Engineering.

Dr J.W.B. Bradfield, Consultant Senior Lecturer in Pathology at the University of Bristol, has been appointed to Bristol University's Chair in Histopathology.

Awards

The National Science Foundation is offering awards to women scientists and engineers wishing to become visiting professors at US academic institutions. Awards may range from one academic term to 24 months, full or part time, with the usual period being one year. For terms of eligibility and further information write to Visiting Professorships for Women Program, National Science Foundation, Washington, DC 20550.

Miscellaneous

The University of Essex is to offer the UK's first MSc Course in Aerosol Science and Technology from October this year. The one-year course will teach the theoretical and practical aspects of aerosol science and technology with specialist options in atmospheric chemistry and aerosols, and the physics of aerosols. For further information contact Irene Lally, Information Officer, University of Essex, Wivenhoe Park, Colchester, Essex.

Recent Publications for Chemists and Biochemists

from the Royal Society of Chemistry

Natural Product Reports



Approx 100 pages per issue.
6 issues per annum
Author and Subject Index (cumulated annually)

Subscription 1985
£125.00 ($242.00)
Rest of World £126.00

Natural Product Reports is a new bi-monthly review journal which commenced publication in February 1984 Natural Product Reports deals exclusively with reviews of natural product chemistry previously covered by a number of books published by the Royal Society of Chemistry – "The Alkaloids", "Biosynthesis", "Terpenoids and Steroids", and "Aliphatic and Related Natural Product Chemistry".
Publication in journal form helps to overcome the problem of overlap and enables reports to be published in a much faster time. Coverage has also been extended to include such areas as chemotaxonomy, enzymology and biosynthetic aspects of biotechnology and advances in physical techniques used for structure determination.
Each issue consists of critical reviews written by leading authorities of worldwide recognition.

Recent Advances in the Chemistry of ß-lactam Antibiotics

3rd International Symposium 1984

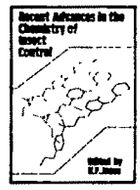


Edited by A G Brown and S M Roberts

Softcover 399pp
ISBN 0 85186 955 6
Price £26.50 ($48.00)

Worldwide sales of anti-bacterial substances amount to ca £6,000M per annum. About 60% of this figure can be allocated to the ß-lactam antibiotics and it can be estimated that ß-lactams account for about 10% of the total drug sales.
Not surprisingly, therefore, many laboratories in academia and the pharmaceutical industry are continuing the search for new and improved anti-bacterial substances based on the ß-lactam template.
This book contains a series of articles which describe the results of research accomplished in recent years.
Contents include:
Recent studies on the Biosynthesis of Penicillins; Do ß-Lactam Antibiotics require a ß-Lactam Ring? The Chemistry of Clavulanic Acid and its Derivatives; The Synthesis of Biological Properties of Recent Cephalosporins, Structure-Activity Relationship Among Some Totally Synthetic Carbapenems
Special Publication No. 52 (1985).

Recent Advances in the Chemistry of Insect Control

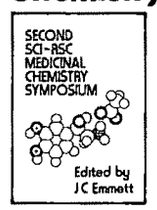


Edited by N F Janes

Softcover 336pp
ISBN 0 85186 965 3
Price £26.00 ($34.00)

This publication covers recent advances in the synthesis and evaluation of highly active compounds in pesticide chemistry such as the avermectins and milbemycins and a range of insect pheromones and antifeedants, aspects of structure activity relationships in insecticides, including pyrethroids (where stereochemistry is highlighted), carbamate propesticides, and juvenile hormone mimics
Contents:
The Biochemical Mode of Action of Insecticides; Stereochemical Aspects of Pyrethroid Chemistry; The Avermectins; Lipophilic Insect Control Agents; Oxime Ethers; New Potent Insect Growth Regulators; Multidisciplinary Studies in the Design of New Insecticides; Some Aspects of Structure Activity Relationships in Pyrethroids; Synthesis and Biological Activity of 2-Indanyl Pyrethroid Esters; New Intermediates for the Synthesis of Pyrethroids; A New Type of Synthetic Pyrethroid Insecticide; Structure Activity Relationships of Methylcarbamoyloximes and Their Sulfenylated Derivatives. Discovery of Thiodicarb; Synthetic Approaches to the Avermectin Toxophore; Synthetic Studies towards the Avermectins and Milbemycins; The Application of Novel Carbanion Chemistry in Milbemycin Avermectin Synthesis; Phytochemical Resources for Pant Protection; Chiral Synthesis: Examples in the Pheromone Field; Synthesis of Some Insect Antifeedants.
Special Publication No 53 (1985)

Second SCI-RSC Medicinal Chemistry Symposium

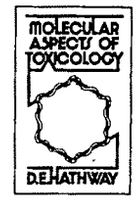


Edited by J C Emmett

Softcover 342pp
ISBN 0 85186 395 1
Price £24.00 ($43.00)

There are many new and developing areas in the subject of medicinal chemistry. This book attempts to cater for some of the wider interests of medicinal chemists and will provide not only interesting reading, but also food for thought for those involved in active research.
Brief Contents:
Mediator Regulation in Cardiovascular Drug Therapy; The use of Computer Graphics in Drug Design; Biological Challenges to Organic Synthesis; Control of Arachidonic Acid Cascade.
Special Publication No. 50 (1984).

Molecular Aspects of Toxicology



by D E. Hathway

Hardcover 319pp
ISBN 0 85186 068 0
Price £27.50 ($50.00)

Toxicology covers a wide field, which comprises chemical toxicology, cosmetics toxicology, environmental toxicology, food and drugs toxicology, industrial toxicology, pesticide toxicology, and the toxicology of atmospheric pollution, and which involves acute and long-term testing as well as special tests for carcinogenicity, irritation, mutagenicity and teratogenicity. In the testing phases, diagnoses are reached through the disciplines of chemical pathology, clinical medicine and pathology, and in the experimental one, explanation is sought through chemical/biochemical and pharmacological methods.
In such a fragmented field as toxicology with so many diverse practices and specialisms, it is hard to gain an idea of continuity.
This book attempts to unify the subject of toxicology and should be useful to chemists engaged in molecular biology and workers concerned with toxicology and cancer research in particular, as well as to pharmacologists and specialists in drug development and occupational hygiene and medicine in general
Brief Contents:
Part I Toxicity of Foreign Compounds; Part II Relation between Dose and Effect and Time; Part III Metabolism; Part IV Pharmacogenetics, Part V Biochemical Lesions; Part VI Chemical Carcinogenesis, Part VII Toxicant Allergy;

Food: The Chemistry of its Components



by T.P. Coultate

Softcover 202pp
ISBN 0 85186 483 X
Price £5.95 ($11.00)

This book gives a detailed account of the chemistry of the principle substances of which our food is composed. Both the macro-components, and the micro-components, are considered. Throughout the book, Dr Coultate's theme is the relationship between the chemical structure of a substance and its contribution to the properties and behaviour of foodstuffs—whether observed in the laboratory, the factory, the kitchen or the dining room.
Contents:
Introduction, carbohydrates, lipids, proteins, colours, flavours, vitamins, preservatives, EEC numbers of food additives. Subject Index. This book will be of particular benefit to students and teachers of food science and related courses in universities, colleges of further education and schools.
RSC Paperback (1984)

Ordering
Orders should be sent to:
The Royal Society of Chemistry, Distribution Centre, Blackhorse Road, Letchworth, Herts SG6 1HN, U.K.

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Opportunities with the Ministry of Agriculture, Fisheries and Food

ADAS - Agricultural Science Service
Harpenden Laboratory

Zoologist

...to identify insects and other invertebrates associated with imported plant material, diagnose the presence of pests from damage symptoms, and give guidance to Regional Advisory Entomologists in identification of native invertebrate crop pests. Frequent contact with the British Museum (Natural History) will be necessary.

You should have a good Honours Degree specialising in invertebrate zoology, agricultural zoology, applied entomology or other appropriate subject, with post graduate experience in insect/invertebrate identification or taxonomy. A special interest in a particular insect group or experience in crop pest diagnosis would be an advantage.

Appointment as Scientific Officer. Ref: SB/42/AL

Food Science Laboratory - Norwich

Chemist

...to join a small team investigating the occurrence of mycotoxins in processed foods, contamination of foods from plastic packaging and environmental contaminants in the food chain.

You should have an honours degree in chemistry or related discipline. Experience in trace analysis would be advantageous.

Appointment as Scientific Officer. Ref: SB/65/AE

Food Science Division - London

Chemist

...to be responsible for supervising and training a small team engaged in the determination of pesticide residues in food. You will be expected to develop and/or modify multi-residue methods and to research the degradation of residues after food processing.

You should have an honours degree or equivalent in a relevant subject. Experience in both capillary and packed column gas-chromotography would be advantageous.

Appointment as Scientific Officer. Ref: SB/66/AE

Food Chemist/ Technologist

...to provide advice on all aspects of the manufacture and composition of foods and beverages. You will assist in revising existing legislation, prepare papers on topics of national and international concern and identify information research requirements. The work involves close liaison with industry, Government Departments and advisory committees. No laboratory work is involved.

You must have a good honours degree or equivalent in Chemistry and at least 2 years' post graduate experience. Relevant experience in food technology would be an advantage but a sound scientific training plus the ability to communicate at all levels are of paramount importance.

Appointment as Higher Scientific Officer. Ref: SB/67/AE

Fisheries Research Directorate - Lowestoft

Physical Oceanographer

...to work on all aspects of physical oceanography affecting the movement and dispersion of biological organisms and pollutants in the continental shelf seas around the British Isles. You will provide advice to the appropriate Divisions on oceanographic problems relating to currents and dispersion in coastal waters.

You should have a good honours degree or equivalent in physical sciences or mathematics and at least 2 years' relevant post-graduate experience with proven research ability in dynamical oceanography. A knowledge of computer data analysis, mathematical modelling or dispersion measurement techniques would be an advantage.

RELOCATION ASSISTANCE MAY BE AVAILABLE FOR THIS POST.

Appointment as Higher Scientific Officer. Ref: SB/93/AD

Senior Programmers

...to be responsible for supporting and developing existing and future computing systems in the Directorate; the design, development and support of large computer applications; management of programmers and liaison with user research sections. The laboratory uses HP1000, HP9000 and Prime minicomputers, Apricot and Torch personal computers, and has RJE links to an ICL2966 mainframe. The main programming language is FORTRAN 77, but increasing use is being made of database packages, including SIR.

HP1000 computers are currently operated on two research vessels and you will be expected to take part in the laboratory's cruise programme, spending up to 4 weeks per year at sea.

You should have a good honours degree in an appropriate subject and at least 2 years' relevant post-graduate experience with knowledge of maintaining and operating a range of computer systems.

Appointment as Higher Scientific Officer. Ref: SB/94/AD

Salaries: Scientific Officer £5905 - £8150; Higher Scientific Officer £7435 - £10,050. Up to £1300 higher in London. Starting salary according to qualifications and experience. SALARIES UNDER REVIEW.

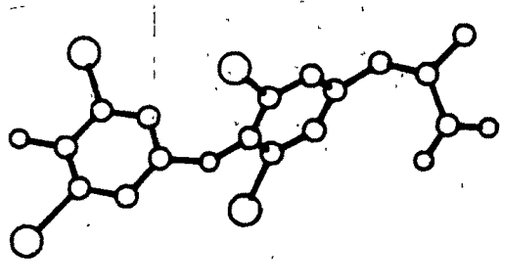
For further details and an application form (to be returned by 14 June 1985) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours).

Please quote appropriate reference.

The Civil Service is an equal opportunity employer

(6611)A

Scientific Civil Service



MODEL CHEMISTS

Chemical Design Limited is a British company which specialises in the development of molecular modelling software. With over **100** installations of the ChemGraf system, we consider ourselves to be the leading software company in the field.

We are currently recruiting **FORTRAN PROGRAMMERS** and **SUPPORT STAFF** with experience of:-

- Numerical techniques
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- Molecular mechanics
- Quantum mechanics
- Protein structures
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We offer the best salaries and conditions for the best people.

If you are interested in the possibility of joining us, please write to our Personnel Manager at the address below.

Chemical Design Limited
The Lodge, Botley Works,
North Hinksey Lane,
Oxford OX2 0NN

Telephone Oxford (0865) 251483

(6566)A

Biochemical Microbiologist or Parasitologist for Section Leadership

£17,000-£21,500 Beckenham, Kent

The Wellcome Research Laboratories is the main centre for the research activities of The Wellcome Foundation Ltd., a major group of pharmaceutical and chemical companies with headquarters in the UK and a turnover close to £800m.

The major objective of the Department of Biochemical Parasitology (Head: Dr. W.E. Gutteridge) is the discovery and development of novel drugs for the microbial and parasitic diseases of Man, including tropical diseases.

We now require an experienced Biochemist to lead the Department's Biochemistry Section which presently consists of 12 post-doctoral scientists and graduate level technicians. In addition to the line management of the Section, you will take responsibility for the co-ordination of its activities with those of the Department's Biology Section and with research chemists involved in programme work. You will deputise for the Head of Department when necessary and undertake personal research work pertinent to anti-infective chemotherapy, across a broad spectrum of human microbial and parasitic diseases.

The successful candidate will possess a Ph.D. in Biochemistry and at least 10 years' post-doctoral research experience, from an academic or industrial environment, in the biochemistry of bacteria, fungi, protozoa or helminths, preferably in relation to chemotherapy. A demonstrated flair for man-management and for organising and leading scientific research teams, and a sound publication record are essential for this key position. The salary will be dependent upon experience, and excellent benefits include five weeks' holiday, pension fund, sick pay scheme, subsidised restaurant and assistance with relocation expenses where appropriate.

Please write with full personal and career details, quoting reference number WRL/337, to: Dr. H. D. Avshu, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS. (6582)A

Wellcome

PROFESSOR & HEAD Biological Sciences Carnegie-Mellon University

The Department of Biological Sciences invites applications from qualified individuals to serve as Professor and Chairperson. We seek an individual with an established reputation in any field of biological research and an ability to provide leadership in the continuing growth of this department. We expect to appoint the successful candidate by 30 June 1986 or before.

Applications or inquiries should be sent to: Professor W.R. McClure, Chairman, Search Committee, Carnegie-Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213. ***Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer.*** (NW1765)A

ENDOCRINOLOGY BIOCHEMISTRY/ PHYSIOLOGY

Postdoctoral Opportunities

Broad range of Endocrine research projects: (i) isolation and identification of pituitary and lung peptides; (ii) physiologic and pharmacologic action, (iii) growth and control of function of fetal adrenals; (iv) role of growth factors in fetal development.

Send curriculum vitae, bibliography, three letters of recommendation, and short statement regarding career goals, interests and experience to: Dr Samuel Solomon, Endocrine Laboratory, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec H3A 1A1. (NW1772)A

AFRC Animal Breeding Research Organisation

CELL BIOLOGIST

ABRO is seeking staff to join its expanding Molecular Biology Group in Edinburgh. Research objectives are to investigate the potential of new techniques for genome manipulation of higher animals. A key component of this research will involve gene transfer into tissue culture cells and the examination of signals determining tissue-specific expression.

Applications are invited from scientists with experience of cell culture techniques Persons familiar with procedures for establishing primary cell lines are especially invited to apply.

Candidates should possess a good honours degree in cell biology or a related subject. For appointment at the Higher Scientific Officer level a minimum of two years post-graduate experience is expected. Candidates for recruitment at the Senior Scientific Officer level will normally be expected to possess a PhD or equivalent qualification. Salary will be according to qualifications and experience on the scale £7,435 – £10,039 (HSO).

Applicants should submit a full curriculum vitae together with the names of three referees to: The Secretary, Animal Breeding Research Organisation, King's Buildings, West Mains Road, Edinburgh EH9 3JQ.

The AFRS and the Grant-Aided Institutes of the AFRS are Equal Opportunities Employers. (6565)A

COMMONWEALTH AGRICULTURAL BUREAUX

The leading world agricultural information service for scientists and technologists

SCIENTIFIC INFORMATION OFFICER

at the

Commonwealth Forestry Bureau

Duties include scanning the world literature on forest products, and to some extent foresty in general, and preparing abstracts for the CAB database, mainly for publication in *Forest Products Abstracts.*

A degree in forestry, wood science or a related subject is essential. Some preference will be given to applicants with a reading knowledge of French, German or Russian.

Salary in the scales £5909 to £8153, £7435 to £10039. Starting salary according to qualifications and experience.

Application form and further details can be obtained from:
Commonwealth Forestry Bureau
South Parks Road,
Oxford OX1 3RD
Tel: No. (0865) 57185

Closing date for applications. 15th June 1985.

(6540)A

WORLD SERVICES TO AGRICULTURE CAB

OVERSEAS DEVELOPMENT ADMINISTRATION
THE TROPICAL DEVELOPMENT AND RESEARCH INSTITUTE, LONDON

DIRECTOR

This post falls vacant in November 1985 on the retirement of the present Director, Dr Malcolm Thain.

The Tropical Development and Research Institute is part of the Overseas Development Administration and has a staff of some 400 which includes about 260 scientific and professional grades. It is financed through the British Overseas Aid Programme and has an important role in helping to increase food production in developing countries and in improving food security through post-harvest technology and pest and vector management. It is concerned with the post-harvest and pest control aspects of a range of other tropical products, and also carries out work for other development agencies such as the European Community and FAO.

The Institute, created in 1983 by the amalgamation of the former Tropical Products Institute and the Centre for Overseas Pest Research, is in a period of transition and one of the new Director's main tasks will be to give it firm leadership during this period. Most of the Institute's component parts will be brought together on a new location within reach of London and ODA Headquarters in 1988 and the Director will be required to supervise this move whilst maintaining the impetus of the Institute's scientific programme.
Candidates for the post should be of proven scientific standing, with experience in scientific administration and a knowledge and understanding of overseas development.

The post is Grade 4 in the Home Civil Service, with a present salary of £26,909, currently plus a London Allowance of £1300 a year. The appointment will be for a period of 3-5 years. There may be the possibility of subsequent conversion to a permanent basis. Assistance with relocation expenses may be available.

Applications, including a full CV and details of referees, should be sent to Miss L J Evans, Overseas Development Administration, Room 634, Eland House, Stag Place, London, SW1E 5DH. The closing date for applications will be 5 July; late applications from overseas will be considered if there is time.

ODA OVERSEAS DEVELOPMENT

An equal opportunity employer
(6581)A

Graduate Molecular Biologist

An energetic graduate is required to join a team of molecular biologists at I.C.I. Pharmaceuticals Division engaged in the cloning of both natural and synthetic genes for pharmacologically active proteins. A good first degree in biochemistry, microbiology or a related discipline is required along with some further research experience. Expertise in cloning DNA fragments into plasmid vectors in colony and blot hybridisation analysis and in nucleotide sequencing is required. Additional expertise in isolation of nucleic acids from mammalian cells and in cloning into phage vectors will be advantageous. The jobholder will use a range of techniques with considerable scope for their development and refinement towards the establishment of rapid and robust methods for application to a variety of target genes.

In return we can offer excellent technical and recreational facilities situated in pleasant parkland surroundings, about 15 miles south of Manchester. Our salaries are highly competitive and generous assistance with relocation is available if appropriate.

Applications quoting reference number ICP 932 and including a detailed c.v. should be addressed to:

ICI **Kim Griffiths, Assistant Personnel Officer, Imperial Chemical Industries PLC, Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire.** (6595)A

INSTITUTE OF CANCER RESEARCH

Applications are invited for the following positions based at Sutton, Surrey:—

(1) A THREE-YEAR POSTDOCTORAL FELLOWSHIP

in the areas of computerised molecular modelling and drug design, with special reference to drug-DNA and drug-protein interactions. There is an active and integrated programme of experimental (X-ray, Chemical and Biophysical) and theoretical studies currently under way in several of these areas. Salary in range £7,520 — £8,920 p.a. plus London Allowance of £1,233 p.a. Post available from 1st October, 1985. **Ref. 300/G/13.**

(2) A COMPUTER SPECIALIST

is required for three years to provide programming and systems support for a number of projects involving crystallography, molecular graphics and molecular mechanics of DNA-drug and DNA-protein interactions. The successful candidate will be required to assume day-to-day responsibility for the Unit's computing facilities, comprising VAX, PDP11 and graphics. A strong scientific and computational background in at least some of these areas is required, with **a PhD in crystallography**, molecular modelling or closely related areas. Salary in range £7,520 — £8,920 p.a., plus London Allowance of £1,233 p.a. Post available from 1st July, 1985. **Ref. 300/G/14.**

Applicants are advised that in the majority of the Institute's premises smoking is prohibited

Enquiries to Dr. S. Neidle (01-643 8901, ext. 268).

Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting the relevant reference. (6590)A

TRANSGENE

As a consequence of continued expansion, TRANSGENE, a leading genetic engineering company based in Strasbourg, FRANCE, has immediate openings for scientists in the following positions:

— MOLECULAR BIOLOGISTS

with research experience in the physiology and genetic manipulation of gram negative bacteria, gram positive bacteria or yeast; with a strong background in the latest cDNA and genomic cloning techniques, to work with a group involved in the expression of genes in heterologous systems.

— IMMUNOCHEMIST

interested in the assay, purification and final characterization of natural and cloned porteins using immunochemical techniques.

— PROTEIN BIOCHEMIST

with practical experience of conventional LC, electrophoretic and HPLC techniques for purification and characterization of natural and cloned products. Experience with carbohydrate analysis an asset.

— MICROBIOLOGIST (Gene Expression)

with an interest in practical problem solving at the molecular biology, fermentation interface.

— FERMENTATION SCIENTIST

to work on the laboratory scale fermentation development of recombinant microorganisms. Experience in computing and computer control of fermentation processes would be an advantage.

TRANSGENE is an independent recombinant DNA research and development company, backed by a group of major French industrial and financial organizations.

Interested candidates are invited to submit, in confidence, a full curriculum vitae, a list of publications and the names and addresses of three referees to:

Dr. J.P. LECOCQ
Scientific Director
11, rue de Molsheim
67000 STRASBOURG
FRANCE
Tel: (88) 22.24.90

(W1872)A

Genetics International (UK) Inc.

We are a rapidly expanding high Technology Health Care Company involved in the development and manufacture of innovative diagnostic systems. We are currently establishing our Quality Control function and we wish to fill the following positions.

Senior Quality Control Technician

Responsible for the control of high volume product and process together with the introduction of Q.C. techniques and methodologies. This person will also assist in the daily running of the Q.C. facility and should be educated to BSc or PhD level in Chemistry/Biochemistry or a related discipline. Previous experience of similar Q.C. work with chemical and/or immunodiagnostic systems (RIA, EIA/ELISA) in the Health Care industry advantageous.

Quality Control Technician — Materials

Responsible for the control of raw materials and components and required to organise and run the Manufacturing stock control/inventory systems for received, in-process and finished goods Qualified to HNC level and ideally with experience of similar areas of responsibility in a manufacturing environment

Quality Control Technicians — Process

Responsible for the analysis of critical materials and for the control of high volume in-process and finished goods using instrument techniques (HPhC, u.v., i.r.).

Educated to BSc level or equivalent in Chemistry/Biochemistry or a related discipline, previous experience of Chemical and/or immunodiagnostic systems (RIA, EIA/ELISA) in a Q.C./Manufacturing environment advantageous.

We are an equal opportunities employer and seek to recruit people for these posts who demonstrate a high degree of initiative, enthusiasm and flexibility with attention to detail. We offer a highly competitive salary and benefits package with real opportunity for career development. Opportunity also exists for participation in the Company Executive Stock Option Scheme.

If you wish to apply for any of these positions please send a letter of application with a full CV and 2 Referees, specifying which position you are interested in to:

Ms F. Minal,
Company Secretary,
G.I. (UK) Inc.,
11 Nuffield Way,
Ashville Trading Estate,
Abingdon,
Oxon. OX14 1RL.

(6608)A

THE ZOOLOGICAL SOCIETY OF LONDON

Institute of Zoology

POSTDOCTORAL RESEARCH ASSISTANT required to join collaborative project on the control of follicular development and ovulation in primates, based at the Institute of Zoology and the Institute of Obstetrics & Gynaecology, Hammersmith. Knowledge of reproductive endocrinology or biochemistry and experience in RIA and cell culture techniques essential

The appointment, MRC-funded and within the MRC/AFRC Comparative Physiology Research Group, is for three years and salary will be at appropriate point of the Society's salary scales (similar to University of London) according to age and experience

Please apply in writing with c.v. and addresses of two referees (enclosing s.a.e.) to the Establishment Officer, Zoological Society of London, Regent's Park, London, NW1 4RY, to arrive before June 15th 1985.

(6541)A

ICI JOINT LABORATORY UNIVERSITY OF LEICESTER RESEARCH TECHNICIAN

Applications are invited for a post of Research Technician (Grade 3) available in the Leicester/ICI Joint Laboratory.

The Joint Laboratory provides an excellent environment for fundamental research in molecular genetics and its staff interact fully with other groups in Biological and Medical Sciences at Leicester and within the sponsoring company. The funding of the laboratory is guaranteed until September 1988 and its extension is subject to periodic review.

The appointee will assist in project involving the application c molecular biological techniques t the study of breast cancer. Previou experience of recombinant DN and/or cell techniques is desirab but not essential.

The post will be for three years, at salary of £5m399 to £6,325 p annum.

Applications, including a cu riculum vitae and the name of tw referees, should be sent to Hele George, ICI Joint Laboratory, Un versity of Leicester, University Roa Leicester LE1 7RH. (6577)A

UNIVERSITY OF OXFORD
NEW BLOOD UNIVERSITY LECTURESHIP IN HIGH ENERGY ASTROPHYSICS

Applications are invited for a new blood University Lectureship in High Energy Astrophysics and related areas tenable from 1 October 1985. This lectureship may be held in conjuction with a fellowship only. It is hoped that a fellowship at another college may be available in due course if a man is appointed.

Further particulars may be obtained from Professor D.E. Blackwell, Department of Astrophysics, South Parks Road, Oxford OX1 3RQ (Tel: 0865-511336/7) to whom applications (eight typed copies or one from overseas applicants) should be sent by 15 June 1985. (6523)A

POSTDOCTROAL POSITION

available to study transmembrane signaling in *Dictyostelium*. Current work is focused on the role of cAMP-induced phosphorylation of surface cAMP receptors in sensory adapation Applicants should have experience in biochemistry, cell biology, or genetics.

Send curriculum vitae and names of three references to: Dr. Peter Devreotes, Department of Biological Chemistry, John Hopkins University, School of Medicine, Baltimore, Maryland 21205. (NW1766)A

A POSTDOCTORAL POSITION

is available immediately to study functional role of insulin in the brain at the cellular level with Drs. DW Clarke and MK Raizada. Experience in cell culture and biochemical techniques is desirable. Experience with immunocytochemistry would be helpful.

Contact: Dr. Mohan K. Raizada, Dept. of Physiology, Box J-274, Univ. of Florida, Gainesville, FL 32610 or telephone (904) 392-3791. (NW1782)A

Senior Pathologist

Internationally respected as a leading name in the field of ethical pharmaceuticals, Smith Kline & French operates intensive and innovative research programmes. This is an opportunity for a man or woman with ambition, initiative, flair and drive to make a significant contribution to our programmes.

We are looking for a person whose experience of the pathology of laboratory animals is coupled with academic ability and an interest in investigative pathology. A veterinary or medical graduate and/or an appropriate research background is desirable. The person should be eligible for MRC Path.

There is considerable scope, the position will be styled to match individual abilities and experience, and research in applied areas will be encouraged. It is a challenge which could interest both experienced pathologists wishing to expand their fields of involvement and also those with a background in experimental pathology and want to enhance their career potential by participating in an expanding department.

There is an attractive salary and benefits include a company car, free BUPA, bonus plan, contributory pension scheme, free life insurance and flexible working arrangements.

Please write for an application form or send a detailed c.v. outlining qualifications and experience, quoting Ref N/122/JW to Tom Forster, Personnel Manager, Smith Kline & French Research Limited, The Frythe, Welwyn, Herts.

SK&F

(6546)A

axon healthcare

Axon Healthcare will shortly have vacancies for the following:

IMMUNOHISTOPATHOLOGIST
HISTOLOGY ASSISTANT
IMMUNOCHEMIST
IMMUNOCHEMISTRY ASSISTANT
ASSAY ASSISTANT
TISSUE CULTURE SPECIALIST
TISSUE CULTURE ASSISTANT

Formal applications, accompanied by a curriculum vitae, should be addressed to Dr. S. J. Starkie, Axon Healthcare, Denny End, Waterbeach, Cambs. Further information and job descriptions may be obtained from Mrs S. Imms or Mrs B. Sennitt on (0223) 861224 or +44 223 861224. Previous applicants are encouraged to apply again. (6465)A

DIRECTOR
LABORATORY OF NEUROBIOLOGY
MEDICAL SCIENCES CAMPUS
UNIVERSITY OF PUERTO RICO

Dr José del Castillo is retiring from administrative responsibilities as Director of the Laboratory of Neurobiology of the Medical Sciences Campus of the University of Puerto Rico. Candidates for this position are therefore being solicited. The Laboratory was established in 1967 as an interdisciplinary, interdepartmental research facility with access to the rich and varied local fauna, both terrestial and marine. It has a distinguished history of productivity and of attracting visiting scientists from throughout the world. There are excellent opportunities for further growth. The Director reports directly to the Chancellor of the Medical Sciences Campus and will be offered a tenure track appointment in an appropriate academic department of the School of Medicine. **Candidates should send their Curriculum Vitae and the names of at least three references to: Search Committee, Laboratory of Neurobiology, 201 Boulevard del Valle, San Juan, Puerto Rico 00901.**

The Search Committee will begin evaluating applications on August 1, 1985. The University of Puerto Rico is an Equal Opportunity/ Affirmative Action Employer. (NW1776)A

ROCHE

The *Clinical Pharmacology* group of the **Department of Clinical Research** is responsible for investigation of the *pharmacodynamics* and *pharmacokinetics* of new drugs in humans.

To expand our team, we are looking for a

SENIOR CLINICAL PHARCOLOGIST

with expert training in clinical pharmacology (internal medicine, pharmacology), and an interest in working in research.

This interesting field of work includes the planning, conducting, and evaluation of clinical pharmacology studies of research compounds in phases I — IV.

Pre-requisites are a knowledge of the methods of assessing drug effects in man, and/or pharmacokinetics, as well as a good command of English and possibly German.

Interested applicants are requested to send complete application documentation to our Personnel Department, quoting reference Nature 74/85.

F. Hoffmann-La Roche & Co. Limited Company, CH — 4002 Basle

(W1869)A

ROCHE

UNIVERSITY OF MANCHESTER

DEPARTMENT OF BOTANY

TWO RESEARCH ASSISTANTS (IA)

Applications are invited for the ab posts in connection with an EEC gr to study (i) regulation of interno elongation and development, and submergence-tolerance of the pho synthetic apparatus, in deepwater ri This crop is particularly important such areas as Thailand a Bangladesh where flooding can ra th water level by 2-5m.

Post 1. (To work with Professor E Cutter and Dr W A Charlton)

Developmental botanist to work the mode of rapid elongation of internodes, its regulation, and the sponses of nodal regions to gravi Experience of light and electi microscopy, and/or hormonal tre ments and extractions, would be advantage.

Post 2. (To work with Dr M J Em

Plant physiologist to look at stability of the photosynthetic ap ratus, following leaf submergen Candidates should preferably h some experience in measuring l fluorenscence induction kinet and/or infra red gas analysis.

Both appointments will be on IA scale (range £7,520 — £8,920) will be for a period of 2 ye beginning July 1st, 1985 or as soor possible thereafter. Candida currently completing a PhD are couraged to apply.

Applications to include full CV the names and addresses of t referees should be sent to eit Professor E G Cutter or Dr. M Emes, Department of Botany, T University, Manchester M13 9PL, later than June 7th. (6572)A

THE DEPARTMENT OF LAND, AIR AND WATER RESOURCES, UNIVERSITY OF CALIFORNIA, DAVIS

announces a position in the area of Soil Science/Plant Nutrition. Tenure track (11 mos. plus 1 mo. paid vacation) position will be divided 30% teaching and 70% research. Appointment will be at the Assistant Professor level.

QUALIFICATIONS: A Ph.D. in a field related to soil or plant science with specialization in mineral nutrition, and strength in mathematics, physics and chemistry. The appointee will develop research on the root-soil interface, involving experimental study and quantitative modeling of chemical and biological processes associated with ion uptake and solute transport. Depending on the appointee's interests, the basic research might be slanted toward a particular application such as nutrient efficiency, stress tolerance, pollutant behavior, soil acidification, symbioses, or control of rhizosphere microbes. The appointee will take part with other faculty in teaching courses in soil science and plant nutrition, advising responsibilities and will develop a graduate course in rhizosphere chemistry and biology.

APPLICATIONS: Applicants should submit curriculum vitae, statements of interests and background in research and in teaching, official undergraduate and graduate transcripts, copies of publications and current manuscripts, and names and addresses of at least three references. Send materials to Dr. D. N. Munns, Chair, Plant Nutritionist Search Committee, Department of Land, Air and Water Resources, Hoagland Hall, University of California, Davis, California 95616, no later than June 13, 1985. Position becomes available July 1, 1985.

THE UNIVERSITY OF CALIFORNIA IS AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER. (NW1771)A

DEVELOPMENTAL BIOLOGIST

CORNELL UNIVERSITY MEDICAL COLLEGE

Applications are invited for a senior or junior tenure-track faculty position. Applicants should have at least two years of postdoctoral experience and maintain a research program in early development or morphogenesis, using genetic, recombinant DNA, biochemical, immunological, and/or cell biological tools to explore mechanisms of pattern specification and determination in a vertebrate or invertebrate system. The position includes supervision of graduate students and teaching in a related area.

Send curriculum vitae, research summary, and three letters of reference to: Dr. Rosemary Bachvarova, Developmental Search Committee, Department of Cell Biology and Anatomy, Cornell University Medical College, 1300 York Avenue, New York, NY10021. An Equal Opportunity/Affirmative Action Employer.

(NW1767)A

POSTIONS OPEN MOLECULAR BIOLOGIST

post-doctoral fellow in second or third year of training. With experience in DNA sequencing or transcription to apply toward the elucidation of the structure and function of fragile sites and their relation to specific cancer chromosomal rearrangements. Salary approximately $20,000 per year, commensurate with experience. **Please submit curriculum vitae, three references, and a brief description of present and future research interests to: Dr. Jorge J. Yunis, Dept. of Laboratory Medicine and Pathology, Box 198 Mayo Memorial Building, University of Minnesota Medical School, Minneapolis, Minn. 55455, U.S.A.** (NW1778)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions:

Research School of Earth Sciences

POSTDOCTORAL FELLOW/RESEARCH FELLOW/ SENIOR RESEARCH FELLOW

NOBLE GAS GEOCHEMISTRY

Applications are invited for an appointment in the School's Geochronology and Isotope Geochemistry Group, the field of interest being noble-gas geochemistry The appointee will work with Dr Ian McDougall, Senior Fellow, in developing research in this field, which is new to the School. The School wishes to make the appointment at the Research Fellow level, but will be prepared to consider applications at the level PDF or SRF

Noble gas investigations are being initiated to assist in elucidation of the geochemical evolution of the mantle, the mantle-crust system, and the atmosphere. Research initially will concentrate on mantle-derived rocks, with subsequent development of studies on fluids in the crustal environment Provision of a suitable high-resolution gas-source mass spectrometer is in hand, and construction of appropriate extraction lines is planned

Persons with suitable backgrounds in the physical sciences, and with particular interests in geochemistry, are encouraged to apply. Experience in noble gas studies on geological samples would be a great advantage.

The appointee will assist in developing the laboratory facilities, and will be expected to undertake discrete projects within this broad field of study. Active collaboration with other workers in isotope geochemistry is envisaged. Enquiries may be directed to Dr I McDougall (062 49 4136).

Closing date: **14 June 1985.** Ref. ES/1.5 1

Faculty of Science
DEPARTMENT OF ZOOLOGY
POSTDOCTORAL FELLOW

The successful applicant will work on a NH & MRC funded project involving gene cloning of sheep parasites particularly larval tapeworms and liver fluke Particular interest will be centred on those genes which code for antigens that are involved either in resistance to reinfection or immunopathological phenomina A principal objective will be to obtain expression of these antigens in prokaryotic systems. Experience in gene cloning techniques is essential and some knowledge of immunological procedures would be highly desirable. The successful applicant will work in the Centre for Recombinant DNA Research, Research School of Biological Sciences (Executive Director· Professor J A Pateman FRS). The position is funded for 3 years. Previous applicants need not reapply as all applications will be considered.

Further information is available from Dr M J Howell, (062) 49 3060 or (062) 49 4225

Closing date: **15 July 1985.** Ref: FS/1 5.1

CONDITIONS OF APPOINTMENT. Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow $A21,411-$24,528 pa; Research Fellow $A24,840-$32,634 pa; Senior Research Fellow $A34,699-$41,390 pa Current exchange rate is $A1 00 = Stg 0.48.

Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time **Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be sent to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. General Information also available from the Secretary General, Association of Commonwealth Universities (Appts) 36 Gordon Square, London WC1H 0PF.**

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W1880)A

MEDICAL RESEARCH COUNCIL

Clinical and Population Cytogenetics Unit Edingburgh

POSTDOCTORAL POSITION

Connective Tissue Development

A 2-year position is available for studying collagen-proteoglycan interactions in the morphogenesis of the avian corneal stroma The research involves connective tissue biochemistry and electron microscopy. **Further details from Dr J Bard, MRC Unit, Western General Hospital, Edinburgh EH4 2XU, UK.** (6550)A

COMMONWEALTH AGRICULTURAL BUREAUX

Applications are invited for the following posts

DIRECTOR

COMMONWEALTH INSTITUTE OF BIOLOGICAL CONTROL

The Commonwealth Institute of Biological Control, backed by the CAB Information and Identification Services, provides a world service in biological control, giving advice, disseminating information and undertaking research and development projects for sponsors. Although best known for the control of insects and weeds by introduced beneficial insects, its work on the use of microbial agents and the control of other pest organisms by biotic agents is expanding.

It is also collaborating in the development of integrated crop protection with emphasis on biological control. Its research programme is carried out, much of it from strategically situated field stations in Europe, tropical America, Africa and tropical Asia, controlled by a headquarters group at Imperial College, Silwood Park, Ascot, Berks

The appointed officer will be stationed at either Silwood Park or at CAB Headquarters and will be responsible to the Director Scientific Services.

Duties

The Director will promote the Institute's activities, actively seeking research contracts and developing a network of callaborators worldwide. He/She will be an authority on biological control of international standing who will provide scientific leadership and develop the Headquarters group as a centre of excellence. They will also have responsibility for developing the training activities and publications of the Institute. The latter include Biocontrol News and Information, books and data banks

Qualifications

Candidates should have a background of research in biological control with a good publication record, extensive knowledge of pest control in developing countries, experience in research administration and an ability communicate with people from diverse backgrounds.

Salary

The salary will be in the scale £20,013 — £22,283

DIRECTOR

COMMONWEALTH INSTITUTE OF ENTOMOLOGY

The Commonwealth Institute of Entomology is a constituent Institute of the Commonwealth Agricultural Bureaux located at the British Museum (Natural History), where a team of specialists are involved in insect indentification and taxonomic research, and at 56 Queen's Gate, South Kensington, London, which houses the publications office and library. After centralisation of the CAB Information Service, currently being planned, the Institute will operate mainly from the British Museum (Natural History)

The main functions of the Institute are.

(1) To provide an authoritative and efficient world service for the identification of insects and mites of economic imporance to agriculture (including forestry and other related disciplines) and make the information available through its publications and otherwise.
(2) To conduct research on applied taxonomy relevant to the identification services.
(3) To undertake training in taxonomy and identification.
(4) To provide information on the nomenclature, biology, distribution, host ranges and status of pest species and of their associated predator/parasitoid complexes

Duties

The Director will be a working taxonomist and will be responsible for day-to-day running of the Institute and for promoting its activities under the general direction of the Director Scientific Services. He/She will keep in close touch with entomological research organisations in the UK and overseas.

Qualifications

Candidates should have a higher degree in Entomology, a wide knowledge of applied entomology, knowledge of current entomological research and entomological taxonomy as well as experience in administration.

Salary

The salary will be in the scale £16,229 — £18,333 plus Inner London Weighting of £1,300 p.a.

Further particulars of these posts are available from:
Director of Administration Services
Commonwealth Agricultural Bureaux
Farnham House
Farnham Royal
SLOUGH SL2 3BN, UK.

to whom applications, enclosing a curriculum vitae and naming three referees, should be submitted *not later than June 10th, 1985.*

(6603)A

ICRISAT

International Crops Research Institute for Semi-Arid Tropics (ICRISAT)
needs

PLANT PATHOLOGIST
at Bulawayo, Zimbabwe (Southern Africa)

To work in a multidisciplinary team for sorghum and pearl millet improvement in nine Southern African Development Coordination Conference (SADCC) countries. The position will require: development of disease resistance; work with national sorghum and millet programs to develop their own effective capability to deal with disease problems on a continuing basis; and keep a constant vigilance, in a collaboration with national programs, for shifts in disease problems, particularly susceptibilities of promising breeding lines. Candidates must be able to furnish leadership for the establishment of productive research and training linkages with all SADCC countries and work closely with the regional, multidisciplinary team.

Qualifications. Ph.D. in Plant Pathology with sound knowledge and experience of disease resistance and field experimentation, and should be willing to travel frequently and conduct research in the sorghum and millet areas of southern Africa. Excellent command of English is required Tropical agricultural experience and a working knowledge of Portuguese would be an advantage

Salary for the position is at the principal staff level, according to qualifications and experience. The position is contractural with initial 2-year appointment and attractive fringe benefits The post is in Bulawayo, Zimbabwe, a modern city with pleasant surroundings, excellent educational facilities, good medical facilities, and frequent transportation service. Position is one of an eight-member team being assembled for sorghum and millet improvement.

Send resume and names and addresses of three referees within 30 days from the date of advertisement to the Director General, ICRISAT Patancheru, P.O. Andhra Pradesh 502 324, India.

(W1870)A

CORNELL UNIVERSITY
Department of Medicine (Div. of Immunology)
New York, N.Y.

POSTDOCTORAL RESEARCH POSITION
(3 yr Appointment) available July 1, 1985

This position is for a Ph.D., or an M.D. with research experience in immunobiology. The research involves human lymphocyte membrane receptors such as the T cell antigen receptor. The successful candidate will be expected to develop into an independent investigator. The position is not associated with any clinical or teaching responsibilities. The yearly salary will be approx. $20,000.
Send CV and letter of support to: David N. Posnett, M.D., Assistant Professor, Division of Allergy/Immunology, Department of Medicine, Cornwell University Medical Center, 1300 York Avenue, New York, NY 10021.
CORNELL UNIVERSITY IS AN EQUAL OPPORTUNITY, AFFIRMATIVE ACTION EMPLOYER. (NW1768)A

SUNDERLAND POLYTECHNIC
Department of Biology
RESEARCH ASSISTANT IN VIROLOGY
Salary: £5181 - £5910 (under review)

Applications are invited for the above 3 year post to study the virology and immunology of astroviruses and enteric adenoviruses.

Candidates should possess, or expect to obtain, a first or upper second class honours degree in microbiology or closely related discipline, and have some experience of virology and cell culture. The appointee will be registered with CNAA for a higher degree.

An application form and further particulars may be obtained from Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland, SR2 7EE or telephone (0783) 76231 Ext. 11.

Closing date: 14 June 1985. (6578)A

ΕΘΝΙΚΟ ΙΔΡΥΜΑ ΕΡΕΥΝΩΝ 1958

THE NATIONAL HELLENIC RESEARCH FOUNDATION

ANNOUNCEMENT

The Institute of Biological Research of the National Hellenic Research Foundation seeks personnel for the following positions:

- One senior scientist to head a group of Genetic engineering — Molecular Biology of eukaryotes-Microbial genetics
- Two research associates to participate in the above group
- One research associate specialized in Cell Biology-Cytogenetics
- One research associate specialized in fluorescence and NMR spectroscopy in biological systems
- One research associate specialized in enzyme technology-Biotechnology

All candidates should hold a PhD and be greek citizens.

Full c.v. with publications and addresses of at least two persons for references should be addressed to. Mrs. D. Stefanou, Secretary, Institute of Biological Research, NHRF, Vassileos Constantinou 48, Athens, Greece, not later than June 15. (W1849)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Wolfson Unit
MOLECULAR BIOLOGIST

An additional molecular biologist is required to join a growing research team working on the molecular biology of protozoan and viral agents of human disease. There will be an opportunity to develop personal research interests. Salary, on appropriate scales, and conditions of appointment are negotiable according to experience and age.

Candidates should send full curriculum vitae, naming three professional referees and describing research interests, to the Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. CLOSING DATE 10 June 1985. (6593)A

MEDICAL RESEARCH COUNCIL
BRAIN METABOLISM UNIT, EDINBURGH
CLINICAL SCIENTIST AT CONSULTANT LEVEL

Applications are invited for a CAREER CLINICAL SCIENTIST post with the Medical Research Council. The post is tenable at this Unit in the first instance. The successful applcant will be an appropriately qualified psychiatrist who has an outstanding backgrond in psychiatric research and has demonstrated the capacity od leading a major programme of clinical research with special emphasis on psychoneuroendocrinology and/or psychopharmacology. An honorary consultant contract will be sought on behalf of the successful applicant whose clinical duties will include responsibilities for the in-patient unit and routine outpatients and emergency duties

The clinical staff of the Unit include 3 other clinical scientists and 1 registrar with facilities on a self-contained Metabolic Ward of 9 beds with access to a further 8 beds in a general psychiatric area of the hospital. The Metabolic Ward is directly linked with the clinical offices and the clinical research suite of the Unit which are housed in a new building at the Royal Edinburgh Hospital The unit pursues an integrated programme of research in clinical and experimental neuroendocrinology, psychoneuroendocrinology, psychopharmacology and nuerophармacology, and has close links with the Departments of Pharmacology and Psychiatry in the University of Edinburgh.

The salary will be in the range £20,200 — £26,080 (Medical Research Council Consultant Grade).

Applications (6 copies), including a detailed curriculum vitae, list of publications and the names of 3 professional referees, should be lodged no later than June 7th, 1985 with the Director, Professor G. Fink, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ, from whom further details about the post may also be obtained. Applicants should include a statement, of about 1,000 words in length, explaining the research programme that they would wish to carry out if appointed. (6429)A

The Biomedical Research Centre
University of British Columbia
Vancouver

Scientific Director

This new research institute established jointly by The Wellcome Foundation Limited, one of the world's major pharmaceutical companies and the Terry Fox Medical Research Foundation, a Canadian charity, will be sited on the campus of the University of British Columbia, Vancouver. The primary focus of its work will be the role of biologically active proteins in the control of cell growth, division and differentiation, with special reference to their evaluation as potential medicinal substances.

The Centre will be closely associated with the University Health Sciences Centre Hospital providing the opportunity to extend the results of basic scientific research to practical application. There will also be access to a pilot fermentation plant and interaction with University programmes will be encouraged.

The Scientific Director will be accountable for the effective utilisation of this custom-built, 35,000 sq.ft. facility, in the fields of scientific research. Leading a team of up to 100 people, with start up funds of Canadian $23m over 5 years, he/she will be responsible for establishing the Centre as a unique venture of international standing.

Candidates must have a record of scientific achievement of a quality which will attract scientists of the highest calibre to the Centre. Experience in an area of cell or molecular biology is essential and a medical qualification would be an advantage. A proven ability to direct or manage in an entrepreneurial environment and a commitment to a practical approach to science will be equally important.

Salary and other benefits will be negotiable and commensurate with the key nature of the post.

The appointment will be made in accordance with Canadian immigration requirements and equal opportunities will be given to male and female applicants.

Applications or nominations are invited by 31st July 1985 and should be forwarded to The Terry Fox Medical Research Foundation, Suites No. 1130-1176, West Georgia Street, Vancouver, British Columbia V6E 4A2, Canada, from where further particulars can be obtained. (NW1784)A

RESEARCH CENTER DIRECTOR

Applications and nominations are invited for a Director, Center for Atmospheric and Biospheric Effects of Combustion, located in the Applied Science Division. The scope of the Center is interdisciplinary and includes four major areas: Combustion and Abatement, Atmospheric Pathways and Chemistry, Radiative Balance, and Ecosystem Pathways and Effects. The Director will coordinate the efforts of a number of scientists, including chemists, physicists, engineers, biologists, geologists, meteorologists, and energy analysts to carry out the objectives of the Center; will be responsible for obtaining research support from governmental and non-governmental organizations; will represent the Laboratory at various related national and international meetings; and will also carry out research within the Center. This position requires a Ph.D., and a record of distinguished leadership, outstanding research accomplishments and an international reputation in one or more of the above areas. Experience and background in program planning, development, implementation, administration and evaluation is required. The incumbent should also have experience in obtaining research funds and have a familiarity with organizations that provide these research funds. Salary will be negotiable. The successful candidate will be appointed a Senior Staff Scientist at the Lawrence Berkeley Laboratory.

Applications and/or nominations should be forwarded to: Lawrence Berkeley Laboratory, One Cyclotron Road, Employment Office 90-1042, Berkeley, CA 94720. Please specify Job A/3330. An Equal Opportunity Employer M/F/H. (NW1777)A

LAWRENCE BERKELEY LABORATORY

The SANDOZ Agrotoxicology Facility, Basel, Switzerland invites applications for a

SENIOR TOXICOLOGIST

The person appointed will, as head of the long-term toxicity group, be responsible to the Director of the Laboratory for the planning, supervision, evaluation and reporting of chronic toxicity studies with newly developed agricultural chemicals.

Applicants should hold a first degree in toxicology, medicine, or veterinary sciences. Broad experience in experimental toxicology is considered essential.

Applicants are requested to send a full c.v. including a list of publications as well as the names and addresses of two persons to whom reference may be made. Please write to:

SANDOZ

Sadoz Ltd., Personnel Department, Ref. 8546 CH-4002 Basel, Switzerland.

(W1868)A

Behavioural Pharmacologist

Roussel Laboratories Limited is part of the international Roussel Uclaf group with extensive interests in pharmaceuticals and associated health-care products. The Swindon research centre is an integral part of the international research organisation with a commitment to the development of new drugs in a number of therapeutic areas.

We seek a Behavioural Pharmacologist to join an established team expanding its research into new remedies for the treatment of central nervous system diseases. Candidates should have a PhD, preferably with one to two years' relevant post-doctoral experience.

Conditions of employment are excellent, including a competitive salary, free life, accident and medical insurance cover, contributory pension scheme and generous assistance with the cost of relocation to this attractive part of Wiltshire.

Further details are available on request, and applications should be made by sending a detailed cv to Rosemary Curtis, Assistant Personnel Manager, Roussel Laboratories Limited, Kingfisher Drive, Covingham, Swindon, Wiltshire SN3 5BZ. Telephone: 0793 24411. Closing date: 21 June 1985.

ROUSSEL

(6609)A

VIRGINIA INSTITUTE OF MARINE SCIENCE
FISHERIES CRUSTACEAN BIOLOGIST

Applications are invited for a tenure track faculty position in fisheries crustacean biology. The position is at the assistant or associate professor level in the Department of Fisheries Science, School of Marine Science (VIMS), College of William and Mary at Gloucester Point, Virginia. Candidates must have a Ph D. in marine ecology, oceanography, or fisheries science Experience is required in crustacean biology, statistics and marine field ecology

The incumbent will be expected to develop and supervise a research program on crustacean biology with initial emphasis on blue crab recruitment, abundance and distribution. The incumbent will also supervise an on-going 30-year monitoring program for collection of data on juvenile blue crab abundance and distribution. Additionally, the incumbent will develop an ecological research program on other crustaceans, including their role in Chesapeake Bay finfish food webs.

VIMS serves as the mandated scientific advisory institution to state and regional fisheries management agencies, and as such the incumbent would be called upon periodically to serve on committees or advise such agencies

The position, in the School of Marine Science, includes contributing to the academic program in the conduct of formal course offerings and direction of graduate student programs and research.

Prior to 1 July 1985, applicants should submit a vitae, statement of research interests, and the names and addresses of three references to: Herbert M. Austin, Chairman, Search Committee, VIMS, Gloucester Point, Virginia 23062.

An EEO/AA Employer. Minorities and women are encouraged to apply.
(NW1775)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF BIOCHEMISTRY AND UNIT OF MOLECULAR GENETICS

COORDINATED PROGRAMME IN PROTEIN ENGINEERING

The SERC Biotechnology Directorate and several industrial sponsors have recently established an initiative to stimulate research in protein engineering Bristol, which is one of the small number of centres chosen for this coordinated programme, will concentrate on the use of phosphoglycerate kinase as a test bed system for the analysis and exploitation of the factors affecting protein thermal stability.

Applications are invited for TWO posts, both of post-doctoral (Research Assistant 1A) status:

1 A Molecular Biologist, preferably with some cloning and/or M13 sequencing experience.

2. A Crystallographer, preferably with some experience of protein stucture determination and/or computer graphics.

Both positions are initially tenable for three years with starting salaries in the range of £7,520 — £8,920 p.a according to age and experience

Applications including curriculum vitae and the names of two referees should be sent to Dr L Hall (Molecular Biology post) or Dr H C Watson (Protein Crystallography post), at the Department of Biochemistry, University of Bristol, Medical School, Bristol, UK, BS8 1TD. To arrive not later than June 24th 1985 (6558)A

UNIVERSITY OF SOUTHAMTPON

Human Nutrition

NEW BLOOD LECTURESHIP

Applications are invited for a New Blood post in the Human Nutrition group of the School of Biochemical and Physiological Sciences.

The successful candidate will be expected to develop research in collaboration with the Chemical Pathology and Human Metabolism group and Child Health group on the metabolism of free radicals, trace elements and other nutrients with particular reference to normal and premature infants. Applicants, who should be under 35 years of age, should preferably have research experience in the field of free radical metabolism, but candidates with research experience in trace element metabolism are also encouraged to apply.

Salary scale: £7,520 × £470 to £14,925 per annum. The initial salary will depend on qualifications and experience.

Further particulars may be obtained from Mr A J Small, Staffing Department, Southampton University, Southampton SO9 5NH to whom applications (7 copies from UK applicants) giving brief curriculum vitae and the names, addresses and telephone numbers of three referees should be sent not later than 14th June 1985. Please quote ref no AJS/85/31 (6526)A

THE UNIVERSITY OF LEEDS

(Leeds General Infirmary

DEPARTMENT OF IMMUNOLO

LECTURER/SENIOR LECTURER

Applications are invited from r stered medical practitioners, pre ably holding a higher degree, for above post, available immediate

Applicants should have MRCP in either Immunology Histopathology. Duties of the p include participation in the dep mental routine diagnostic serv teaching and research. Preference be given to candidates with an inte in the immunohistology of immu logical processes in disease, and demonstrable ability to teach at levels. Application will be made to Leeds Western Health Authority an honorary contract at Consul level.

Salary on the clinical scale Lecturers/Senior Lecturers Readers with honorary consul contract (£20200 — £26080) (un review) according to qualificati and experience.

Informal enquiries may be mad Professor G Gowland, Departmen Immunology, The General Infirm Leeds LS1 3EX (Tel 0532 432799 3540)

Application forms and further ticulars may be obtained from Registrar, The University, Leeds 9JT quoting reference no. 9 Closing date for applications 15 J 1985

(6434)

ST MARY'S HOSPITAL MEDICAL SCHOOL

(University of London)
Norfolk Place,
London W2 1PG

DEPARTMENT OF BIOCHEMIST

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for above post to work on a MRC fun project on the microdissection microcloning of the mouse X-chr osome. The project involves the struction of detailed molecular m of individual regions of the m X-chromosome for the isolation characterisation of interes genetic loci, in particular (X-linked muscular dystrophy). applicant should have experienc recombinant DNA technology.

The post is available immedia Salary is on the 1A scale (star point, £7,520 plus £1,233 pa Lon Allowance) for research staff.

Enquiries to Dr S D M Brow (01) 723-1252 ext 333. Applicat including full cv and names addresses of 2 referees to Assis Secretary (Personnel) at ab address by 31 May 1985. (6534)

MONASH UNIVERSITY
Melbourne, Australia

FOUNDATION CHAIR OF FORENSIC MEDICINE

Applications are invited for appointment to the Foundation Chair of Forensic Medicine. By agreement between the Attorney-General of the State of Victoria and Monash University the Professor will also hold the position of Director of the newly-established Victorian Institute of Forensic Pathology. It is expected that the appointee will take up duty as soon as practicable in 1986 so that the appointee can advise the Council of the Institute on the appointment of professional and technical staff and on further planning of the Institute which it is envisaged will be completed for occupancy in 1987.

Applicants should be medical graduates with qualifications registrable in Victoria and should hold specialist qualifications in Pathology. Applicants should also have extensive experience in the area of Forensic Pathology and be able to provide leadership and direction in the service activities of the Institute and in the teaching and research activities of the University.

Salary: $A64,773 per annum which includes a clinical loading of $A9,825 per annum. Superannuation, travel and removal allowance, and temporary housing assistance.

Information on application procedure and further particulars may be obtained from the Registrar, Monash University, Clayton, Victoria 3168, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF

Applications should reach the Registrar not later than 31 July 1985. Council reserves the right to make no appointment or to appoint by invitation at any stage.

An equal opportunity employer.
(W1885)A

INSTITUTE OF CANCER RESEARCH
TECHNICIAN

A vacancy exists in the Institute's Radiotherapy Research Unit at Sutton, Surrey for a Technician to take part in a research project on experimental tumour therapy. The work will involve in vivo and in vitro studies on human tumour xenografts and cell lines, using both radiotheraphy and chemotheraphy. The post would be suitable for graduates in biological sciences or biochemistry, experience in tissue culture would be an advantage Starting salary in range £5,738 — £7,417 pa, plus London Allowance of £623 pa, dependant on age and experience.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting Ref 01/B/88 (6571)A

Environmental Chemist

Negotiable salary + car

The Burmah Group is a major British industrial enterprise with a turnover of £1.7 billion. Our wide-ranging activities include oil exploration and production, the marketing of lubricants and fuels, speciality chemicals, shipping, and an investment division.

Reporting to the Group Environmental Officer, you will be responsible for obtaining and analysing occupational hygiene samples to ensure employees are not exposed to chemical harm. In addition to operating a monitoring programme and developing new analytical methods, you will set up and run a computerised archiving system.

You should have a good chemistry degree and some years' industrial experience. Self-motivated with the ability to tackle the unknown, you must be an able communicator at all levels. A willingness to travel extensively to Burmah sites spread throughout the UK is essential. An understanding of microcomputers and programming ability would be an advantage.

A competitive salary is accompanied by attractive large-company benefits including a car.

Please send a full cv, or ring or write for an application form to Mr R J Doncaster, Recruitment Officer, Burmah Oil Trading Limited, Burmah House, Pipers Way, Swindon, Wiltshire SN3 1RE.

(6610)A

Burmah

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDLESEX HA1 3UJ

TECHNICIAN

A vacancy exists within our Divison of Immunochemical Genetics for a Technician.

The work of the division is concerned with the molecular basis and genetic regulation of carbohydrate antigens and exemplified by certain of the human blood group antigens.

The successful applicant will be required to assist in the production of monoclonal antibodies, genetic complementation, and gene clonings experiments. Previous experience in any of these areas would be an advantage, but a good grounding in tissue culture techniques is essential.

Applicants should have a degree, HNC or equivalent in a Biological subject. Salary will be on a scale £6,361 — £8,719 inclusive of London weighting, depending on qualifications and experience.

Applicants should apply to Miss B A Shaw, Personnel Section, quoting reference: 131/2/4290. Closing date: 14th June 1985.
(6606)A

MOLECULAR BIOLOGIST/ BIOCHEMIST:

Applications are invited from post doctoral scientists to join a multidisciplinary team investigating the mechanism of carcinogenesis in mouse and human epithelial cells. Main research interests are oncogene transfection of keratinocytes, alterations in cell genotype/phenotype and regulation of epidermal differentiation by mesenchymal factors at different stages of malignant transformation. The candidate must hold a PhD in Biochemistry, Molecular Biology or Molecular Genetics and have a strong interest in Cell Biology and Molecular Genetics. Experience with recombinant DNA techniques, cellular transfection and in situ hybridisation would be advantageous. Excellent research facilities are provided and the position is available from July 1985 for 1½ years in the first instance with a possibility of further prolongation. The salary will be in the range 30,000 — 50,000 DM per annum depending on age and experience.

Applications including curriculum vitae, research experience and interests, list of publications and the names of two referees should be sent to German Cancer Research Centre (DKFZ), Personalabteilung, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG.

For further information ring Prof. N.E. Fusenig: country code and 06211-484507. (W1874)A

Assistant Agronomist
Sudan

An Assistant Agronomist is required to supervise all crop research activities in the Yei district of Southern Sudan, together with conducting surveys on the agronomic problems and constraints in agricultural production with a view to establishing an adaptive on-farm research programme.

Responsibilities will also include establishing crop storage experiments, participating in the technical agronomy programme together with the supervision of sub-stations and assisting in the analysis of data.

Applicants should be British Citizens with a post graduate degree or diploma in Tropical Agronomy, with three to seven years experience of handling grain legumes, preferably in a humid tropics environment. Experience in on-farm trials with smallholder farms is desirable.

The appointment is on contract to ODA on loan to the Government of Sudan for a period of two years. Salary (UK taxable) is in the range £13,650 to £17,150 pa, which includes an element in lieu of superannuation. A variable tax-free Foreign Service Allowance, currently in the range £4,520 to £9,085 pa, is also payable.

The post is wholly financed by the British Government under Britain's programme of Aid to the developing countries. Other benefits normally include paid leave, free family passages, children's education allowances, free accommodation and medical attention.

For full details and application form, please apply, quoting ref. AH309/AF/N, giving full details of age, qualifications and experience to: Appointments Officer, Overseas Development Administration, Room 351, Abercrombie House, Eaglesham Road, EAST KILBRIDE, Glasgow G75 8EA. (6556)A

ODA OVERSEAS DEVELOPMENT
Britain helping nations to help themselves

CROP PHYSIOLOGIST POSITION

The Department of Agronomy and Range Science invites applications for an Assistant or Associate Professor/Assistant or Associate Agronomist position at the University of California Agricultural Experiment Station, Davis, CA. Research interests should focus on whole plant physiology processes that relate to agronomic crop performance in California. The candidate will be expected to develop an active research program which complements existing multidisciplinary efforts. Teaching duties will include a graduate course in crop physiology/ecology and participation in graduate seminars on a rotating basis. Advising duties will be in an appropriate discipline. A Ph.D. in plant physiology or related field is required. The level of appointment will be commensurate with the candidates experience. Recent graduates are especially encouraged to apply. **Send a statement of research interests, curriculum vitae, transcripts and the names and addresses of *three* references by August 15, 1985 to: R.L. Travis, Search Committee Chairman, Department of Agronomy and Range Science, University of California, Davis, CA 95616. Phone: (916) 752-6187 or (916) 752-1703.**

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW1780)A

UNIVERSITY OF BIRMINGHAM
Faculty of Medicine and Dentistry
DEPARTMENT OF PHYSIOLOGY

Applications invited to work on the mechanisms of insecticide resistance by studying nerve insensitivity to pyrethroids and DDT in genetically designed strains of house-fly. The work will be carried out under the supervision of Dr M P Osborne in collaboration with Rothamstead Experimental Field Station.

The post is for three years from 1.10.85, financed by the Agricultural and Food Research Council. Salary Grade IB £6,600 - £7,520 with superannuation.

It is expected that the appointed person will have a Good Honours Degree in some aspects of Biological Sciences with a special interest in Neurophysiology and/or Entomology. Suitably qualified candidates will have the opportunity to register for a PhD.

Enquiries to Dr M P Osborne, Department of Physiology, Medical School, University of Birmingham B15 2TJ. Telephone 021-472 1301, ext 3350.

Applications (three copies) to Assistant Registrar, Medical School, University of Birmingham B15 2TJ, by 10.6.85. Quote reference RA/Phy/MO. (6580)A

UNIVERSITY OF GLASGOW
DEPARTMENT OF PATHOLOGY
WESTERN INFIRMARY

A PhD graduate with 3 years post-doctoral research experience in protein chemistry is required for a three year Arthritis and Rheumatism Council project grant in the University of Glasgow. The aim of the project is to isolate and characterise a plasma protein which interferes with the complement-mediated solubilisation of antigen-antibody complexes. Salary starts at point 4 on the University scale 1A. Applications with CV and names and addresses of two referees to Professor K Whaley, Department of Pathology, Western Infirmary, Glasgow G11 6NT by June 14th 1985. (6597)A

UNIVERSITY OF CAMBRIDGE
COLLEEN MACLEOD PROFESSORSHIP OF ANIMAL WELFARE

Applications are invited for this newly established Chair.

Present pensionable stipend £20,995.

Applications (10 copies) marked 'Confidential' should be sent to the Secretary General of the Faculties, from whom further information may be obtained, at the General Board Office, The Old Schools, Cambridge, CB2 1TT. Names of two referees may be submitted is desired.

Closing date 7th June 1985. (6557)A

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF METALLURG AND MATERIALS SCIENCE
RESEARCH ON DIFFUSIC IN THIN OXIDE FILMS

A postdoctoral appointment for th years is available for study of dif sion processes in the formation of t oxide films. The technique which volves the use of radon atoms Kirkendall markers, has been appl to anodic oxide films and recent i provements have shown that it is s ficiently sensitive and precise for it be applied to the passive films, tarn films and other low temperature, th mally activated film growth proces

The investigations are relevant i only to oxidation and corrosion p cesses but to the generation of ox films for integrated solid state el tronic devices. The technique is available elsewhere in Europe a funds are available for the impro ment of the present equipment. T programme includes an element collaboration with research work in Paris who are using complement techniques for the study of simi phenomena.

Applications are invited fr suitably qualified people. Please w or telephone Professor J S L Lea Department of Metallurgy a Materials Science, The University Nottingham, University Park, N tingham, NG7 2RD. Tel 0602 506 ext 2116. (657

UNIVERSITY OF SOUTHAMPTON
CHAIR OF OCEANOGRAPHY

Applications are invited for the Ch of Oceanography which will beco vacant on the retirement of Profes H Charnock, FRS, on 30 Septem 1986. Applicants should ha research interests in the physical related marine sciences and ability to foster work in a mu disciplinary field.

Further details may be obtair from the Secretary and Registr The University, Southampton, S 5NH, to whom applications copies from persons in the U should be sent before 12 July 198 (6524)A

IMPERIAL COLLEGE
London SW7 2AY
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a po doctoral research assistant for newly created Imperial Coll colloid and interface group to w with Drs M H George and J A Bar on the immobilisation of enzymes catalysis in copolymer micell Previous experience in orga synthesis and/or polymer chemis would be desirable.

Applications should be sent soon as possible to Dr M H Geor Department of Chemistry, Imper College, London SW7 2AY. (6594)A

ASSISTANT PROFESSOR, BIOCHEMISTRY

Applications are invited for one 12-month, tenure-track position which will begin on or before 1 January 1986 Preference will be given to individuals with chemical and biochemcal expertise and research interests in the structure-function relationships of biological macromolecules. Despite these preferences, application from individuals having other research interests and expertise will be considered. Applicants, who should have a Ph.D and postdoctoral experience in biochemistry or a related field, will be expected to develop an active, externally funded research program, and to participate in the undergraduate and graduate teaching programs of the department Normal teaching assignments are a one semester lecture or laboratory course per academic year. Applicants should include curriculum vitae, copies of publications, graduate and undergraduate transcripts and a brief statement of current, and future research interests. Three people, well acquainted with the applicants's research and teaching ability, should be asked to send letters of reference. Review of applications will begin in June 1985; interviews will be scheduled in late summer or early fall 1985. Inquiries and applications should be directed to. **Roger E. Koeppe, Biochemistry Department, 454 PSII, OKLAHOMA STATE UNIVERSITY, Stillwater, Okla. 74078.** *Equal Opportunity/Affirmative Action Employer.* (NW1769)A

Scientists for Research into Arthritis

The Boots Company PLC has, at its Nottingham Headquarters, a well-established research group concentrating on the identification and development of more effective drugs to treat arthritic diseases.

Continuing growth in this major research field has created the need for two more Research Scientists to join our team. There is considerable challenge — playing a key role in the development and implementation of novel experimental models which simulate chronic inflammation in humans.

You should have a Degree in biochemistry, cell biology or pharmacology with a relevant PhD or three years research experience. You will find wide scope for your initiative in these rewarding posts, with excellent opportunities for career growth.

We are offering a range of benefits expected of a large Company, including profit sharing, contributory pension and staff purchase schemes. Generous relocation assistance will be available where applicable. (6601)A

Please write, with full career details, to: T. W. Flower, Employment Manager (Technical), The Boots Company PLC, Station Street, Nottingham NG2 3AA. Telephone (0602) 506255. Ext. 243.

The Boots Company PLC

THE DEPARTMENT OF PEDIATRICS, University of California, San Diego, Division of Pediatric Immunology and Allergy, has an opening for a **FACULTY MEMBER** with a special interest in clinical immunology/allergy, with research experience in neonatal immunology and cellular aspects of allergic disease. Preferred experience in Pediatric Intensive Care Unit. This position will be at the level of Assistant Professor. Position includes clinical supervision and teaching of housestaff and medical students. Applicants should be board certificate or board eligible in pediatrics, experience in teaching and clinical service; with research background. Salary commensurate with skills, knowledge, and University of California policy. Candidates apply by June 1, 1985 with C.V. to Robert N. Hamburger, M.D., Professor of Pediatrics, Department of Pediatrics, UCSD, M-009-D, La Jolla, CA 92093. EEO/AA. (NW1783)A

DEPARTMENT OF BIOLOGY YORK UNIVERSITY

Applictions are invited for a tenure track position as Assistant Professor in Population Biology (Animals or Plants) or Behavioural Ecology. Applicants must have demonstrated a strong potential in some area of ecological research. In accordance with Canadian Immigration Requirements, this advertisement is directed to Canadian citizens or permanent residents. **Applications, including a curriculum vitae, recent reprints and names of at least three referees should be sent to Professor A.S.M. Saleuddin, Chairman, Department of Biology, York University, 4700 Keele Street, 246 Farquharson Building, North York, Ontario M3J 1P3. Closing date is December 31, 1985 or when the position is filled.** (NW1773)A

UNIVERSITY OF WISCONSIN-MADISON AQUATIC BOTANIST

RESEARCH ASSOCIATE (postdoctoral) position available by 1 June, 1985, for laboratory and lake studies on integrated control of Eurasian watermilfoil and impact of control strategies on water quality. Additional experience in water chemistry, limnology, or mycology desirable. Funding for two years. Salary competitive and commensurate with qualifications

Send résumé, including the names of three referees, to: Dr. John H. Andrews, Department of Plant Pathology, 1630 Linden Drive, Madison, Wisconsin 53706.

An affirmative action-equal opportunity employer (NW1781)A

RESEARCH POSITION IN MOLECULAR/CELL BIOLOGY

Post-doctoral, Assistant or Associate Research Professor level position are available starting immediately in the Department of Cell Biology. The person/s appointed will be member of a research group investigating the role of oncogenes, their possible interactions with carcinogens in normal and HBV-infected hepatocyte transformation in culture or *in vivo*

Applicants should have experience or knowledge in the following areas: general cell culture techniques; recombinant DNA techniques; virology

Salary is negotiable and will be based upon the candidate's previous training and experience (BAT IIA — BAT IA). Permanent positions will be made available to persons with the appropriate background.

Send CV and names of 2 referees before 13 June 1985 to: Prof. Dr. Dieter Paul, Institut of Toxicology, University Hamburg Medical School, Grindelallee 117, 2000 Hamburg 13 Federal Republic of Germany. (W1871)A

FhG

DIRECTOR OF FOOD RESEARCH

£26,909 (Under Review)

Applications are invited for the post of Director of Food Research. The person appointed will be directly responsible to the Secretary to the AFRC for the control, development and management of research at the Council's Food Research Institute at Bristol, Norwich and Reading The Director will be required to contribute more widely to the continuing development of a food research strategy for the Agricultural and Food Research Service.

The post will be based initially at one of the Institute sites where the Director will be supported by a small administrative team

Candidates should be highly qualified in food science or the related underpinning sciences, with a distinguished record in research, a knowledge of the food industry and considerable experience, at a senior level, in the management of research. Non-contributory pension scheme. The successful applicant may qualify for assistance with removal expenses

The AFRC is an Equal Opportunity Employer.

Further particulars and application forms can be obtained from John Pettinger on 01-580 6655 (Ext 244). Completed applications should be returned by 21 June 1985 to Mr J M Y Dickens, Chief Personnel Officer, AFRC, 160 Great Portland Street, London W1N 6DT.

(6537)A

MRC IMMUNOCHEMISTRY UNIT
Associated with DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

MOLECULAR IMMUNOLOGIST

Applications are invited for a short-term non-clinical scientific post at postdoctoral level tenable for 3 or 5 years in this MRC Unit.

The project to be investigated would be subject to discussion with the successful applicant, but would be concerned with the application of protein chemistry and recombinant DNA techniques within the general field of effector mechanisms in immunity and autoimmunity.

The appointment will be from 1 January, 1986, or later by arrangement.

Remuneration will be at an appropriate point on the scale for University non-clinical academic staff. **Further information may be obtained from Professor R. R. Porter, F.R.S., Department of Biochemistry, South Parks Road, Oxford OX1 3QU (Telephone: 0865-56733), to whom applications should be sent by 5 July. Ref: BC/129.** (6576)A

PLANT GENE TECHNOLOGY and DNA RESEARCH

POSTDOCTORAL POSITION

Candidates with a background in plant physiology and/or DNA research are invited to join an industry-funded independent research group at the Institute of Molecular Biology of the Austrian Academy of Sciences in Salzburg.

We offer a salary and benefits commensurate with experience according to Austrian Academy of Sciences scheme. The initial appointment will be for one year with possible extention up to four years. Resume, list of publications and three letters of reference should be submitted to CHEMIE LINZ AG, Personalwirtschaft, POB 296, A 4021 Linz, Austria.

CHEMIE LINZ AG

(W1878)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals, Houndmills, Basingstoke, Hants. RG21 2XS, UK

or

Paul Siman, Bio/Technology, 65 Bleecker St., New York, NY 10012.

DURHAM UNIVERSITY

DEPARTMENT OF MATHEMATICAL SCIENCES

Applications are invited for the post of

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

in Elementary Particle Theory tenable for two years from 1 August 1985 or later by arrangement under the direction of Drs E F Corrigan and D B Fairlie for investigations in the Structure of Gauge Theories. There is scope for working on a variety of different topics.

Initial salary in the range £7,520 - £8,920 per annum plus superannuation.

Applications (3 copies) naming 3 referees should be sent by 7 June 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may be obtained. (6589)A

THE SCHOOL OF PHARMACY

University of London

'NEW BLOOD' LECTURESHIP TOXICOLOGY/ NEUROTOXICOLOGY

Candidates with a strong biochemical, molecular biological or pharmacological background are invited to apply. Applicants must be willing to apply their expertise to a study of mechanisms of toxicity/neurotoxicity.

Further particulars from Dr G M Cohen 01 837 7651.

Salary in range £7,520 — £14,925 pa + LA £1,233 pa.

Applications including 2 copies of CV and names of three referees to be sent by 28 June to Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX. (6575)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF ORGANIC CHEMISTRY

TWO POSTS OF LECTURE

Applications are invited fro suitably qualified candidates for t above posts. They are both availat from 1 October 1985 and one is fo fixed-term of three years.

Salary on the scale for Lecture (£7,520 - £14,925) (under revie according to age, qualifications a experience.

Application forms and further pa ticulars may be obtained from t Registrar, The University, Leeds L 9JT quoting reference no 45/3 Applicants from overseas may apr in the first instance by cable with t names of three referees preferat living in the UK. Closing date f applications 21 June 1985. (6584)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

ACADEMIC DEPARTMENT OF MEDICINE

TECHNICIAN

Required to work with a gro studying the development physiology of the liver and relationship to inborn errors metabolism. Appointment for thi years in the first instance.

Salary on Whitley Council Sca according to qualifications a experience.

Further details and applicati form available from the School Off RFHSM, Rowland Hill Stre London, NW3 2PF (794-0500 4262). Please quote ref NM/T. (6551)A

Nous sommes l'un des tout premiers groupes pharmaceutiques mondiaux et nos activités en France sont en forte croissance

Pour renforcer le potentiel technique et de créativité de notre Centre de Recherche situé à RENNES nous créons de nouveaux postes

Pharmacologue Cardiovasculaire

Au sein du département **Biologie** et en synergie avec les autres chercheurs et techniciens du centre :

- vous concevrez et mettrez au point les modèles " Physiopathologiques " et " thérapeutiques ".
- vous réaliserez et suivrez les études et travaux sur le métabolisme myocardique.

Vous possédez une formation universitaire en Pharmacie ou Sciences (Physio) et un doctorat en Sciences Biologiques et une expérience d'au moins 3 ans en pharmacologie dans le domaine cardiovasculaire.

Chimiste Organicien

Au sein du département **Chimie** et en synergie avec les autres chercheurs et techniciens du centre :

- vous concevrez et réaliserez la synthèse de nouvelles molécules thérapeutiques dans le domaine cardiovasculaire
- au vu des résultats pharmacologiques, vous optimiserez les structures explorées.

Vous possédez une formation de type ENSC (option chimie organique) et un doctorat (chimie organique et / ou thérapeutique) et une expérience d'au moins 3 ans en synthèse (chimie thérapeutique) dans l'industrie pharmaceutique ainsi que des connaissances en méthodes de " Drug Design ".

L'environnement international du groupe vous permettra d'évoluer et implique la connaissance de l'anglais.

Merci d'adresser votre candidature sous réf. 770 M en indiquant le poste souhaité à J.M. REMY- C.R.E.S.
4, rue du Chesnay - Beauregard BP 45 35760 SAINT-GREGOIRE (W1888)A

Beecham pharmaceutique

laboratoires SOBIO

UNIVERSITY OF DURHAM
INDUSTRIAL RESEARCH LABORATORIES

Applications are invited for the following one years post available from dates to be arranged.

SENIOR EXPERIMENTAL OFFICER

To work on the synthesis and characterisation of polymers. Applicants should have a strong background in synthetic polymer chemistry and have experience with standard physicochemical techniques used for polymer characterisation.

SENIOR RESEARCH ASSISTANT

Postdoctoral Senior Research Assistant in polymer synthesis and characterisation for work on the synthesis of co-polymers for use in the manufacture of toner particles. Applicants should have experience in synthetic organic chemistry and familiarity with polymer synthesis is desirable but not essential.

The projects will be undertaken in collaboration with a leading international company. Salaries in the range (under review) £7,520 – £12,150 with superannuation.

Applications (three copies) naming three referees should be sent by 15 June 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars are available. (6568)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY

MICROBIOLOGICAL CORROSION

Applications are invited for two posts to carry out a research programme into the mechanisms and importance of microbial corrosion in seabed sediments.

The two posts are:

1 POST DOCTORAL RESEARCH FELLOW

with a PhD or equivalent industrial experience in corrosion science, preferably with experience of cathodic protection and corrosion monitoring.

2 RESEARCH ASSISTANT

with a degree, or equivalent experience, in microbiology or a related subject; experience in microbial corrosion would be an advantage.

Appointment will be made with immediate effect and will be for two years.

Salaries within range £6,600 – £9,860 per annum on the Range IA or IB scales for Research and Analogous Staff with placement according to experience.

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 14 June 1985. (6519)A

MOLECULAR NEUROBIOLOGY IN SWITZERLAND

POST DOCTORAL POSITION

The Molecular Biology Department of Hoffmann-La Roche Inc., Basle Switzerland has an opening for a post-doctoral fellow to perform basic research on the genetic basis and regulation of:

NEUROTRANSMITTER RECEPTORS AND ION CHANNELS

The department (Director Dr. H. Bujard) offers an excellent research environment and the opportunity for interaction with many research laboratories in molecular biology, neurobiology, electrophysiology and pharmacology. The position is available from July 1985 for 1-2 years. Costs of moving to and from Basle will be covered. Salary conforms with the Swiss National Science Foundation standards.

Applicants should submit a curriculum vitae and a statement of research activities to Dr. H. Mohler, Prof. of Biochemistry, Research Department, Building 70, Hoffman-La Roche, 4002 Basle, Switzerland. Phone 061/27/6202. (W1879)A

Faculty Position in Neurophysiology

Brain Research Institute, Zürich

The Brain Research Institute, University of Zürich, invites application to a tenured faculty position at the full professor level (Extraordinariat). Preference will be given to candidates with a strong research record in vertebrate system physiology at the single neuron level. The candidate is expected to interact with other neuroscience research groups at the Medical Faculty, particularly those studying neurotransmitters and developmental biology. Some teaching in neurophysiology courses for medical students is required. The candidate should acquire proficiency to teach in German within one year.

Send curriculum vitae, including a synopsis of past research experience, and a statement of research objectives to the Chairman of the Search Committee, Dr. V. Henn, Neurological Clinic, University Hospital, 8091 Zürich, Switzerland. Deadline August 15th. (W1886)A

N.S.W. STATE CANCER COUNCIL
SENIOR RESEARCH SCIENTIST

Applications are invited for the position of Senior Research Scientist within the Carcinogenesis Research Facility, N.S.W. State Cancer Council in the school of pathology, University of N.S.W. the Carcinogenesis Research Facility is a newly founded research laboratory intending to study cellular and molecular aspects of chemical carcinogenesis.

Scientists with strong credentials for, and commitment to, developing and independent research programme in the molecular biology or biochemistry of chemical carcinogenesis are invited to apply. Candidates interested in combining genetic and molecular approaches to the study of neoplastic cells, in gene expression/regulation as it relates to carcinogensis, or in macromolecular changes induced by chemical carcinogens will be given preferential consideration. Applicants must have appropriate qualifications, a record of research publications and experience in establishing an independent research programme.

The post is available immediately and has an annual salary within the range $29,826 to $34,779 p.a. The appointment is for 5 years in the first instance. Consideration will be given to assistance with costs involved in relocation to Sydney.

Interested applicants should submit a full curriculum vitae, list of publications, brief statement of research interests and the names of 3 references to:-

Dr. Gordon C. Hard
Head, Carcinogenesis Research Facility
N.S.W. State Cancer Council
School of Pathology
University of NSW
PO Box 1
Kensington NSW 2033

Applications close on 30th June, 1985. (W1887)A

LUDWIG INSTITUTE FOR CANCER RESEARCH (LONDON BRANCH)

Haddow Laboratories,
Clifton Avenue,
Sutton, Surrey SM2 5PX

THREE YEAR APPOINTMENT
RESEARCH ASSISTANT

is required to work in a group studying the processes of growth, differentiation and neoplastic cell formation in cultured mammary gland epithelial cells. The successful candidate will assist in the first instance in determining the alterations in growth control that allow benign mammary epithelial cells to become malignant. Previous experience with sterile techniques in tissue culture and/or microbiology are essential and prior knowledge of protein purification and/or molecular transfection techniques would be advantageous. Salary will be in the range of £5,738 — £8,492 pa plus £623 pa London Weighting, depending on previous relevant experience.

Applications with details of career and two referees should be sent to the Administrator, at the above address no later than 31st May, 1985. (6533)A

UNIVERSITY OF NEWCASTLE UPON TYNE
CANCER RESEARCH UNIT
RESEARCH ASSOCIATE/ SENIOR RESEARCH ASSOCIATE

Applications are invited from graduate or post-doctoral scientists to work on a research project investigating the activation of oncogenes in cancer progression financed by a grant awarded by the North England Cancer Research Campaign. The Cancer Research Unit has already interest in the relationship of DNA repair to cancer matastasis and in the cloning of DNA repair genes. Collaborative efforts will be encouraged. Candidates should have experience in recombinant DNA techniques. Additional experience in cell biology would be an advantage. The post is available for three years. Commencing salary according to qualifications and experience on Range IA scale £7,520 — £12,150 or up to £13,625 on Range II scale £11,205 — £14,925.

Applications giving a full curriculum vitae and names of three referees should be submitted to Dr G V Sherbet, Cancer Research Unit, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, not later than Friday, 21st June 1985. (6531)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF AERONAUTICS

RESEARCH ASSISTANT

A Research Assistant is required to conduct a study of the flow in the wake of lowflying aircraft. The work will include experiments which will be performed in the larger wind tunnels in the department. The theoretical aspects of this research will be concerned with modelling the vortex patterns in the wake and predicting the flow produced by the aircraft close to the ground

Applicants should have a good degree in a relevant subject and experience of wind tunnel or related research. The salary will be in the range £7,520 — £12,150 pa (Under review) plus London weighting £1,233.

Further details can be obtained from Dr J K Harvey, Department of Aeronautics, Imperial College, London SW7 2BY, to whom applications should be sent. (6604)A

UNIVERSITY OF LONDON INSTITUTE OF NEUROLOGY

Applications are invited for a post-doctoral position available for up to 4 years in a group investigating transmitter amine metabolism in the freely moving rat by new techniques and studying relationships and finding to milieu, feeding, receptors and behaviour

Applicants should have expertise in relevant aspects of experimental psychology and be interested in neurochemistry and/or pharmacology

Initial salary within range £8,376 — £10,611 (including London Weighting).

Apply with CV and names of two referees to: Professor G Curzon, 33 John's Mews, London WC1N 2NS within a month of the appearance of this advertisement. (6536)A

SYDNEY — AUSTRALIA
THE UNIVERSITY OF NEW SOUTH WALES
SCHOOL OF ANATOMY

RESEARCH OFFICER (REF.220)

The appointee will work under the supervision of Associate Professor J Stone on the development of mammalian retina Applicants should have a PhD or equivalent. A background in Neuroanatomy, Neuroimmunology, Neurophysiology or Neuroembryology would be an advantage. The position is available initially for one year from early January 1986, with prospects of renewal for a further year.

Further information from Associate Professor Stone — (02) 697 2471.

Salary: $A22,614 — $A25,905.

Please submit written application to the General Staff Office, PO Box 1, Kensington, NSW, Australia 2033, by August 2 1985

Equality of employment opportunity is University policy. (W1881)A

UNIVERSITY OF LEEDS
DEPARTMENT OF BIOCHEMISTRY
PROTEIN SEQUENCING FACILITY

Applicants are invited for a post of Post-Doctoral Research Fellow in the above department to assist in the day-to-day operation of the sequencing facility and participate in research work. Knowledge of automated sequencers would be an advantage. Applicants should have a PhD or equivalent experience in a relevant area.

The post is available for a fixed term of up to four years.

Salary on the IA Range for Research and Analogous Staff (£7,520 — £12,150)(under review) according to age, qualifications and experience.

Informal enquiries may be made to Dr J B C Findlay (Tel 0532 431751 ext 7180).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT quoting reference no 83/61. Closing date for applications 13 June 1985. (6563)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF IMMUNOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT

in the Department of Immunology to work with Dr Peter Stern on the molecular genetics of MHC Class-I products expressed by human teratocarcinoma cells. At least a basic knowledge and experience of modern recombinant DNA techniques would be advantageous. The initial salary within the range £6,600 - £7,980 per annum

Applications, together with the names of three referees, should be received not later than 24 June, 1985, by The Registrar, The University, PO Box 147, Liverpool L69 3BX from whom further particulars may be obtained.

Quote Ref: RV/289/N. (6587)A

POSTDOCTORAL/RESEARCH ASSOCIATE Position available immediately for studies of nuclear structures and functions. One (1) project involves primary transcripts, heterogeneous nuclear RNA and small nuclear RNA. The other deals with the identification and characterization of the genes of the blood group antigens. Background in biochemistry, molecular biology and DNA Recombinant Technology is essential. Applicant will join in active research program in Recombinant DNA Technology. Job security with opportunity for advancement, competitive salary, excellent benefits Send Curriculum Vitae and letters of reference to Dr Oscar Pogo, Head, Department of Cell Biology, Kimball Research Institute, New York Blood Centre, 310 E 67 St, New York, NY 10021. Equal Opportunity Employer (NW1753)A

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place, London W2 1PG

BIOCHEMISTRY DEPARTMENT

PhD

MRC funded PhD place available to work with Dr John Hardy on neurotransmitter release and uptake by preparations from post mortem human brain. Many neurological and psychiatric disorders are thought to involve specific defects in neurotransmission.

The purpose of this project will be to study various aspects of neurotransmitter release and uptake in post mortem tissue. Specifically, isolated nerve endings (synaptosomes) will be prepared and used to study the distribution of the different transmitter uptake systems in the human brain. Many clinically prescribed drugs are thought to have their actions of modulating neurotransmitter uptake and release by human brain synaptosomes will be examined.

Enquiries to Dr John Hardy. Tel 01-723 1252 ext 117. Applications in the form of a cv with the names and addresses of two referees to the Assistant Secretary (Personnel) at above address by 3 June 1985. (6573)A

DURHAM UNIVERSITY

DEPARTMENT OF GEOLOGICAL SCIENCES

LECTURESHIP IN GEOPHYSICS

Applications are invited for a Lectureship in Geophysics tenable in any area of solid-earth geophysics, but with some preference for candidates in marine geophysics or seismology.

Applicants must have previous research experience in geophysics.

It is hoped to fill the post from not later than 1 October 1985.

Salaries on the scale £7,520 — £14,925 p.a with superannuation.

Application (3 copies) naming three referees should be sent by 31 May 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE. From whom further particulars maybe obtained. (6529)A

MEDICAL RESEARCH COUNCIL LABORATORIES THE GAMBIA

VIROLOGIST

For the MRC Laboratories, Fajara, The Gambia. You will join a team embarking on a study of acute respiratory infections in Gambian children and will be responsible for viral isolations and identifications. Previous experience of respiratory virus isolation is required and more general experience in microbiology would be an advantage. An initial contract of 2 years will be offered which may be renewed. Salary will be determined by qualifications and experience.

Application forms and further details may be obtained from Miss K S Douglas, MRC, 20 Park Crescent, London W1N 4AL. The closing date for applications is 28 June 1985. (6579)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

DEPARTMENT OF NEUROLOGICAL SCIENCE

GRADUATE RESEARCH ASSISTANT

(preferably a cellular immunologist) required from 1 September 1985 to help run a fluorescence-activated cell sorter (FACS) in a largely MRC-funded human cellular immunology group. There is some routine analytical and maintenance work, and considerable scope for functional studies on FACS-purified cells in culture. Simple engineering/electronic skill/interest an advantage (but not essential) for this challenging post which is initially available for one year with very good prospects of renewal for a further five.

Salary on scale £6,600 - £12,150 plus £1,233 London Allowance, according to qualifications and experience.

Further details available from School Office, Room G650, RFHSM, Rowland Hill Street, London, NW3 2PF, (01-794-0500 Extn 4262) to which full curriculum vitae and names and addresses of 2 referees should be sent by 7 June 1985. Please quote reference JND/GRA. (6527)A

UNIVERSITY TÜBINGEN FRG

POSTDOCTORAL CO-WORKER

We are looking for a postdoctoral coworker in the field of molecular mechanisms of infection immunity.

Apply to W G Bessler, Arbeitsbereich Immunologie der Universitat, D7400 Tübingen, FRG (W1876)A

AFRC — FOOD RESEARCH INSTITUTE - BRISTOL
Langford, Bristol BS18 7DY

SCIENTIFIC OFFICER

Applications are invited for the post of Scientific Officer (Band II) to join the Hygiene Section and participate in research on the incidence, origin and behaviour of food-spoilage and food-poisoning microorganisms associated with the processing of poultry-meat products.

The work will include both factory surveys and laboratory studies involving the isolation and identification of relevant organisms and investigation of their properties.

Qualifications: Degree in microbiology or HNC with relevant post-qualifying experience.

Salary: £5,909 - £8,153 pa.

Application forms from the Personnel Officer at the above address. Please quote reference no 85/5.

The Agricultural and Food Research Council is an equal opportunities employer. (6592)A

PHILIPPS-UNIVERSITÄT MARBURG

Im fachbereich Humanmedizin in der Humangenetik mit humangenetischer Poliklinik ist zum Herbst 1986 eine

Professur (C4)

für Humangenetik
(Nachfolge von Herrn Prof. Dr. Wendt)

zu besetzen.

Für den Stelleninhaber ist die Leitung der ersten Abteilung in einem neu zu gründenden Zentrum für Humangenetik vorgesehen Nach Berufung soll eine C3 — Professur für die zweite Abteilung ausgeschrieben werden. Außerdem ist ein Funktionsbereich fur klinische Cytogenetik und Pränataldiagnostik als zentrale Einrichtung vorgesehen.

Gem. Erlaß des Hess. Ministers fur Wissenschaft und Kunst ist alternativ offenzuhalten, daß die Humangenetik in das Zentrum fur Frauenheilkunde und Geburtshilfe oder in das Zentrum fur Kinderheilkunde organisatorisch eingebunden wird.

Der Bewerber soll auf einem aktuellen Forschungsgebiet der Humangenetik international anerkannt sein und über eigene Erfahrungen mit molekulargenetischen Techniken verfügen. Es wird erwartet, daß die Fortführung der klinischen Genetik auf dem in marburg erreichten hohen Niveau gewahrleistet bleibt.

Fur die Ernennung zum Professor sind grundsätzlich padagogische Eignung und-als Nachweis der Befähigung zu wissenschaftlicher Arbeit — neben der Promotion die Habilitation oder gleichwertige wissenschaftliche Leistungen erforderlich. Im übrigen gelten die Einstellungsvoraussetzungen des § 39 a HUG.

Berwerbungen mit den üblichen Unterlagen (das Schriftenverzeichnis ist nach Originalarbeiten, Übersichtsartikel und Fallstudien zu gliedern Bei gemeinschaftlichen Veroffentlichungen soll der Bewerber ausführen, worin seine Miturheberschaft besteht) sind bis zum 30.6.1985 zu richten an den Präsidenten der Philipps Universität Marburg, Beigenstraße 10, D 3550 Marburg.

POSTDOCTORAL POSITION
Massachusetts General Hospital/
Harvard Medical School

2-3 year position available immediately to study regulation of transcription of mammalian genes by hormonal and other factors. Particular focus on interaction of glucocorticoid receptor with DNA and transcription factors. preference will be given to applicants with strong background in molecular genetics, including experience with standard techniques of molecular biology. **Send curriculum vitae and three letters of reference to: Dr D D Moore, Dept. Molecular Biology, Wellman Building, Massachusetts General Hospital, Boston, Ma. 02114.**
equal opportunity/affirmative action employer (NW1774)A

ST. GEORGES UNIVERSITY SCHOOL OF MEDICINE

St. George's University School of Medicine, Grenada seeking additional faculty for the following subjects:

BIOCHEMISTRY PHYSIOLOGY
PHARMACOLOGY NEUROANATOMY
HISTOLOGY PATHOLOGY

Applications from both recent graduates and PhD or MD or more mature individuals. Those taking early retirement may find a period in the Caribbean attractive.

For histology and pathology young recent graduates PhDs or MDs who would like Caribbean environment for a year or two Positions are demonstrators. We wish to fill some positions next August, others New Year.

Return tourist air fare paid for appointee and wife

For further info apply to:

The Vice Chancellor,
849 Lullwater Parkway,
Atlanta,
GA 30307.
United States of America.
Tel: 404-378-0104. (W1873)A

AFRC INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

The Institute has a programme of research into infectious diseases of cattle and pigs. Two "New-Initiative" appointments, each of 3 years duration, have become available within the programme.

(1) MOLECULAR BIOLOGIST

Required to join a team carrying out research into the molecular basis of virulence in *Salmonella* pathogens. Research emphasis will be on determining the distribution and precise function in various *Salmonella* serotypes of a plasmid DNA sequence that appears to play a major role in virulence. Ref: 565.

Applicants should have a first or upper second class honours degree and PhD. Experience of immunology would be an added advantage.

(2) IMMUNOLOGIST OR VIROLOGIST

Required to join a project investigating the potential of monoclonal antibodies as prophylactic and therapeutic agents against respiratory syncytial virus infection. Experience in production and characterisation of monoclonal antibodies would be an advantage. Ref: 566.

Students completing PhDs in Autumn 1985 will also be considered.

For further information contact David Baird (ref 565) or Jim Stott (ref 566) on 0635 22 411.

Appointments will be to Higher Scientific Officer grade, salary scale £7,435 — £10,039 (under review) depending on qualifications and experience. Non-contributory pension scheme.

The Institute is set in pleasant rural surroundings with canteen and recreational facilities.

The AFRC is an equal opportunities employer.

Application forms from: Secretary, IRAD, Compton, Newbury. RG16 ONN, quoting appropriate ref. (6562)A

KINGSTON POLYTECHNIC
Faculty of Science
School of Applied Physics
CEGB LECTURESHIP IN APPLIED PHYSICS

Applicants should have worked in an area of physics applications, eg in applied optics, environmental measurement, development of instrumentation, which would be of interest to CEGB research.

The person appointed will be required to contribute to the teaching of the School of Applied Physics up to degree level. He or she will also be a recognized member of the appropriate research team at the CEGB Research Laboratories, Leatherhead, with access to a comprehensive range of equipment and instrumentation, and will be expected to carry out research both at CEGB and at the Polytechnic.

Salary range LII/SL £8,226 — £14,739 including London allowance.

Details and application forms (quoting ref PHB2) from Personnel Officer, Kingston Polytechnic, Penrhyn Road, Kingston Upon Thames KT1 2EE. Tel 01-549 1366 ext 287.

Re-advertisement — previous applicants need not re-apply. Closing date 1/7/85. (6547)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY
LECTURESHIP

Applications are invited for a newly-created Wellcome Trust Lectureship for 5 years (subject to review after three years).

Applicants should have specific interest in research on membrane-bound receptors. The successful applicant will be expected to develop his or her own research programme and also to collaborate in related research in the Department, and to participate in the teaching programme.

Applicants shall not be more then 35 years old, nor holders of a tenured post. Salary on the scale £7,520 — £14,925 plus £1,233 London Allowance.

Further particulars may be obtained from the School Office RFHSM, Rowland Hill Street, London, NW3 2PF (01-794-0500 ext 4262) to which applications, including a curriculum vitae, the names of three referees and a 500 word summary of the research proposed should be submitted by 14 June. Please quote reference JAL/WL/2. (6552)A

The Department of Pediatrics at the University of California, San Diego is seeking a candidate for a 75% to 80% time faculty position at the Assistant Clinical Professor level in the Division of Adolescent Medicine. Position will have the responsibility of attending in community Adolescent Medicine Clinics, and work on inpatient and outpatient, and teaching residents. Candidate must be able to obtain California license and be board eligible or certified in Pediatrics. Experience or fellowship training in Adolescent Medicine is desirable. Salary will be commensurate with experience and U.C.S.D. policy.

Interested persons should forward curriculum vitae and the names of three references by June 1, 1985 to: Marianne Felice, M.D., Director, Adolescent Medicine, Department of Pediatrics, UCSD Medical Center, Box 3548, San Diego, California 92103. *The University of California, San Diego is an Equal Opportunity/Affirmative Action Employer* (NW1770)A

IMPERIAL COLLEGE
(University of London)
Physiological Flow Studies Unit
POST-DOCTORAL RESEARCH ASSISTANT
FLOW IN DEFORMABLE POROUS MATERIALS

Support is available for a post-doctoral research assistant to undertake studies of flow in deformable porous materials. The work, which has wide relevance, will involve experimental studies and numerical analysis. The research is supported by the BP Research Centre, Sunbury (with whom close contact will be maintained) but will be carried out at the Physiological Flow Studies Unit, Imperial College. The appointment is for one year but the possibility exists of extension for a further year.

Applications (including curriculum vitae and names of two referees) should be submitted not later than 27th June, 1985 to Prof. C.G. Caro, Physiological Flow Studies Unit, Imperial College, London SW7 2AZ. For further information, apply to Prof. Caro (589 5111 ext. 4082) or Dr K H Parker (589 5111 ext. 4085). (6521)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
London W1P 6DB
(University of London)
THE COURTAULD INSTITUTE OF BIOCHEMISTRY, AND THE DEPARTMENT OF ANATOMY AND BIOLOGY AS APPLIED TO MEDICINE
RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to work on the molecular basis of limb development in the chick embryo, for two years from 1 September. The work is supported by the Nuffield Foundation. Previous experience in cDNA cloning technology is not essential but would be an advantage. Applications are welcomed from those with postgraduate experience as well as new graduates.

Applications, together with the names of two referees, should be sent to either Professor R K Craig at The Courtauld Institute, or Professor L Wolpert, Department of Anatomy and Biology as Applied to Medicine. (6535)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMICAL MEDICINE
Applications are invited for a post as
LECTURER IN APPLIED BIOCHEMISTRY

in the above Department based in Ninewells Hospital and Medical School. Candidates should have a good honours degree in Biochemistry or Chemistry, and a higher degree with postgraduate experience in either immobilisation of enzymes, microbiological degradation of biochemicals or related topics. The Appointee will be expected to undertake research and contribute in particular to an established MSc Course in Analytical Biochemistry.

The appointment will be made on the Lecturer scale (currently £7,520 — £14,925, but due for review) with point of entry dependent on qualifications and experience.

Further Particulars are available from The Personnel Officer, The University, Dundee DD1 4HN, with whom applications (6 copies containing full career details and the names of three referees) shold be lodged by 29th June 1985. Please quote reference EST/16/856(J). (6518)A

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF OCCUPATIONAL HEALTH & HYGIENE
CHAIR OF OCCUPATIONAL HEALTH AND HYGIENE

Applications are invited for the Chair of Occupational Health & Hygiene which will fall vacant on 1 October 1985, on the retirement of Professor R I McCallum. Candidates should be registered medical practitioners and hold a higher medical qualification. The successful candidate will be accorded Honorary Consultant status by the Newcastle Health Authority and will be appointed to the headship of the University Department of Occupational Health & Hygiene for an initial period of five years.

Applications (15 copies), giving the names of not more than three referees must be lodged with the Senior Assistant Registrar, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, not later than 14 June 1985, from whom further particulars can be obtained. (Applicants from outside the British Isles may submit one copy only) (6553)A

ANIMAL VIRUS RESEARCH INSTITUTE

HIGHER SCIENTIFIC OFFICER BAND 1

Structure and function of the Bluetongue virus genome.

Applications are invited for the above post which will be concerned with genetic analysis, cloning and sequencing of bluetongue virus genomes.

This is a new position within the Division of Molecular Biology, which is one of three Divisions within the Institute and comprises some 20 -25 scientific staff.

The appointee would work closely with Dr P Mertens in the Biochemistry Department, who has already established the coding assignments of bluetongue virus type 1 (See Virology 135, 207-217 1984), which is a highly virulent strain causing disease in sheep. Clones of the virus genome will be obtained both for sequencing studies and also for use as diagnostic probes for the detection of bluetongue virus RNA segments. The position would suit someone with a strong background in molecular biology. Previous experience with viruses is not essential. (6494)A

KINGSTON POLYTECHNIC

School of Applied Physics

RESEARCH ASSISTANT IN FIBRE OPTICS

Required to investigate the Interaction of optical signals with lattice vibrations (Brillouin scattering) in optical fibres. The work is directed towards the development of fibre-optic sensors and is a collaborative project between the School and the CEGB Research Laboratories, Leatherhead.

The Assistant will be based at Kingston but will work in association with a research group at Leatherhead and have access to facilities there.

Candidates should have a good Honours degree or should be expecting to graduate shortly in Physics or a related science and will be expected to register for MPhil/PhD with CNAA

Salary range £5,859 — £6,588 including London allowance.

Details and application forms (quoting ref PHB1) from Personnel Officer, Kingston Polytechnic, Penrhyn Road, Kingston Upon Thames KT1 2EE Tel 01-549 1366 ext 287 (6548)A

ICARDA

International Center for Agricultural Research in the Dry Areas

HEAD COMMUNICATIONS AND DOCUMENTATION

The International Center for Agricultural Research in the Dry Areas (ICARDA) invites applications for the position of Head Communications and Documentation to be based at the Headquarters in Aleppo, Syria.

RESPONSIBILITIES:

Direct the Department of Communications and Documentation which is primarily responsible for gathering, producing, and disseminating information on the Center's research and training activities. Involves writing and editing, production of internal and external newsletters and other publications, graphic arts, audio visual presentations, printing and computerized storage and retrieval of scientific information.

QUALIFICATIONS:

Bachelor of Science or advanced degree in agricultural journalism or closely related field, at least 10 years in responsible position in agricultural information/communication work; some international experience preferred; demonstrated competence in supervision of a staff and management of overall agricultural information operations. Knowledge of French desirable.

CONDITIONS OF APPOINTMENT;

International tax-free salary based on background and experience; use of car; allowance towards housing; annual home leave; non-contributory medical insurance scheme and pension fund.

Candidates should submit two copies of their Curriculum Vitae and samples of work, plus names of two referees quoting REF. COMM/1/85. to:

Dr. Mohammed A. Nour,
Director General,
ICARDA, P.O. Box 5466,
Aleppo, Syria.

(W1877)A

UNIVERSITY OF LEEDS

DEPARTMENT OF CHEMICAL PATHOLOGY
(In collaboration with Department of Genetics)

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post to work on the DNA sequence diversity of the Y-chromosome of the mouse The appointment will be made from 1 October 1985 for a fixed period of up to two years

Candidates with experience of recombinant DNA techniques would be preferred but previous work with mammals is not a requirement.

Salary on the IA Range for Research and Analogous Staff (£7,520 — £12,150)(under review) according to age, qualifications and experience

Informal enquiries to Dr A D Steward (0532 432799 ext 3927) or Professor D J Cove (Genetics — 0532 431751 ext 599) are invited

Application forms and further particulars may be obtained from The Registrar, The University, Leeds LS2 9JT quoting reference number 84/16 Closing date for applications 13 June 1985 (6564)A

THE UNIVERSITY OF LANCASTER

LOW TEMPERATURE PHYSICS

A postdoctoral Research Associate is needed to participate in an SERC-funded research programme on the breakdown of superfluidity in liquid ^{4}He at mK temperatures The post is tenable from 1 July (or as soon as possible thereafter) for up to three years. Salary within the first two points of Research 1A scale (£7,520 — £7,980 under review) Informal enquiries may be made to Dr P V E McClintock, tel no 0524 65201 ext 4178.

For further particulars and application form, please write to the Establishment Office (quoting reference L208/C), University House, Bailrigg, Lancaster LA1 4YW, where applications (six copies) naming three referees should be sent to arrive NOT LATER THAN 21 JUNE 1985. (6602)A

AN ACTIVE FERTILITY AND IN VITRO PROGRAMME IS DUE TO OPEN AT THIS HOSPITAL ON 1st AUGUST 1985

Applications are invited for the following position, for which date of commencement will be by mutual agreement

TECHNICAL ASSISTANT/EMBRYOLOGIST

The successful applicant will be expected to undertake both routine laboratory and research work Research is currently undertaken in collaboration with MRC (Carshalton) and the Institute of Animal Physiology (Cambridge)

Experience in mammalian embryology would be desirable and a PhD or MSc in reproductive physiology or embryology would be an advantage, but is not essential

The Hospital offers a generous salary and excellent benefit package.

Applicants should forward a Curriculum vitae, including the names of three referees to: The Personnel Department, Humana Hospital Wellington, Wellington Place, London NW8

Closing Date 22nd June 1985 (6599)A

Humana Hospital Wellington

Wellington Place London NW8 9LE

Please mention

nature

when replying to these advertisements

ASSISTANTSHIPS

KINGSTON POLYTECHNIC
Faculty of Science
RESEARCH ASSISTANTSHIPS

Applications are invited for the following Research Assistantships:-

Post 1 (Ref GP2)

SYNTHESIS OF NOVEL CROSSLINKED POLYMERS

This post is with an established group developing new kinds of thermosetting polymers and studying the relationship between their structure and properties.

Post 2 (Ref GAS 1)

MECHANISTIC INVESTIGATION OF THE FLAME RETARDANT AND SMOKE SUPRESSANT ACTIVITY OF SPECIFIC POLYMER ADDITIVES

This study involves polymer synthesis and analysis of an assessment of flammability. Techniques include thermogravimetry, IR and NMR spectroscopy, capillary GC/MS.

Posts 1 and 2 are the result of Kingston Polytechnic being identified as a centre to receive special research funding in science and engineering from the National Advisory Body.

Post 3 (Ref RD1)

INORGANIC AND PHYSICAL CHEMISTRY

This post involves the study of paramagnetic vanadium compounds with organic molecules and is directed towards the development of new organic reactions in the presence of transition metal catalysts.

Post 4 (Ref CHJW 1)

PHOTOCHEMISTRY OF PESTICIDES

This post is to work on research into the effect of light on pesticides The research relates to the fate of pesticides and related materials when exposed to sunlight in the environment, and will provide the opportunity to gain experience of a variety of radiochemical techniques and of modern spectroscopic methods of structure determination

Applicants should hold or expect to gain an Honours degree or equivalent qualification.

Research Assistantships are normally made for a period of 3 years Salary scale £5,859 — £6,588 including London Allowance

SERC STUDENTSHIPS

Applications are invited for the following SERC studentships:

Post 5 (Ref QC 1)

CHEMISTRY RESEARCH

The research interests of the department include aspects of organic, organometallic, inorganic and polymer chemistry, as well as photochemistry and analytical chemistry

Post 6 (Ref RD 2)

INORGANIC AND PHYSICAL CHEMISTRY

This post is to study the reactivity of metal co-ordinated carbonyl ligands with anionic nucleophiles in the presence of group 1 metal-crown ether complex cations and is directed towards the catalysed synthesis of oxalate esters

Applicants for these posts should hold or expect to gain at least an upper second class Honours degree or equivalent qualification SERC studentships are normally for a period of 3 years and candidates will be expected to register for a higher degree.

Application forms and further details for all the above posts are available from the Staffing Officer, Kingston Polytechnic, Penrhyn Road, Kingston Upon Thames KT1 2EE. Tel. 01-549 1366 ext 287. Please quote reference no. when requesting details. (6549)P

nature classified

NEW YORK OFFICE
Garth Walkes
65 Bleecker Street
New York, NW 10012
Tel: (212) 477-9600

LONDON OFFICE
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

TORONTO OFFICE
Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1L1
Tel (416) 690-2423

UNIVERSITY OF OXFORD
DYSON PERRINS LABORATORY
Organic Chemistry

POSTDOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited from chemists who have experience in the synthesis of natural products and in the field of enzymology.

The appointment is on the RA1A scale and would be for one year from September 1985, with starting salary up to £8,920 pa according to age and experience plus USS.

Applicants with the names and addresses of two referees should be sent to Professor J E Baldwin, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY as soon as possible (6542)P

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY AND UNIT OF MOLECULAR GENETICS

POST DOCTORAL/ POST GRADUATE RESEARCH ASSISTANT

Applications are invited for a SERC funded Research Assistantship to join a research group actively working on the molecular genetics on Drosophila melanogaster. The work will involve the characterisation of a gene which controls pigmentation of the cuticle using spontaneous and hybrid dysgenesis induced mutants. DNA cloning, sequencing and in situ hybridisation methods will be employed

The laboratory is well funded and this position is supported for 3 years. Starting salary up to £7,980 p.a (Range 1A) Candidates with a PhD and experience in nucleic acids and/or genetics are preferred; however graduates with a good honours degree and pertinent research experience will be considered at the RA1B level. This position can be taken up immediately

Curriculum vitae along with the names and telephone numbers of three referees should be sent to Dr William Chia, Department of Biochemistry, University of Bristol, Bristol BS8 1TH. Tel 0272 24161 ext 1190 as soon as possible. (6567)P

FELLOWSHIPS

MONASH UNIVERSITY
Melbourne, Australia

POST-DOCTORAL FELLOWSHIPS

Monash University is offering a number of Post-Doctoral Fellowship positions commencing in 1986 and tenable for up to two years in any department of the University. Fellows will be expected to collaborate in their research with a member of the University staff. intending applicants should contact the Chairman of the Department in which they wish to work to discuss possible areas of mutual research interest.

Appointments will be in the Research Fellow I range ($A22,614 — $A25,246), but an appointment at Research Fellow Grade II level ($A26,236 — $A34,467) may be considered in exceptional circumstances. Reference No 90162

Applications will only be accepted on the official Application Form. This and other details available from the Registrar, Mr J D Butchart, Monash University, Clayton, Victoria 3168, Australia Applications close 30 August 1985.

An Equal Opportunity Employer. (W1884)E

THE UNIVERSITY OF SHEFFIELD
FACULTY OF MEDICINE

J G GRAVES MEDICAL RESEARCH FELLOWSHIP

Applications are invited for the above Fellowship tenable for a period of three years from 1 October 1985. The Fellow will be required to undertake full-time medical research in one of the medical or dental departments or hospital. Initial salary according to qualifications and experience within the range £7,520 - £8,920 a year. Additional funds may be available for the Provision of laboratory assistance and materials.

Further particulars from the Personnel Department (Academic Staffing), the University, Sheffield S10 2TN to whom applications (10 copies), including the names and addresses of three referees, (indicating the line of research proposed), should be made by 17 June 1985. Quote ref: R269/G. (6588)E

LA TROBE UNIVERSITY
Melbourne, Australia

LA TROBE RESEARCH FELLOWSHIPS 1985/86

Applications for Research Fellowships are invited from persons who have gained doctoral or equivalent qualifications within the last five years A project must be proposed by an applicant and should ideally be in collaboration with a La Trobe University research worker or team of the applicant's choice, that is, it should complement an existing area of research at La Trobe.

Fellowships will be awarded for an initial period of 12 months with the possibility of renewal for a further maximum period of 12 months.

Salary offered is within the range of $A22,614 — $A26,236

A Fellowship will not be awarded to an applicant who holds a permanent appointment

Prospective applicants should note that application must be made on the University's form which will be sent on request together with a copy of the Conditions of Award.

Enquirers should briefly indicate the research discipline of interest to them The closing date for applications is 30 September 1985 For further information, write to the Staff Officer, La Trobe University, Bundoora, Victoria 3083, Australia (W1883)E

FELLOWSHIPS continued

EMBO
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION
SHORT TERM FELLOWSHIPS
in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 102240, F.R.G. (W1577)E

UNIVERSITY OF YORK
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship for work, in association with Professor R E Hester, on the spectroscopic determination of secondary structure in proteins. Laser Raman spectroscopy will be the principal techniques used in this work, a part of which will take place at the SERC central laser facility in the Rutherford Laboratory, near Oxford. Applicants should have experience in protein biochemistry and/or vibrational spectroscopy.

The post is available immediately for a period of two years. Salary within the IA range — £7,520 to £12,150 pa, with USS.

Two copies of applications, with full curriculum vitae and naming two referees, should be sent as soon as possible to Professor R E Hester, Department of Chemistry, University of York, Heslington, York YO1 5DD. Please quote reference number 11/6206. (6591)E

LUDWIG INSTITUTE FOR CANCER RESEARCH
(Cambridge Branch)
POST-DOCTORAL FELLOW

Applications are invited for a post-doctoral fellowship in the laboratory of Dr A R MacLeod to work on the molecular biology of human cytoskeletal tropomysins. The Cambridge branch of the Ludwig Institute is situated adjacent to the Laboratory of Molecular Biology and offers the opportunity to join a young group working in a stimulating academic environment. The position is tenable from 1 January 1986 or as soon as possible thereafter for a period of two years with the possibility of a year's renewal. Salary is commensurate with experience on the Grade II (non-clinical) scale (£11,205 max).

Applications including a CV and names and addresses of two referees should be sent to The Administrator, Ludwig Institute for Cancer Research, MRC Centre, Hills Road, Cambridge CB2 2QH, England. (6473)E

STUDENTSHIPS

UNIVERSITY OF GLASGOW
BIOTECHNOLOGY UNIT
SERC CASE STUDENTSHIP — ICI

To clone and study expression of resistance and production genes from Streptomyces for an antibiotic of interest to ICI. The project is supervised jointly by Dr I S Hunter (Glasgow) and Dr P Groves (ICI, Corporate Lab, Runcorn).

Applicants should have, or expect to obtain, a first or upper second class honours degree in a related biological science. Interviews will be conducted towards the end of June.

Applications (CV and names of two referees) should be sent to Dr I S Hunter, Institute of Genetics, Church Street, Glasgow G11 5JS, before June 13th, 1985. (6596)P

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
Wolfson Unit
PhD STUDENTSHIPS

Available immediately for research on the molecular biology/immunology of the viral agents of hepatitis and dengue.

For further details send full curriculum vitae, naming three professional referees, to the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT (6539)F

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE
(UNIVERSITY OF LONDON)
KEPPEL STREET, LONDON, WC1E 7HT

MRC Studentship

Applications are invited for a three year MRC Research Studentship tenable from October 1985. The project will be concerned with comparative studies on the immunology of *Schistosoma mansoni* and *S. japonicum* with emphasis on the identification of the "protective" antigens and cloning of the corresponding antigen genes.

This work will be carried out in part at Winches Farm Field Station, but mainly in the School's Wolfson Unit for Molecular Medical Microbiology and Parasitology in London.

Applicants, who must hold, or hope to obtain in June 1985, a first or upper second class honours degree in Molecular Biology, Microbiology or Biochemistry, will be registered for a PhD degree.

Applications consisting of full C.V. and naming two referees should be sent to Dr Q.D. Bickle, London School of Hygiene and Tropical Medicine, Winches Farm Field Station, 395 Hatfield Road, St. Albans, Herts AL4 0XQ. (6605)F

Brighton Polytechnic

DEPARTMENT OF PHARMACY
Applications are invited for the following SERC CASE Awards:

1 Difussion of antibiotic molecules through biopolymer gels, in conjunction with Beecham Pharmaceuticals Research Division;
2 Evaluation of the ciliotoxicity of pharmaceuticals, in conjunction with the Wellcome Foundation.

Applicants should possess a good honours degree in Pharmacy, Physiology, Biochemistry or a related discipline. Normal SERC conditions apply and candidates will be expected to register for a higher degree.

Further information and forms of application may be obtained from Personnel Department, Brighton Polytechnic, Mithras House, Telephone: Brighton 693655 Ext. 2536. Closing date 4 June 1985. (6612)F

QUEEN MARY COLLEGE
(University of London)
SCHOOL OF BIOLOGICAL SCIENCES
SERC CASE STUDENTSHIPS

Applications are invited for SERC-CASE Studentships in the following areas.

1. Microbial Ecophysiology/Biochemisty (Dr J P Robinson)

Investigation of the anaerobic decomposition of chlorinated hydrocarbons by bacteria in land-fill will be carried out in collaboration with Dr R Sleat, BioTechnica Ltd, Cardiff. The work will involve characterisation of anaerobic decomposition processes using a wide variety of microbiological and analytical techniques including GLC and HPLC

2. Plant Biochemisty (Dr P Heathcote)

The isolation, purification and characterisation of acetyl-CoA carboxylase from spinach leaf chloroplasts will be undertaken in collaboration with Dr A P Slabas, Unilever Research. The possible regulation of leaf and seed lipid synthesis by different forms of enzyme will be investigated

Applications including a current curriculum vitae and the names of two referees should be sent as soon as possible to Dr J P Robinson or Dr P Heathcote, School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS. Applicants should have or expect to have a first or upper second class honours degree in a relevant subject area (6574)F

Continued on page 22

UNIVERSITY OF KENT
AT CANTERBURY

BIOLOGICAL LABORATORY

Applications are invited for the following two SERC CASE studentships under the supervision of Dr R B Cain.

(i) in collaboration with Castrol Research Laboratories, Pangbourne, to investigate the metabolic fate in soil and activated sludge of synthetic two-stroke motor lubricants with particular reference to the enzymes involved in degrading branched chain hydrocarbon residues.

(ii) in collaboration with the Glasshouse Crops Research Institute, Littlehampton, to examine the physiology, biochemistry and enzymology of lignin and lignocellulose degredation by edible fungi, including the common mushroom *Agaricus*

These projects both complement existing research programmes with research grant support. Applicants should either hold, or expect to obtain in 1985, at least an upper second-class Honours degree in an appropriate subject area Applications with the names of two referees, or with references enclosed, should be directed without delay to the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory. University of Kent, CANTERBURY, CT2 7NH from whom further details are available Please quote ref· PG12/85 (6561)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF ORGANIC CHEMISTRY

SERC CASE AWARD

Applications are invited for an SERC CASE studentship (Cambridge Research Biochemicals collaborating company), to work on a project entitled "New Methods for the Conjugation of Peptides".

The successful applicant will carry out studies leading to the degree of PhD under the supervision of Dr I J Galpin. The work will involve solid phase peptide synthesis and conjugation of synthetic peptides to protein carriers in order to facilitate antibody generation.

Although biological aspects of the programme will be carried elsewhere, the applicant will acquire a good knowledge of the synthesis and manipulation of peptides and proteins and will join a group working on various aspects of peptide chemistry

Applicants should possess or expect to receive a First or Upper Second Class Honours Degree in Organic Chemistry, although Honours Graduates from other disciplines, such as biochemistry maybe considered providing they have sufficient background and knowledge of organic chemistry.

Applications, together with the names of two referees should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote ref RV/283 (6559)F

UNIVERSITY OF LONDON

Institute of Psychiatry

DEPARTMENT OF PSYCHOLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited for a research studentship, leading to a PhD D,tenable for 3 years from October 1985. Applicants should hold or expect to receive a first or upper second class Honours degree and have a background in psychology or a branch of neurobiology. The successful candidate will join a research group that aims to apply the techniques of contemporary neurobiology to the study of the control of behaviour by the brain. The group collaborates closely with the Departments of Biochemistry and Neuropathology and training is available in a range of appropriate techniques.

Applicants should write to Professor J A Gray, Department of Psychology, Institute of Psychiatry, De Crespingy Park, Denmark Hill, London SE5 8AF, giving details of their curriculum vitae, the names of two referees and an indication of their preferred field(s) of research. Closing date for applications: 7 June 1985 (6520)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

SERC CASE STUDENTSHIP

Applications are invited for a studentship leading to the degree of PhD on "Controlled proteolysis of the sweet protein — thaumatin" The collaborating body of Tate and Lyle, plc and the industrial supervisor is Dr P B Rodgers Candidates should possess or expect to receive a 1st or upper 2nd class degree in Biochemistry or a related subject.

Applications, together with the names of two referees should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool L69 3BX Quote Ref: RV/279 (6538)F

UNIVERSITY OF SOUTHAMPTON

Faculty of Medicine

RESEARCH STUDENTSHIP

Applications are invited for a University Research Studentship for 3 years in the Clinical Pharmacology Group The student should have (or expect to obtain) a First or Upper Second Class Honours Degree in Pharmacology, Biochemistry or related Biological Sciences and will register for a PhD. The project will investigate the processes involved in hormone production (including prostaglandins and leukotrienes) in gastrointestinal disease. Further information can be obtained from Dr K Hillier, 0703 559122 Ext 4264. The grant will be at the appropriate MRC scale rate.

Applications giving full cv and the names and addresses of two referees should be sent as soon as possible to Dr K Hillier, Clinical Pharmacology Group, University of Southampton Bassett Crescent East, Southampton, SO9 3TU. Please quote ref no AJS/85/32. (6525)F

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DEPARTMENT OF PHYSICS

SERC PhD STUDENTSHIP

Applications are invited from graduates with a class I or II 1 Honours degree in Physics, Materials Science or Electronic Engineering, for an SERC Information Technology award, to study luminescence in extremely thin layer structures of amorphous silicon which show quantum size effects Comparative studies on GaAs superlattices will be included. The successful candidate will join an established group in the field of amorphous semiconductors especially interested in the study of photo-and electro-luminescence, using lasers and related opto-electronic techniques. We are chiefly interested in recombination mechanisms and photo induced defects This work is being extended to include quantum size effects in both amorphous silicon and crystalline III-V semiconductors. The group collaborates actively with a number of workers in the device field The project will involve a wide range of solid state techniques with emphasis on the physics of these materials. Tenable from 1 October 1985.

Applications with the names of two referees should be sent to Professor I G Austin, Department of Physics, The University, Sheffield S10 2TN by 6 June 1985. Quote ref: R268/G. (6585)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MEDICAL CELL BIOLOGY

PhD STUDENTSHIP ON THE MOLECULAR MECHANISMS OF CELL MOTILITY

A University of Liverpool postgraduate studentship (same conditions as MRC studentship) is available from October 1985 to work in a group studying the molecular mechanisms of non-muscle cell motility. Interests of the group include the cellular regulation of actin filament assembly and the mechanisms by which the spatial organisation, polarity and anchorage of filaments is determined during cell motility.

The project will involve the development of experimental approaches to the study of possible myosin-independent movement resulting from the polymerisation of actin filament bundles and the Donnan-induced shape changes in cross-linked actin filament networks. Studies with purified proteins will be correlated as far as possible with the cellular behaviour of actin filament networks in cells. Informal enquiries may be made to Dr P Sheterline, Tel 051-709 6022 ext 2842.

Applications, together with the names of two referees, should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool L69 3BX.

Quote Ref: RV/288/N. (6586)F

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

SERC CASE STUDENTSHIP IN PROTEIN MODIFICATION AND DESIGN

Applications are invited for a three-year Postgraduate Case Studentship with G D Searle and Co Ltd, leading to the degree of PhD at the University of Nottingham, from Students who have or expect to obtain a First or Upper Second Class Honours Degree in Molecular Biology, Biochemistry or a related biological subject.

The proposed Project is directed towards an investigation of two animo transferase enzymes and will offer training in a range of biochemical, molecular biological and genetic techniques. In particular it will involve oligonucleotide directed mutagenesis, recombinant and DNA technology protein purification, enzymology and computer modelling.

The research will be supervised by Dr R E Glass in collaboration with Dr M G Hunter of G D Searle and Co Ltd. The student will be expected to spend part of the time in G D Searle's Laboratories at High Wycombe

Applications, including two copies of a curriculum vitae and the names of two referees should be sent by 14th June 1985 to Dr R E Glass, Department of Biochemistry, University of Nottingham, Queens Medical Centre, Nottingham, Notts, NG7 2UH. Tel 0602 700111 ext 3395

Informal enquiries should be directed to Dr M G Hunter. Tel 0494 21124 ext 3401. (6569)F

JOHN INNES INSTITUTE

GRADUATE STUDENTSHIPS IN PLANT VIROLOGY

Three studentships are offered for prospective PhD students in the Department of Virus Research of the John Innes Institute. The research topics are (i) Strain differentiation in Beet Yellow Virus (Dr R Hull), (ii) Molecular biology of a tobravirus (Dr J W Davies in collaboration with Dr K R Wood in Birmingham University), (iii) Changes in host gene expression in virus-infected plant tissue (Dr A S Maule).

The John Innes Institute is affiliated with the nearby University of East Anglia and graduate students working here are entitled to use all the academic and social facilities of the University as well as the excellent facilities of the Institute.

Prospective applicants should write to the project supervisor for further details. The financial arrangements for the studentships will be the same as those of the usual Research Council Studentships Applications, with full cv, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH. (6543)F

UNIVERSITY OF DURHAM

DEPARTMENT OF ZOOLOGY

RESEARCH STUDENTSHIP

Applications are invited from candidates, preferably with an interest in Cell Biology, who hold or expect to be awarded at least 2:1 honours in Biology or Biochemistry for a North of England Cancer Research Campaign Studentship to study the relationship between the thermal sensitivity of tumour cells and the biochemistry of the tumour cell membrane. The award is for 3 years from October 1985 and the successful candidate must register for a higher degree.

Applications (curriculum vitae and 2 academic referees) should be sent by 31 May 1985 to Professor K Bowler, Department of Zoology, Science Laboratories, South Road, Durham, DH1 3LE. (6528)F

THE UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ZOOLOGY

RESEARCH STUDENT IN NEUROBIOLOGY

Applications are invited for a research studentship, which has been awarded by SERC for research in Invertebrate Neurobiology. Research interests are in the physiology of synaptic transmission between visual neurones in insects, and in the roles which identified neurons play in the control of behaviour. The studentship is tenable for three years from October 1985. Candidates are required to obtain a 1st Class or Upper Second Class Honours Degree in Zoology or Physiology.

Applications, with the names of 2 academic referees, or requests for information to be sent as soon as possible to: Dr P J Simmons, Department of Zoology, The University, Newcastle Upon Tyne NE1 7RU. (6532)F

UNIVERSITY OF BRADFORD

SERC/CASE RESEARCH STUDENTSHIP IN CHEMISTRY

Applications are invited for the above studentship in collaboration with UKAEA, Windscale, from 1 October, 1985 leading to a PhD. The project involves the application of Raman microscopy to chemical problems associated with nuclear reactors and the feasibility of using remote control Raman microscopy for the in-situ study of radioactive materials. Applicants must hold or expect to gain a first or upper second class Honours Degree in Chemistry or its equivalent.

Applications, including the names and addresses of two academic referees and a CV should be sent to: Professor D A Long/Dr H G M Edwards, Molecular Spectroscopy Unit, School of Chemistry, University of Bradford, W Yorks BD7 1DP. (6583)F

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PLANT BIOLOGY

OPPORTUNITIES FOR POSTGRADUATE STUDY COMMENCING OCTOBER 1985

Studentships are available for PhD study in the following research areas or projects.

1) NERC (CASE) with Dr B Leadbeater, on the turnover of organic matter in the sea by heterotrophic chrysomonads (in collaboration with Marine Biological Association).

2) SERC (CASE) with Dr M T Jackson and Prof J A Callow, on the physiology and genetics of the non-blackening character of wild potatoes.

3) SERC (CASE) with Dr B Leadbeater and Dr T R Bott (Dept of Chemical Engineering), on the use of ozone as a biocide in water systems (in collaboraton with Ozotech Ltd).

4) SERC (CASE) with Dr B Leadbeater, on the physiology of movement and structure of the haptonema in coccolithophorid algae (in collaboration with Marine Biological Association).

5) Department Studentship in Plant Genetic Resources, to work on one of the following aspects of plant genetic resources; isoenzymes and genetic diversity of potato, regulation of metabolic activity in recalcitrant seeds, anther culture in breeding of Alliums, promotion of germination in hard seeds in relation to genebank activities. Applications in this case should be addressed to Prof J A Callow.

Applicants should possess a first or upper second class honours degree in an appropriate biological subject. Applications (2 copies including cv) should be addressed directly to the Supervisors indicated and further details may be obtained from them at the following address. Dept of Plant Biology, University of Birmingham, PO Box 363, Birmingham B15 2TT. (6544)F

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

SERC STUDENTSHIP

October 1985

Applications are invited for a studentship expected to lead to a PhD degree for research on inherited biochemical lesions resulting in ketoacidosis in humans. The work will involve cultured skin fibroblasts from affected and unaffected individuals. Applicants will need a first or upper second class honours degree in Biochemistry or a closely related subject.

Applications including curriculum vitae and names of two academic referees to Dr B Middleton, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH. (6530)F

UNIVERSITY OF KENT AT CANTERBURY

Biological Laboratory

The following research studentships for postgraduate research are available in the Biological Laboratory Applicants must have, or hope to obtain, an upper second or first class honours degree

SERC Quota Studentships*

1 Molecular Biology of Microtubules Differential Expression of a Tubulin Multi-Gene Family DR K GULL
2 The Bioenergetics of Methanogenic Bacteria DR I SALMON
3 Regulation of Bacterial Secondary Metabolite Production DR A W BUNCH.

SERC CASE Studentships*

4. Disulphide Bonds and Conformation of Recombinant Proteins DR R B FREEDMAN with DR J E FITTON, ICI Pharmaceuticals, Alderly Edge
5 Differential Gene Expression in a Eukaryotic Microbe DR K GULL with DR R COX, MRC Laboratory, Mill Hill

SERC Biotechnology Directorate Studentships

6 Physiology of Immobilised Steroid-Transforming Fungi PROFESSOR A T BULL
7 Catalysis by Redox Enzymes in Water/Oil Microemulsions. DR R B FREEDMAN and DR B H ROBINSON

NERC Studentships

8 Use of Monoclonal Antibodies for Distinguishing Hyphae of Root Infecting Fungi DR P JEFFRIES and DR K GULL

Applicants should indicate the projects for which they wish to be considered and should submit a curriculm vitae together with the names of two referees to the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH from whom further details are available on request Please quote ref PG11/85 (6554)A

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DEPARTMENT OF BIOCHEMICAL GENETICS

SERC/CASE RESEARCH STUDENTSHIP

Applications are invited from candidates with, or expecting to gain, a good honours degree in Biological Sciences for this 3 year project leading to a PhD degree The project is concerned with the study of gene expression in differentiation and will involve the use of viral vectors to introduce cloned genes into recipient cells and is in collaboration with ICI Pharmaceuticals Division, Alderley Edge, Cheshire.

Applicants should have a strong interest in Molecular Biology and submit their cv with the names of two referees as soon as possible to Dr John Brennand, Department of Biochemical Genetics Lab 5, Paterson Laboratories, Christie Hospital, Wilmslow Road, Manchester M20 9BX. (6560)F

UNIVERSITY OF LEICESTER

Leicester Biocentre

POSTGRADUATE STUDENTSHIP

Applications are invited for a studentship in the Leicester Biocentre, tenable from October 1st 1985.

The research would be in the challenging area of Molecular Biology and Genetic Engineering of Micro-organisms. The Biocentre is currently placing emphasis on exploiting this rapidly advancing technology for application to commercial processes.

Applicants should write enclosing curriculum vitae and names of two referees to: Mrs Sylvia Dexter, Secretary, Leicester Biocentre, University of Leicester, University Road, Leicester LE1 7RH. Tel No: Leicester (0533) 551234 Ext 277.

Previous applicants for this year's studentships at the Biocentre need not apply. (6598)F

BIRMINGHAM GENERAL HOSPITAL

University Department of Medicine

SERC — CASE RESEARCH STUDENTSHIP

The project is to investigate factors influencing the response of patients with chronic suppurative chest disease to antibiotic therapy. These include antibiotic penetration and ß-lactamase production and will necessitate the development of suitable immunological assay systems which will be undertaken, in part, at the laboratores of Beecham Pharmaceuticals.

Candidates should possess, or expect to obtain, a first or upper second class honours degree in a suitable subject. Three copies of the application should be sent as soon as possible to Dr D. Burnett, Clinical Teaching Block, General Hospital, Steelhouse Lane, Birmingham, Telephone: (021) 236 9922, who can also supply further information (6545)F

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Further information can be obtained from Professor J L Knill, Department of Geology, Imperial College, London SW7 2BP. Tel: 01-589 5111 ext 5501 to whom applications, with names of at least two referees, should be sent by 1 July 1985. (6522)O

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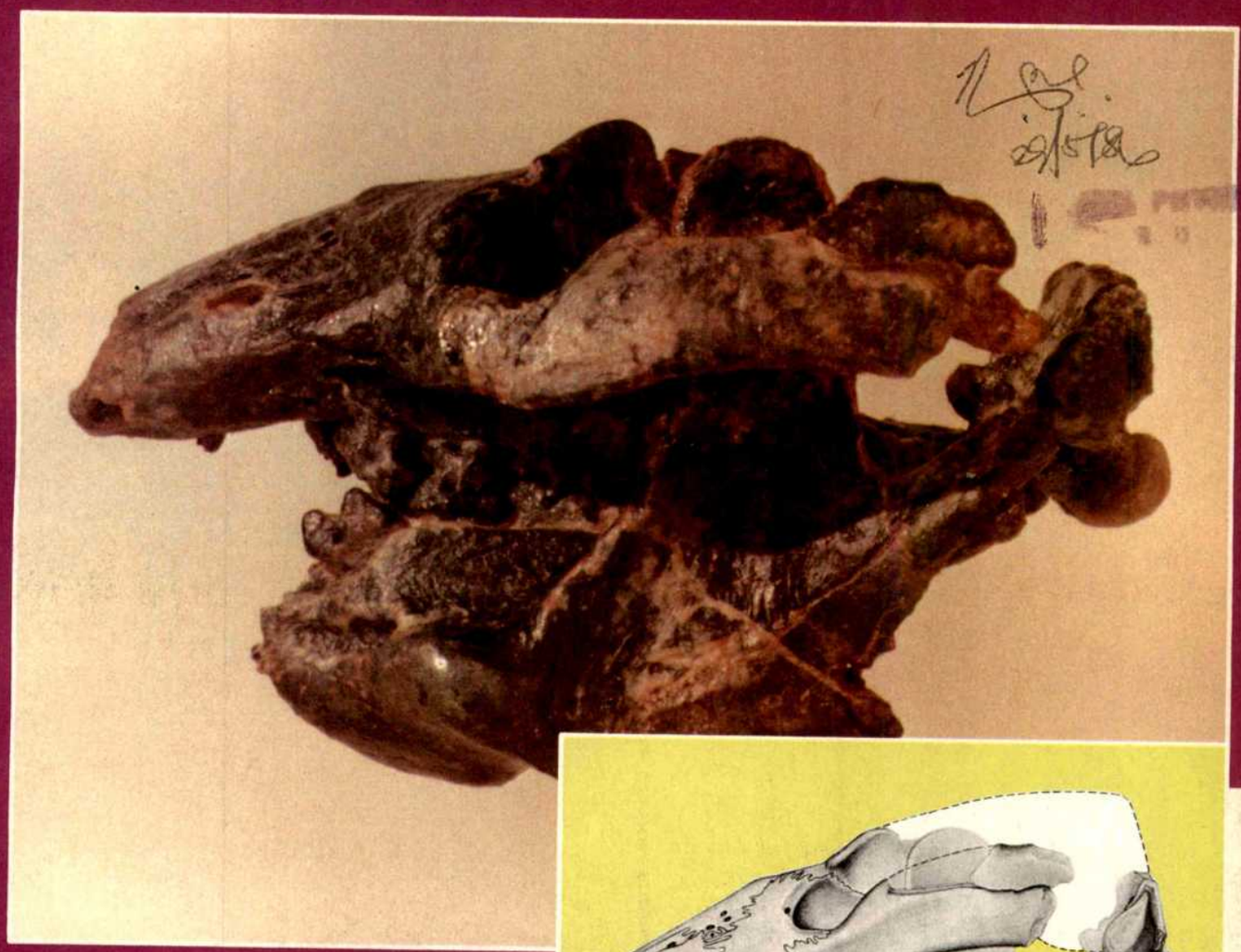
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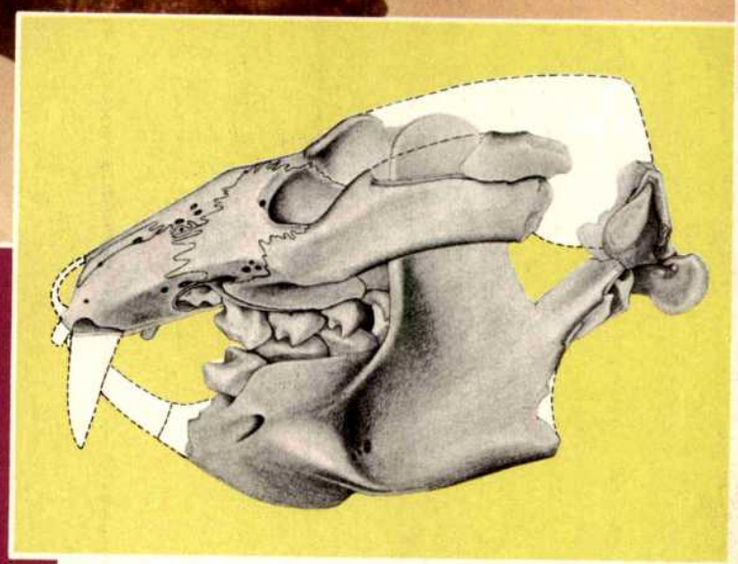
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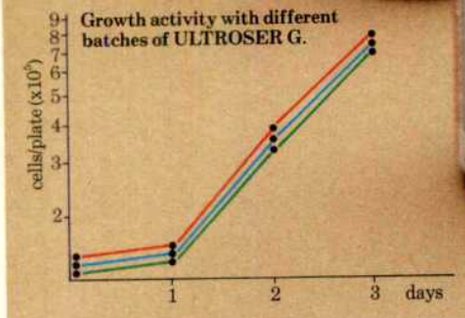
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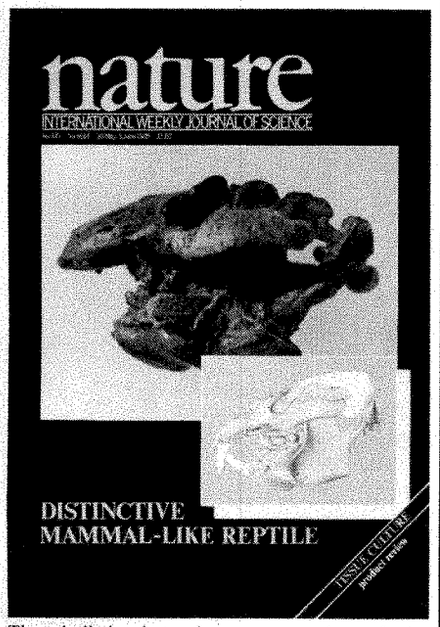
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NATURE VOL. 315 30 MAY 1985

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The skull is that of a new member of the Tritylodontidae — the last of the great mammal reptiles of the Jurassic. Its distinct morphological features have clarified evolutionary relationships between members of the family and these are discussed on p.398.

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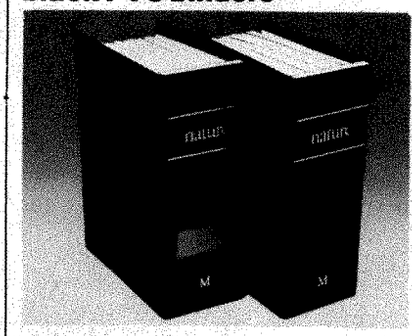
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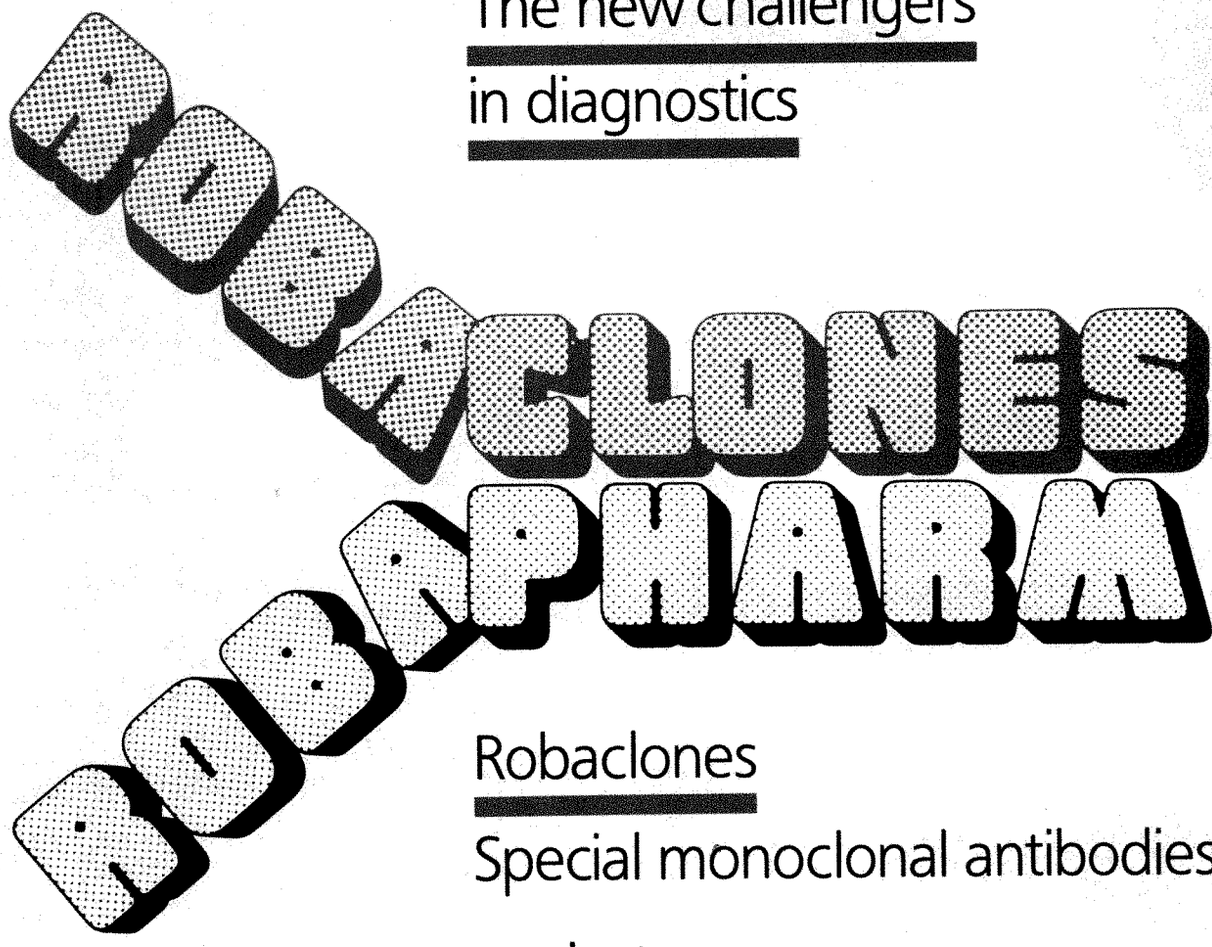
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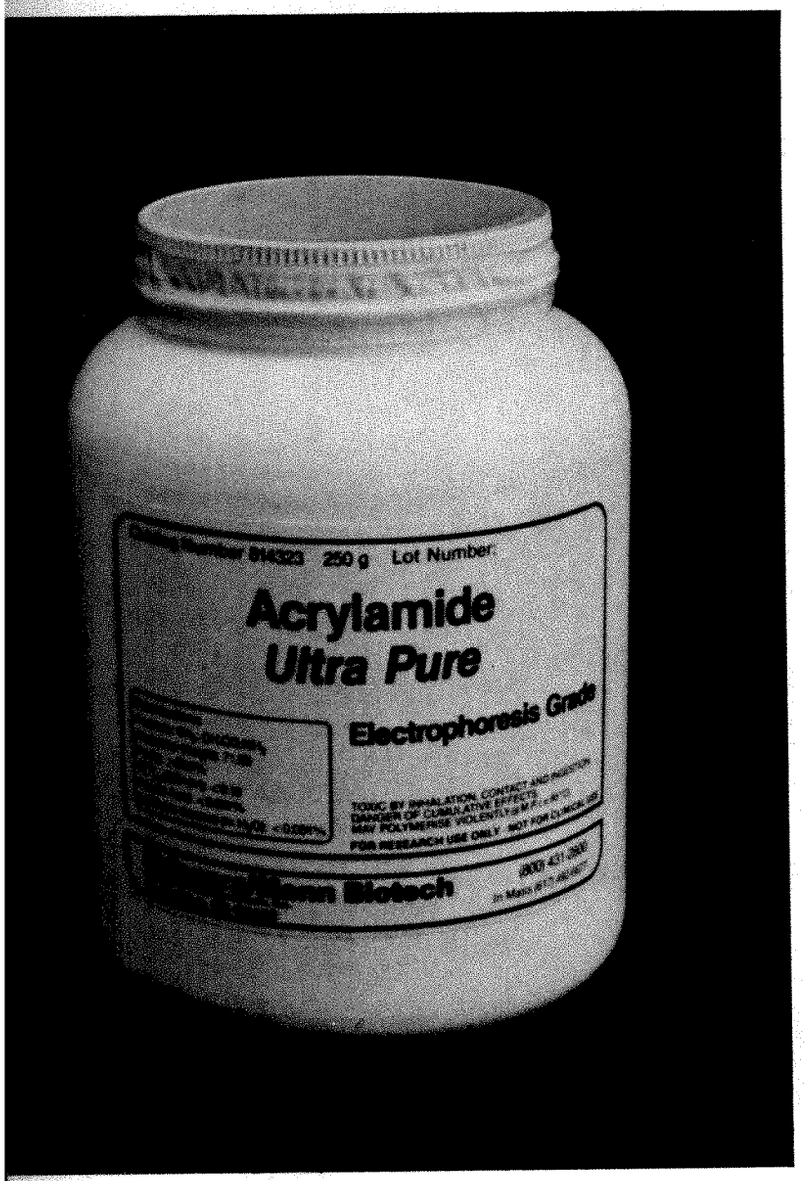


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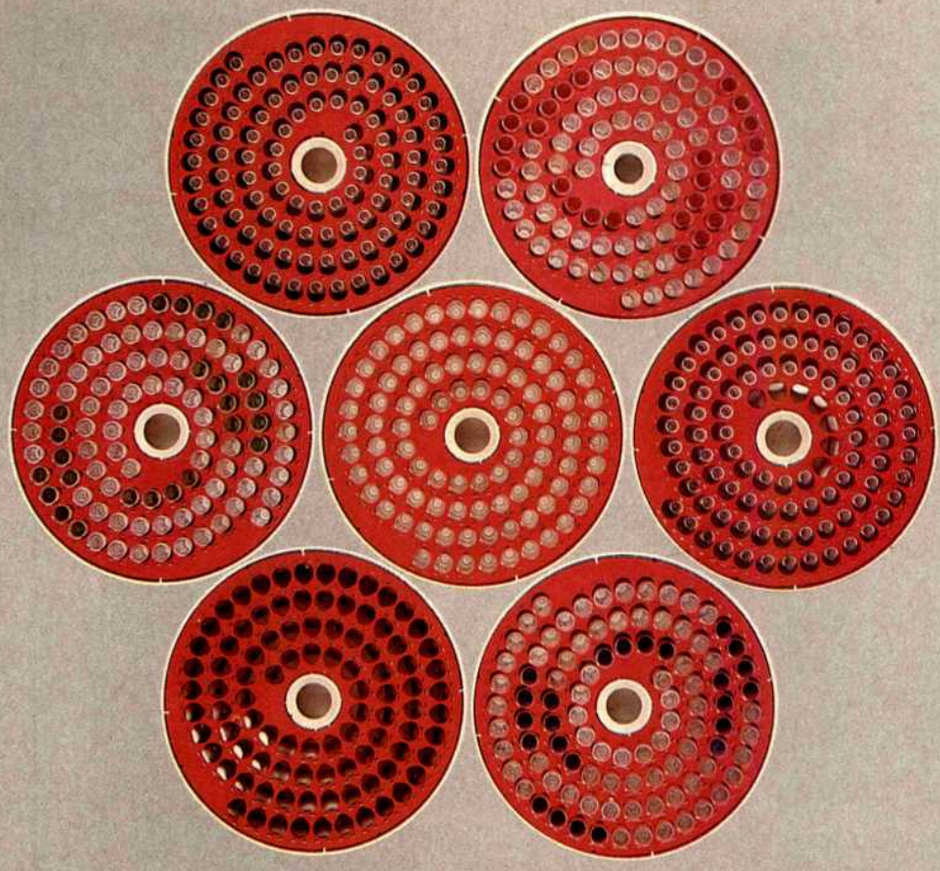
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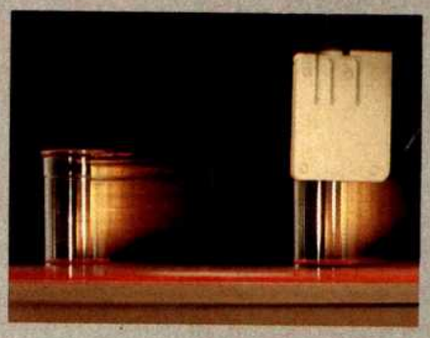
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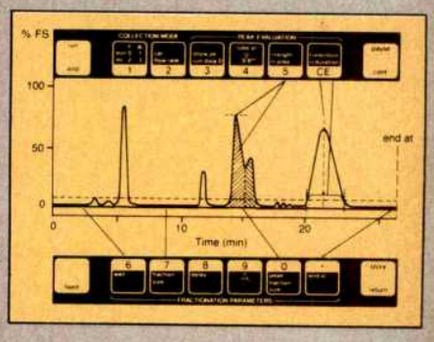
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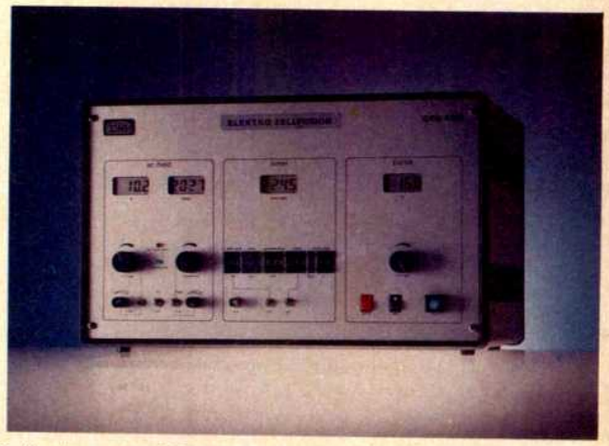


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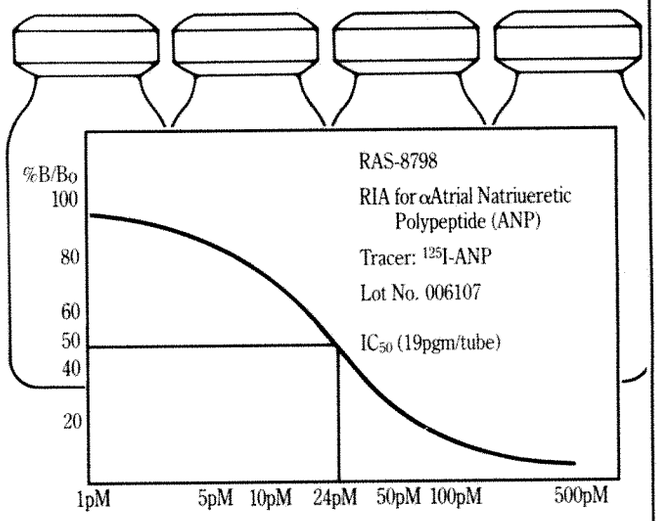
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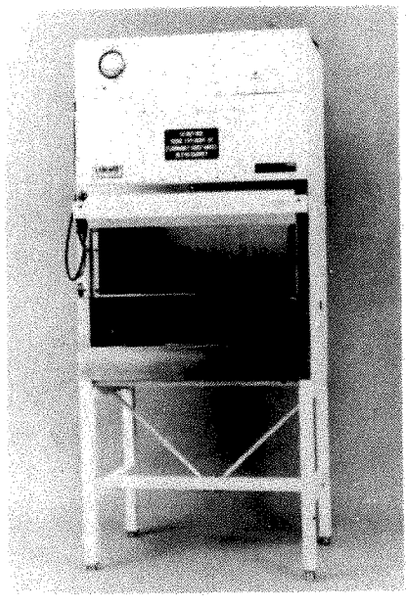
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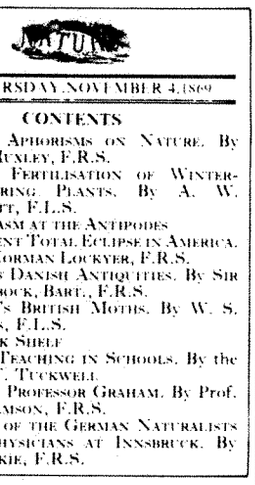
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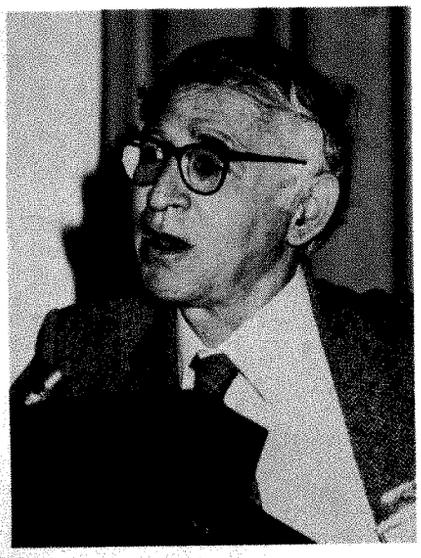






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Dr. A. Klug, MRC Laboratory of Molecular Biology, Cambridge.

AFTERNOON

Antibodies
Dr. J. Birch, Celltech Ltd., Slough.
Dr. M. Neuberger, MRC Laboratory of Molecular Biology, Cambridge.
ROUND TABLE DISCUSSION

Day 2 Wednesday, June 26th

Biosensors
Dr. M. Flanagan, University College, London
Professor I.J. Higgins/Dr. A. Turner, Cranfield/Leicester.
Micro-organisms
Dr. S. Emtage, Celltech Ltd., Slough.

Micro-organisms continued
Professor D. Hopwood, John Innes Institute, Norwich.
Dr. B. Carter, Searle R&D, High Wycombe.
ROUND TABLE DISCUSSION

Day 3 Thursday, June 27th

Mammalian systems
Professor P. Tiollais, Institut Pasteur, Paris.
Dr. A. Levinson, Genentech, San Francisco.
Dr. R. L. Brinster, University of Pennsylvania

Plants
Professor M. Fowler, Wolfson Institute, Sheffield.
Professor J. Schell, Max Planck Institut, Köln.
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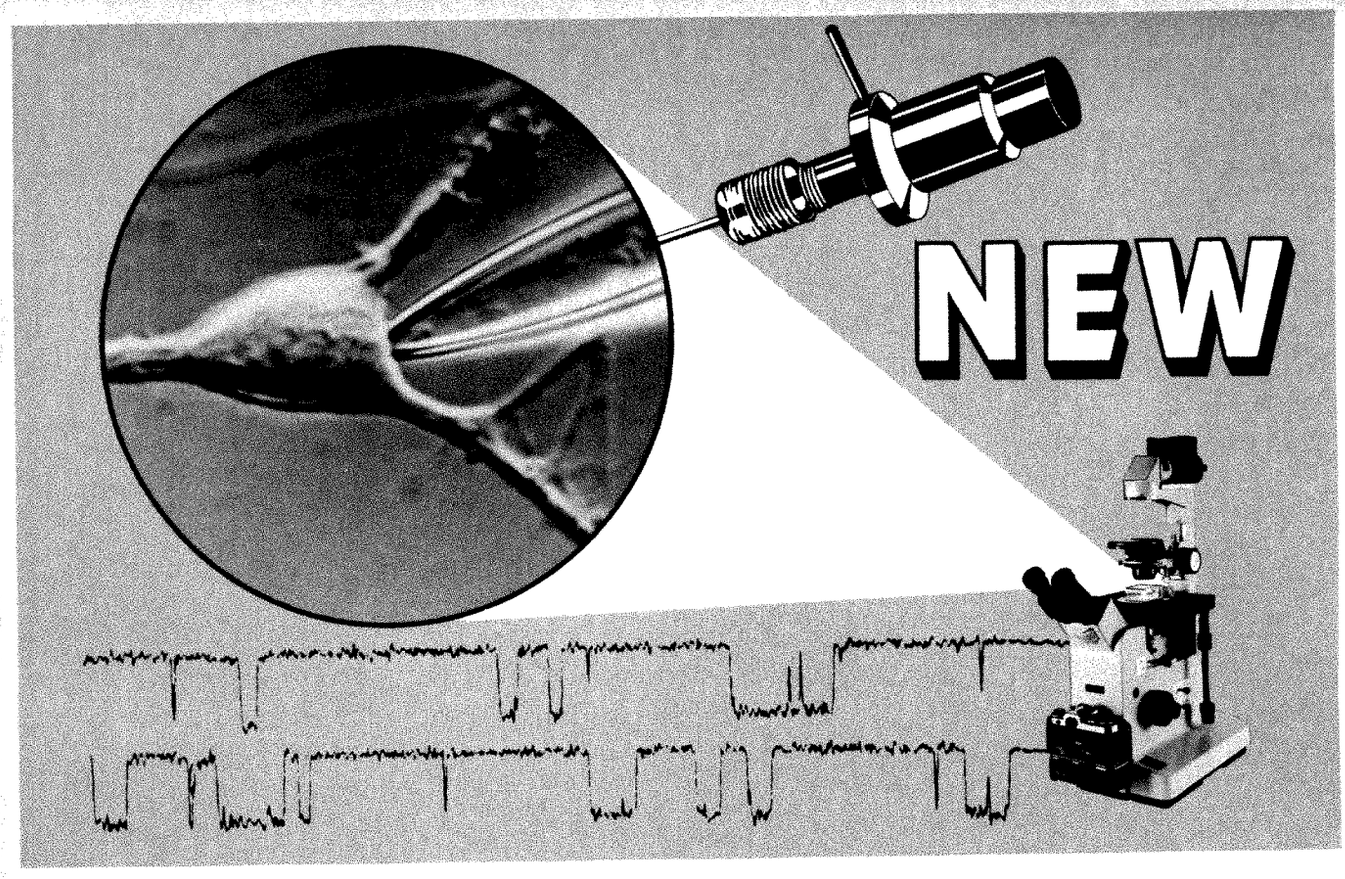
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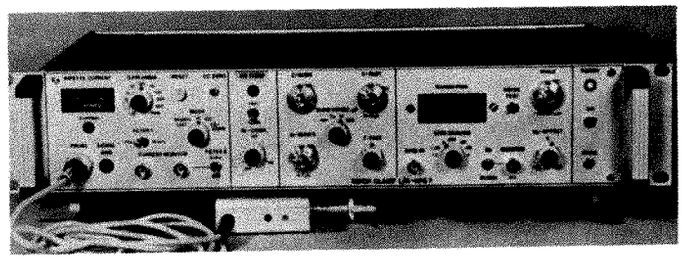
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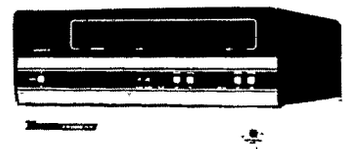
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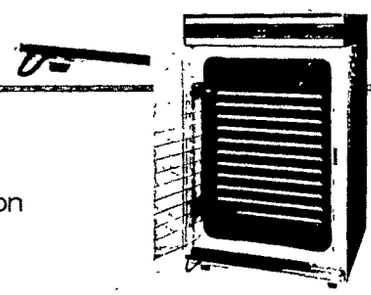


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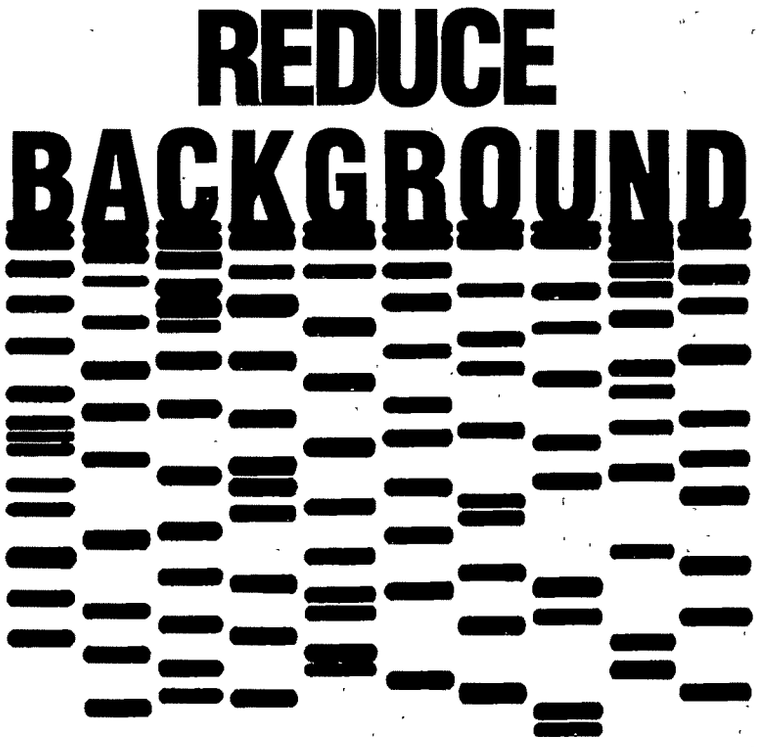
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More crisis for UK research

Now it seems that Britain will stay in high-energy physics for the time being, high-energy physics has an obligation to mitigate the squeeze on general research.

THE Kendrew committee, which has been looking into British membership of CERN, the European nuclear physics laboratory at Geneva, seems about to create a dilemma for everybody in sight — the British Science and Engineering Research Council, which pays the British subscription, the Advisory Board for the Research Councils, which has just presented the British government with a demand for extra cash, and even the management of CERN, the essence of the committee's conclusion seems to be that Britain should not immediately pull out of the European high-energy physics club, but instead demand that the cost of membership should be reduced from the end of this decade, when CERN will be equipping with superconducting magnets the electron collider now being built. Precisely what the committee thinks should happen if these negotiations come to nothing should be clear when its report is published. It will be particularly valuable to know why the committee has decided against withdrawal. Is high-energy physics after all worthwhile, or is it merely that the British research community will need the best part of five years to win what it can from past investment in CERN?

That the outcome is a compromise will be an embarrassment. Although high-energy physics is a steadily shrinking part of the budget of the Science and Engineering Research Council, it is still uncomfortably large. Last year, the advisory board was saying that the research council might have to withdraw from some whole field of science if the squeeze persisted. The Kendrew committee sprang from the sense that high-energy physics, the most conspicuous target for economizers, is too important to be abandoned just like that. The chief consequence of the Kendrew compromise is that there is no immediate prospect of relief. The reappraisal of all activities that has occupied the research council for the past six months will have to continue. Nor will there be much comfort in the recent guileful letter to Kendrew from Dr Jeremy Bray, the Labour Party spokesman on science and technology, pointing out that a future government might have different views about science budgets.

Speculations prompted by that kind of prospect are neither here nor there. Any British government would find itself in the same plight on public spending as does the present. Extra money for research, such as the advisory board has now asked for (£10 million extra next year and in each of the two succeeding years) would have to come from the British government's present spending programme. It would have been more interesting, but dangerous, if the Kendrew committee had resolved flatly to say that the research community cannot afford high-energy physics out of the present budget, and had thrown the onus of grappling with the consequences onto the government.

The advisory board, whose role is to divide the science budget each year, will now be particularly under stress, if only because some of its members are at best agnostic on high-energy physics. But continued membership of CERN squares badly with economies being made elsewhere. Out of a total budget of £631 million, the research councils will have to spend £9 million this year and £11 million next year on persuading research people to retire early. That is simple waste. The advisory board is also acutely aware, to judge from this year's submission to the government, that its own economies will have to take effect before there can be much change in the pattern of spending within the universities on the support of research, which is estimated (a little optimistically) to be worth £550 million a year. Only in the academic year beginning in 1986 will there be the first signs of how the budgets of individual universities will be changed to take account of the importance attached to the research they do. So the muttering has begun that part of the university budget should be handed over to the research councils, which is a recipe for setting academics at each others' throats.

This is where the physics community, and the management of CERN, can help. Both have in the past been dealt with generously, and may by now have earned an obligation to do something for fields more recently made vigorous. The truth about CERN is that it is every bit as splendid a laboratory as its founders intended. It is a model of international collaboration, and it is successful in the work it does. For the past decade, conscious of its cost, the laboratory has also managed to keep ahead technically by finding the money with which to build a new accelerator by closing down an older machine, or by using it as a component for a larger one. But the laboratory has some defects. It has a larger permanent staff than might otherwise be necessary, perhaps because it is international. Much worse, it happens to be in Switzerland, one of the most prosperous countries in Western Europe and that which determines what people working there are paid. Nobody suggests that the new electron accelerator should be moved somewhere else (which would be impossible) or that people working at CERN should be underpaid, but this privileged laboratory should now help solve the problems that have arisen in Britain, and even elsewhere.

What can CERN do? Economizing is an obvious must, but cheeseparing would not save anything like as much as Kendrew appears to be looking for. Broadening the base of collaboration on the second phase of the electron collider is a much better course to follow. Neither the United States nor the Soviet Union is at present a paying member of the club, even though people from each country work on CERN collaborations by a kind of gentlemen's agreement, on the ground that reciprocity eventually accrues. Why not now formalize these arrangements? If CERN has shown that European collaboration in high-energy physics works, why should it not use its present position on the crest of a wave of success to show that international collaboration can also succeed? With the US Congress in the thick of trying to reduce the US budget deficit, this might be just the time for such a move. One of the curious ironies of this field, over several generations of particle accelerators, is that the practitioners are forever saying that machines will eventually have to be built and operated internationally — but not just yet. □

Biological manuscripts

From the beginning of next week (20 May), Miranda Robertson, Biological Sciences Editor of *Nature*, will be based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees). □

US universities

Task force quests for financial wizardry

Washington

THE Congress's Science Policy Task Force was told last week that between $5,000 million and $20,000 million might be needed to bring US university research facilities up to acceptable modern standards, and that the existing partnership between universities, industry and government "may not be adequate" for the task. While the general sentiment has been heard before on Capitol Hill, this particular warning was surprising because it was uttered not by some university president making a cap-in-hand appeal for a new laboratory, but an assistant director of the White House's Office of Science and Technology Policy, Dr Bernadine Healy.

In two days of hearings on "government and the research infrastructure", the task force heard a variety of prescriptions for improving universities' access to state-of-the-art equipment. In Healy's view, both government and universities must take their share of the blame for the accumulated deficit of research hardware. Universities, she said, have often behaved like dependants of the government, "abdicating their responsibility for infrastructure and biding their time until federal facilities and programmes were resumed". The government, for its part, has "attempted not to invest in the research enterprise, but to procure packets of research results at the lowest possible prices".

The Science Policy Task Force, a bipartisan group established within the House of Representatives' Committee on Science and Technology, has been hearing from witnesses in government and academic life since mid-April. The hope is that a preliminary report will be completed by May next year, with a final version available the following October. Witnesses so far have fallen into two clearly defined camps: those who believe that the present system for provision of research facilities is working well, and those who feel that catastrophe is imminent.

The latter group argues that universities grew accustomed to 15 per cent annual increases in their operating research budgets during the heady 1970s, and even during the early years of the first Reagan presidency. As long as the hefty increases continued, universities were cushioned from the effects of the lack of investment in buildings and large items of equipment: most of the federal programmes specifically geared to improving research infrastructure had dried up in the early 1970s. Now that the days of 15 per cent annual increases are seemingly over, the price of years of underinvestment in the infrastructure will have to be paid.

Healy, speaking with the advantage of inside knowledge of the White House's soon-to-be-completed study of the universities chaired by David Packard, said that a multi-billion dollar programme might improve conditions, but that changes in attitudes would also be needed to put things to rights. Specific proposals include the following:

- the proportion of the $20,000 million civilian research and development budget spent in universities should be increased above its present level of 30 per cent;
- unrestricted donations of equipment and contributions to renovations should be encouraged (presumably by favourable tax exemptions);
- amortization periods for both equipment and buildings assumed in federal grants should be reduced from their present unrealistically high levels (50 years and 15 years respectively) to something closer to reality (say, 20 years and 6-8 years);
- and (as a strong hint) the government should stop trying to "micro-manage" equipment purchases and reduce the burden of excessive documentation.

The need for innovative financial approaches was echoed by Dale Corson, chairman of the government/university/industry research round table sponsored by the National Academies of Science and Engineering. Corson asserted that obsolescence of research instruments is limiting productivity, and that in engineering, in particular, this state of affairs is increasingly driving away potential recruits into the field.

Corson argued that the problem has arisen largely because of the increased cost of research instruments; he proposed that more use should be made of shared facilities and that more facilities should be financed jointly with state governments. Besides exploring new ways of providing equity, the traditional form of financing for research facilities, universities should also look at new ways of debt financing. Corson asked for the removal of legal obstacles that, in essence, prevent the promise of federal grants from being used as collateral against loans.

Summing up in a virtuoso exhibition of Washington bureaucratese, Corson asked that these initiatives be brought together in a national programme that will "regularize the facilities appropriation process" and will "leverage federal funds to the maximum degree possible". Corson's proposal will doubtless be aired in July at a conference on academic research facilities sponsored by the government/university/industry research round table and federal agencies.

Tim Beardsley

Growth hormone

FDA ban on pituitary product

Washington

FEARS that growth hormone extracted from human pituitary glands may spread the rare neurological condition known as Creutzfeldt-Jakob disease (CJD) have prompted the US Food and Drug Administration (FDA) to stop the use of all human pituitary products. Those most immediately affected are the 2,500 hypopituitary dwarfs in the United States, who depend on the hormone to maintain normal rates of growth; clinical research on other pituitary products has also been suspended. FDA is now under strong pressure to approve the general use of a recombinant human growth hormone product manufactured by Genentech Inc. that has been under FDA review for the past 18 months.

The two commercial companies that have now withdrawn their human-derived growth hormone products, KabiVitrum and Serano Laboratories, point out that the evidence against them is at best indirect. There have been three recent deaths attributed to CJD; among young men who received crude pituitary extracts as children; suspicions were aroused because the disease is normally found only in those over 40. Only one diagnosis has been confirmed, however, and no autopsy was carried out on one of the suspected cases. In the other two cases, there are other complicating factors that could explain infection by a slow-acting virus such as that believed to cause CJD; one was a diabetic who received frequent insulin injections, for example.

The deaths occurred among patients who had been treated under the National Hormone and Pituitary Program of the National Institutes of Health (NIH). Since the programme started, the purification methods used have improved markedly, and all growth hormone manufactured since 1977 has been through a final stage of column chromatography using Sephadex. The commercial manufacturers, who also use column chromatography, point to a study by Professor A.G. Dickinson of the University of Edinburgh which indicated that virus deliberately introduced into pituitary tissue was not detectable after the column chromatography stage of purification.

One argument against a link between CJD and growth hormone is that the hormone is produced in batches that are used to treat several hundred children. Even if only one batch had been contaminated, therefore, many more than three cases would be expected. There are not known to be any living growth hormone recipients with CJD symptoms, and a recent survey of 300 recipients in Switzerland found no cases of CJD.

Erol Caglarcan of Serano accuses FDA of using "unnecessary pressure" and says the Serano product, which has the trade name Asellacrin, will still be used outside the United States. Serano hopes in time to market a genetically engineered growth hormone being developed by Celltech in England, but that product is not yet at the stage of clinical trials. KabiVitrum's recombinant product, developed under licence from Genentech, is already in clinical trials in Europe: the company is correspondingly less concerned about FDA's action in banning human growth hormone.

The pressure on FDA to approve Genentech's recombinant product as quickly as is seemly arises because testing the pituitary samples for presence of the CJD infective agent has to be done by bioassay, using squirrel monkeys or chimpanzees. Besides being expensive, results of tests now being mounted by NIH will not be available for two years. In any case, negative results from such tests would not provide much reassurance, given that so little is known about the disease. If Genentech's product can be shown to be safe, FDA might be tempted to abandon pituitary-derived growth hormone permanently.

A decision to abandon pituitary products would, however, affect other substances besides growth hormone. Dr A.F. Parlow of the University of California at Los Angeles, who has supplied pituitary products to NIH since 1977, is concerned that research into other pituitary hormones could be set back; clinical trials of prolactin and thyroid-stimulating hormone, for example, have had to be put on ice. Parlow believes that FDA will have to embark on a convincing demonstration that viruses can be effectively excluded by modern purification methods in order to prevent a complete halt in pituitary research.

Genentech first produced a recombinant growth hormone product several years ago, but — perhaps because of the ready availability of the human-derived product — progress towards FDA approval has been slow. The Genentech hormone has an extra methionine residue tagged on that is not found in the natural substance, and in early clinical trials many patients developed antibodies to the product.

Last year, FDA asked Genentech to provide further safety data on a trial population to be monitored for a full year; that study is now under way but the data requested by FDA will not be complete until the start of 1986 at the earliest. Recently, however, FDA officials have been saying publicly that the Genentech product, which has now been improved, is likely to be approved within the next two or three months

Genentech, for its part, is playing it cool, saying the decision is entirely up to FDA; a recombinant growth hormone without the extra methionine is also believed to be in the pipeline. **Tim Beardsley**

French science budget

Fabius goes for growth

M. HUBERT Curien, French minister for research and technology, at last has his figures.For the past few weeks he has been waiting to hear from the Prime Minister, M. Laurent Fabius, how much money there is in the kitty for French research for the next three years, to put some substance in his "three-year plan" for research — an instrument which parliament must vote on and which should give a new impetus to the political emphasis on science in France. The Prime Minister's answer is four per cent real growth per year in civil research and development spending to 1988, excluding defence research spending.

And that is just the government side. There will also be increased incentives to industry to carry out research (a doubling of tax allowances on research and development budgets) which will give industry another FF 600-700 million (£60-70 million) a year to spend, so that overall it is estimated that French national research and development spending will rise over the next three years from 2.25 per cent of gross national product now to 2.6 per cent in 1988. At its nadir in 1979-80, this fraction had touched 1.8 per cent.

Thus the growth in French research spending should continue, provided there are no global budget "corrections" in future, corrections which rather reduced the true impact of the ambitious "law for science" introduced in 1982 by M. Curien's predecessor, M. Jean-Pierre Chevènement. But Chevènement also increased the number of jobs for scientists, and this trend is to continue. While British research councils have lost around a fifth of their posts since 1981, their French counterparts have gained something around 3 per cent a year. Curien's plan offers 1,400 new jobs for scientists over the next three years, less annually than last year's 1,000, but still a substantial figure.

So where are the French after all this attention to science? Doing fairly well, but could do better, particularly in industry, is the informed verdict in Paris.

Briefly, the tally is thought to be this. First, a reversal of the decline of support for French science in the 1970s. The decline was brought about initially by President Pompidou's massive indifference, and continued by successive administrations until Pierre Aigrain, science minister under President Giscard d'Estaing, reversed the trend. Second, M. Chevènement, under President Mitterrand, made science a political touchstone, and changed the whole French political mentality towards science, and of scientists towards industry. Third, the research councils — such as the Centre National de la Recherche Scientifique (CNRS) with its 10,000 scientists — were accorded looser legal ties, which allowed them to make profitable associations with industry, set up affiliates and so on. And fourth, science and technology were given weight, and budgets, in the regional administrations of France, which gave an opportunity for a more flexible response to local industrial and social needs. A fifth bonus, the new impetus given to universities to compete with one another for students and research cash, has come too late to yet have any real impact.

Failures, however, include a poor response from industry, too much centralization of decision-making and too little realistic assessment of the successes and failures of the policy.

There is hope, however, that Curien will be able to correct many of these failings. The new three-year plan, agreed at ministerial level but not yet voted upon, is one of his instruments. A practical man, Curien seems to be taking many of the right steps to put the rebirth of French science back on course. **Robert Walgate**

Australia's NDP in disarray

Canberra

AUSTRALIA's single-issue Nuclear Disarmament Party (NDP), whose 8,000 members include many former Labor Party supporters disaffected by the Hawke Labor government's decision in June last year to mine and export uranium, has lost its only parliamentary representative-elect, Ms Jo Vallentine, who resigned last week, announcing that she would set up a new anti-nuclear political party to be known as Peace and Nuclear Disarmament Action (PANDA).

This move follows NDP's first national conference in Melbourne last month in which Senator-elect Vallentine, former Labor Senator Ms Jean Melzer and narrowly-defeated NDP Senate candidate and rock singer, Mr Peter Garrett, staged a walkout, claiming that NDP was in danger of falling under the domination of entryist members of the Socialist Workers Party, a Marxist grouping of Trotskyite tendency. The conference was to have given some direction to NDP policy, because the party had only six months to organize before last December's half-Senate elections.

One of the chief reasons given by the breakaway group for leaving NDP was the party's "infiltration" by people who were already members of other political parties: their proscription is likely to be a feature of membership of PANDA. The members of Ms Vallentine's West Australian branch of NDP were canvassed by a postal ballot that ended in a vote to cut all ties with the national body and form a separate party. Breakaway groups in most other states are holding similar ballots whose results will be known next month. Jeffrey Sellar

US investment

Universities and South Africa

Washington

THIS spring has brought to many US college campuses demonstrations, reminiscent of the 1960s, against apartheid in South Africa. With the demonstrations has come a reconsideration of an old issue: should universities invest in companies that do business in South Africa?

According to several recent surveys, fewer than a dozen universities have completely divested themselves of holdings in such companies. And even as pressure mounts to divest, a new study by the Investor Responsibility Research Center, an organization founded 13 years ago to provide institutional investors with information on the ethical behaviour of corporations, may stiffen the resolve of those university boards that have resisted so far: according to the report, "divestment will have a detrimental effect over the long term on portfolio performance".

Most of the hundred or so universities that have taken some action on investments in South Africa, mostly in response to protests in the early 1970s, have adopted policies of partial, selective divestment, usually selling off stock in companies that fail to adhere to the so-called Sullivan principles. These rules, developed by Reverend Leon Sullivan of Philadelphia, a member of the General Motors board of directors, prescribe basic fair employment practices for companies operating in South Africa. The universities that have opted for partial divestment argue that a selective policy is the best method of influencing corporate behaviour; with across-the-board divestment, influence is lost.

Those calling for total divestment now say that this selective policy simply has not produced results. According to a recent survey by the American Council on Education (ACE), only about 100 of the 350 US companies operating in South Africa adhere to the Sullivan principles; and in any case there is no evidence that other foreign companies or South African companies have been influenced by the example of these 100. Bishop Desmond Tutu, who won the Nobel peace prize for his stand against apartheid, has called for a more stringent set of principles: companies in South Africa should recognize black labour unions, invest in black education and training, and should freely hire blacks without regard for the country's restrictive laws governing the movement and residence of the 24 million South African blacks.

The most militant protests this spring have taken place at Columbia University and the University of California at Berkeley, both of which already have policies of partial divestment. Students have occupied buildings and there have been a number of arrests.

And while both of those universities have agreed to reconsider their policies, others are being more outspoken in their belief that the chief responsibility of university financial administrators is to make money for the university. Derek Bok, president of Harvard, which has maintained a selective divestment policy since 1981, spoke for many when he said recently: "Despite our revulsion toward apartheid, the fact remains that Harvard's resources were entrusted to us for academic purposes and not as a means for demonstrating our opposition to apartheid or to other manifest injustices and evils around the world." The ACE survey noted that even among those universities choosing total divestment, the justification was that colleges "should not benefit from corporate practices that cause social injury"; they rejected the notion that they were taking a political stand.

US investment in South Africa is estimated at $14,000 million. About one-third of the companies on Standard & Poor's 500-company stock index do business in South Africa.

Besides the efforts on campus, a number of states and municipalities have ordered divestment. A bill now before the US Congress would ban bank loans and computer sales to the South African government.

Stephen Budiansky

Major universities that have divested stock in companies operating in South Africa

	Policy	Value of stock divested	Year action taken
Boston University	Partial	$6 6 million	1979
Brown University	Partial	4.6	1984
City College of New York	Total	7.1	1984
Columbia University	Partial	2 7	1979
Dartmouth College	Partial	2 0	1985
Harvard University	Partial	50.9	1981
		1 0	1985
Michigan State University	Total	7 2	1979
Ohio State University	Partial	0.3	1978
Rutgers	Partial	7 0	1985
Swarthmore College	Partial	2.2	1981
Tufts University	Partial	0.1	1979
University of California, Berkeley	Partial	4.0	1979
University of Michigan	Partial	41.0	1983
University of Pennsylvania	Partial	0.8	1983
University of Wisconsin	Total	11.0	1978
Yale University	Partial	1 6	1979
		4 1	1984

Universities that have adopted a policy of total divestment have sold all holdings in companies that do business in South Africa Partial divestment refers to a policy of selling stock in companies that have failed to adhere to the Sullivan principles or other standards of conduct or that refuse to provide information. Source: American Committee on Africa.

Age discrimination alleged

Washington

HOFFMANN-La Roche employees laid off earlier this year in what the company described as an austerity measure have sued, alleging age discrimination. The suit, so far joined by 250 laid-off workers all over age 40, accuses Roche of pursuing a deliberate policy of singling out older employees for dismissal. More than 1,000 employees were dismissed in a move to save $50 million a year for the next five years. Lawyers for the plaintiffs say that as many as 600 of these employees could be eligible to join the suit, which they hope to have certified as a class action.



The suit also alleges that the company's policies governing promotions, transfers and assignments favour younger workers at the expense of those over 40.

Federal law prohibits age discrimination in employment. Companies that violate the act are liable for back pay, compensation for loss of future earning power, attorneys' fees, and, if "wilful" violation of the act can be shown, double damages.

Leonard Flamm, an attorney for the plaintiff, says that most of the layoffs occurred at Nutley and Belvidere, New Jersey.

Hoffmann-La Roche says that it intends to defend all such lawsuits brought against it and maintains that all decisions to terminate employment were "nondiscriminatory". No date has been set for preliminary hearings. Stephen Budiansky

Japanese psychiatry

Abuse for visiting scientists

Tokyo

WESTERN research workers studying such topics as neurotransmitter receptors and the mode of action of antidepressants are not used to being thought of as brutal oppressors of society, nor do they conduct research in fear of assault. But, as two visiting lecturers found recently, for workers in these fields there are real dangers in Japan.

The two lecturers, Tim Crow of the Clinical Research Centre in the United Kingdom, and D. van Kammen of the University of Pittsburgh, were due to give talks on "Genes and viruses in schizophrenia" and "Episode markers in schizophrenia" at the seventh congress of the Japanese Society on Biological Psychiatry at Gifu, in central Japan. Soon after their arrival in mid-April, however, the speakers were told that the meeting had been cancelled because of threats made by "anti-psychiatrists". Instead, a smaller private meeting was arranged at a hotel in nearby Nagoya. But, twenty minutes after Dr Crow's talk began, news came that a group of 70 demonstrators were on the way to the new location; the talk was abandoned and the meeting hall evacuated.

Behind these strange events lies a 16-year feud within Tokyo University's hospital and accusations of serious abuses within Japan's psychiatric hospitals. These accusations resulted in an inspection visit earlier this month from Disabled People's International (DPI), based in Stockholm and, two weeks ago, from the International Commission of Jurists (ICJ) and the International Commission of Health Professionals (ICHP), both based in Geneva.

The Gifu meeting had itself attracted attention from anti-psychiatrists because lurid publicity had just been given to the ethics of research performed at Gifu University. In February 1984, a schizophrenic woman patient admitted to a local hospital had received an abortion; the fetus's brain had then been examined in the university to look at the effects of drugs the patient had been taking. Accusations followed this year, however, that the patient had wanted to have a child and that she had been forced into the abortion in order to provide research material for a "human experiment". Although the matter was taken up by the Diet, investigations have not so far revealed malpractice.

The "anti-psychiatrists", however, see this experiment as just one more example of the "research supremism" they claim permeates the psychiatric community and which regards psychiatrically-ill patients as being without rights, mere fodder to further scientific careers.

This view has been propagated by a radical group of doctors who, in a truly bizarre episode, established themselves within Tokyo University's hospital in the wake of the student revolt in the 1960s. At the end of the troubles, in 1969, some radical doctors, opposed to the current form of psychiatric education and treatment, seized the in-patient psychiatric ward and used violence to prevent those who did not support them from entering the ward. Since then most of the university's neuropsychiatrists have not been able to enter the building or see the remaining patients.

An attempt was made to solve the problem in 1972. Relatives of confined patients were asked to consider moving them elsewhere; some, but not all, did so. Eventually, as doctors and nurses left, and radical doctors from elsewhere began to work in the ward, the point was reached when there were no officially-authorized staff left. The university, however, continued to supply drugs, food and equipment and to avoid confrontation on the grounds that that would not be in the interest of the remaining patients.

This strange situation persisted until 1978, when the Diet appointed an investigatory committee. The committee put pressure on the university to solve the problem without suggesting how it might be done.

The university eventually chose to avoid a fight and to legitimize the occupiers' position. University posts were given to the radical doctors, the professor was in turn allowed to make a once-a-week token visit to the ward, and a single patient was sent there from the out-patient department.

Relations between the "orthodox doctors" in the out-patient department and the "radical" doctors in the in-patient ward have not however, improved. Out-patient doctors complain of frequent abuse and physical assaults from radicals who enter the out-patient ward, often even in front of patients undergoing treatment. No evidence of unethical conduct by members of the out-patient group has been presented to justify these assaults; instead it seems as though they are being made to answer for abuses that have occurred elsewhere — the very abuses that have led to appeals to the United Nations and visits from human rights groups.

The most notorious incident occurred last year at the Utsunomiya hospital where it was alleged that patients were regularly beaten, two of them to death, one for trying to escape and one for complaining about the food, and that unqualified staff and some patients were forced to act as nurses. The director was eventually arrested, although the case has never been completely cleared up.

The official government view, echoed by many psychiatrists, is that the Utsunomiya case is a rare exception. One person who believes otherwise is lawyer Etsuro Totsuka, who for the past few years has been working almost without pay investigating psychiatric abuses. He stresses that there is an abnormally large number of psychiatric patients in Japan (more than 300,000), many of them hospitalized for exceptionally long periods and most of them confined against their will (more than 80 per cent, against only 5 per cent in the United Kingdom). Hospitals, the great majority of them private and generating large profits, are usually built in remote areas where land is cheap but rehabilitation of patients through integration back into the community almost impossible.

Two main factors seem to lie behind these trends. First, Japan's Mental Health Law Article 33 allows a hospital administrator to commit people without their consent so long as parents or guardians agree; expert opinion from doctors is not required. Thus, as an editorial in the *Asahi* newspaper put it, there need be no surprise at the abnormally large number of people in mental hospitals because "decisions on whether or not to put the mentally ill in hospital are made, in many cases, by the hospital managers, who stand to profit from hospitalization".

The second factor is the considerable stigma still attached to the mentally ill. All too often, families with mentally-ill kin seem to want to dump them where they cannot be seen. Complaints of ill-treatment are thus likely to be few as people prefer not to be identified as having a mental patient in the family.

Detailed reports are not yet available from the three groups that made visits to Japan following lawyer Totsuka's appeal to the United Nations through the Fund for Mental Health and Human Rights that he helped set up. But James Donald of DPI has made an interim statement that "there appears to be no requirement that the decision to commit be made by a qualified expert there is no requirement for independent review of the decision no effective independent counsel is provided to the patient; and there appears to be no access to information on care and treatment of patients once they are put inside and consequently no protection from abuse". Lack of appropriate laws and regulations, and the absence of government supervision, lack of financial incentives to medical professionals for treatment in the community, and lack of general recognition of these rights by the larger community are all blamed for the current state of affairs.

In the eyes of foreign experts, legal reform is thus clearly indicated. Within Japan, however, the issue has so far excited little interest, except among those whose views have already become extreme. What is particularly lacking is a broad-based reform group, like MIND in the United Kingdom, even though there is a movement towards community care among psychiatrists. Even if the true scope of psychiatric abuse can be assessed and, if abuse exists, legal reforms made to remedy it, it is hard to see psychiatrists and "anti-psychiatrists" being reconciled.

Alun Anderson

Poland

University advances checked

THE Polish government's plans to reform the 1982 Higher Education Act will go ahead, in spite of strong opposition from the academic community. Last week, Warsaw University held a massive protest meeting and launched a last-minute appeal to the Sejm (Parliament) against the changes. Nevertheless, it now seems obvious that the government and party authorities were determined from the beginning to press ahead with the amendments, and the public discussion, launched at the end of December, was merely a placebo to the university community.

The amendments, which would annul the autonomy won during the Solidarity era and would give the Ministry of Science and Higher Education virtually unlimited disciplinary powers over the universities, have been strongly opposed not only by the academic community, but also by the government-sponsored "Patriotic Movement for National Rebirth" (PRON), launched during the martial law period in order (it was hoped) to attract the support of the masses to the ruling junta (WRON — the similarity of the acronyms was hardly coincidental) PRON in fact has not picked up the expected mass support, although it has consistently tried to woo the academic community, backing, for example, a proposal to convert the Bialystok "filial" of Warsaw University into an independent university by 1991.

Originally, it appears, the state and party authorities had hoped to amend the law without public outcry. Unfortunately, in October 1984, a confidential draft of the proposed changes was leaked to the underground press. Although Dr Benon Miskiewicz, the Minister of Science and Higher Education, denounced the alleged leak as a "provocation", he shortly afterwards laid his own proposals for change before the Main Council for Higher Education (a kind of parliament of the universities, with one elected delegate from every higher educational institution in Poland, except the Catholic University of Lublin which elected to remain aloof when the Main Council was constituted in 1981). Although 60 per cent of the delegates to the council are party members, they strongly opposed the changes, on the grounds that to revise the law so soon after enactment would tend to "destabilize" the universities. Largely as a result of the main council's opposition, a "public debate" on the amendments was launched at the end of the year by the Committee for Social and Political Affairs, which reports directly to the cabinet.

Articles appeared in the press from leading academics condemning the amendments, and virtually the only organization that favoured them was the party-approved Association of Polish Students (ZSP), which under the proposed changes would become the only representative organization of Polish students. (At present, ZSP has very little active support). Referenda organized among university staff and students produced almost embarrassingly high votes against the changes. Nevertheless, the usefulness of such referenda and articles was questioned by a document signed by the underground Solidarity leadership of Warsaw's eight universities and higher colleges, which, by the end of February, could be found lying about in classrooms and libraries. This claimed that the alleged public discussion was merely a means of allaying public concern, while the authorities pressed ahead with the changes.

At about the same time, a ministry spokesman admitted that the issue was simply whether or not the universities "belonged to the state", and that if they did, the changes must, willy-nilly, go through. (My tape-recording of this interview was subsequently confiscated by the Polish customs (see *Nature* 14 March, 1985, p.120).) After the referenda ended in mid-March there were several weeks of seeming impasse. Then, on 13 May, during a special plenary meeting of the Central Committee of the Polish United Workers' Party on the role of the intelligentsia, Politburo member Jozef Czyrek stated explicitly that the universities were indeed "state owned and socialist" and that therefore the changes must go through, to ensure that "anti-socialist groups" did not exploit university autonomy for their own ends.

A week later, on 21 May, the government press spokesman, Mr Jerzy Urban, announced that there would be no further consultations with the public and that although he could give no date, the amendments would soon be laid before the Sejm. Warsaw University's 3,000-strong protest meeting the next day, which included an address by the former leader of the university's Solidarity chapter, Maciej Geller, and from which all outsiders were excluded by student "guards", made it clear that the university community will fight to preserve its hard-won autonomy.

Vera Rich

Synchrotron source

Italy drives a hard bargain

ITALIAN science minister Luigi Granelli is not at all happy with the Franco-German *fait accompli* that led, effectively, to the choice of Grenoble in south-east France as the site for the planned European Synchroton Radiation Facility (ESRF). Mr Granelli may have to accept the Grenoble site — Italy had proposed Trieste — but France and Germany may see little sign of his lira unless, in the accepted diplomatic tradition, Italy receives something in exchange. It now seems that he is not to be bought off with a promise that a European tritium-handling facility — needed for the time when the Joint European Torus, JET, or its successor, the Next European Torus, NET, need to use tritium to reach nuclear ignition — will be built at Ispra, north of Milan. It had been thought that this was the *quid pro quo* that would yield Italian aquiescence to, and money for the Grenoble site, but this is "not quite right", Mr Granelli said in London earlier this month.

At present there is deadlock in the European council of ministers, which meets next on 4 June, over ESRF, Granelli intimated, as "everyone is using their veto" to prevent France and Germany moving forward. Italy has proposed a site in Trieste, a region which the government is keen to develop in order to maintain its historical toehold at the top of the Adriatic, and offered more financial support than any other single country (including France or Germany). And still Italy must have something in Trieste to balance its support for Grenoble, Granelli insisted.

"There could be the large laboratory in Grenoble, and something different in Trieste", he said. "We should look at the wider context and consider making two or three laboratories." He was prepared to be flexible about what those laboratories would be. Denmark for example, which had offered a site at Risø for ESRF, would be happy with a laboratory for oceanography.

"The council should always consider a number of facilities at the same time, then the opposition doesn't become so stiff", said Granelli. The tritium handling facility could not go into the package with ESRF, however, because "from all points of view it is not an Italian request. It's a common interest for the European Community."

Granelli would like to see big machines for Europe discussed in a more flexible way in future "as long as the Commission of the European Communities is involved". There could be arrangements where two or three countries might agree on a facility but the Commission should also be made a partner, to allow other European states to participate in a smaller way through the Commission.

Professor Paolo Fasella, director-general for research at the Commission in Brussels, agrees that this would be a useful formula. "In a slow and practical way we're already investigating it", he said. The Commission had made initial approaches to national laboratories such as the Rutherford Appleton Laboratory and the Daresbury Laboratory in the United Kingdom and Frascati in Italy, for part-participation on behalf of the Commission's "stimulation programme". "We want to develop collaboration in a flexible way", said Fasella.

Robert Walgate

Ganges pollution

Cleansing river due for cleaning

New Delhi

INDIA'S holy river Ganges, which has been washing away the sins of devout Hindus for centuries, is itself to have a clean-up. The Indian government has set a five-year deadline for the first phase of cleaning the 2,500-km river, at a cost of £180 million. The river carries 468,000 million cubic metres of water a year, a quarter of the country's surface water.

The task has been assigned to the newly formed Ganga Authority, headed by Prime Minister Rajiv Gandhi himself. The eight other members include planning and environment ministers of the central government and chief ministers of three of the eight states through which the Ganges flows. There is also a 17-member steering committee under the chairmanship of environment secretary Dr T.N. Koshoo.

Pollution of the Ganges starts at Hardwar, where it enters the plains of Uttar Pradesh, 350 km from its source in Gangotri in the Himalayas. From Hardwar to Calcutta, the whole of the river has been declared by the Central Pollution Control Board (CPCB) as unfit even for bathing, and unsuitable for drinking without massive treatment. The board says the water has become so saline and so contaminated with toxic heavy metals that some parts of the Ganges basin are now infertile and the groundwater is polluted.

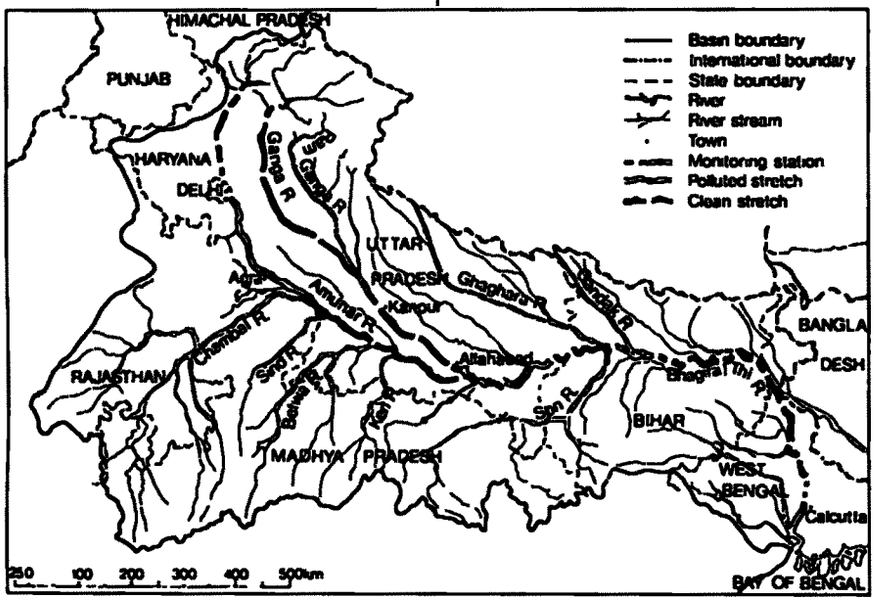
The Ganga Authority aims to restore the water quality under a plan drawn up by CPCB. Dr Nilay Choudhuri, CPCB's chairman, says that 80 per cent of the pollution is caused by raw sewage discharged directly into the river from 100 cities on its banks. The rest is caused by untreated effluent, wallowing and bathing of cattle, pesticide and fertilizer run-off and incompletely cremated dead bodies.

Apart from Hardwar, the most polluted places are Benaras, Kannauj, Allahabad, Patna, Calcutta and Kanpur, where the Ganges water is thick and turbid for 10 km because of effluent from tanneries, textile mills and chemical plants. Monkeys in Benaras have become addicted to opium in wastes discharged from a nearby factory making morphine from opium. On two occasions, the Ganges has caught fire because of oily effluent from a refinery in Barauni and discharges from a foundry forge plant in Hardwar.

Stopping the discharge of sewage into the river will reduce pollution of the Ganges by 75 per cent according to Dr Koshoo. Twenty-seven cities with populations of more than 100,000 dump 900 million litres of municipal waste each day. The plan is to renovate existing sewerage systems and pumping stations and to build new drains to divert the wastes to what are called Resource Recycling Units (RRU), where sewage will be digested to produce methane gas that will generate electricity. The residue will be sold as manure. Liquid remnants will be cleansed by biological aerators and the water produced will be used for irrigation and for growing algae and fish for sale.

According to Choudhuri, each RRU serving a population of 100,000 is expected to make an annual profit of £850,000, a sufficient incentive for private companies to take over the operation of the units after they have been built with government funds. In the second phase, the plan will be extended to the remaining 63 cities, and community cattle-sheds will be set up to collect animal wastes for processing at RRUs.

Unlike the Thames or the Danube, the cleaning up of the river Ganges is complicated by the fact that it is a sacred river to the Hindus. Millions of pilgrims immerse themselves in the river during festivals such as the Kumbha Mela and return home with a vessel filled with Ganges water.



Choudhuri says that a mobile laboratory found 10,500 coliform counts in 100-ml samples taking during a Kumba Mela in Allahabad. In Hardwar, 17.5 million litres of human and industrial waste pour daily from five drains, one of which is just upstream from where 10,000 people bathe every day and a million bathe during the Kumba Mela. At Benaras, 70,000 bathe daily in water that has been turned into an open sewer with the outfall of 120 million litres a day of sewage from 66 drains. Cremation in Benaras is the wish of every Hindu, so 400 half-burnt bodies float in the river on any day, and 9,000 cattle are thrown in each year. It is intended that electric crematoria will be built at several places along the river bank.

Even more alarming is the fact that the alluvial soil in the Ganges basin is showing signs of salinity in the state of Haryana, alkalinity in western Uttar Pradesh, acidity in West Bengal and calcareousness in north Bihar. Irrigation of Ganges water adds 17 million tonnes of salt a year to the soil, according to CPCB. The Ganges basin receives 3,000 tonnes of pesticides a year and one-third of all the fertilizer used in India.

K.S. Jayaraman

Polio eradication

Washington

A PLAN to eradicate poliomyelitis from the Americas by the year 1990 has been announced by the Pan-American Health Organization (PAHO). PAHO believes the goal can be achieved by using its staff and volunteers to coordinate separate national efforts to encourage immunization.

The total cost of eradicating polio from North and South America is estimated at $100 million: much of the infrastructure required is, however, already in place and only about $30 million will be needed in aid from developed countries, much of which is already committed. Since 1977, when the World Health Organization instituted an expanded immunization programme to fight several common infectious diseases, the incidence of polio in the Americas has fallen from around 2,300 cases a year to 489 cases in 1984.

Dr Carlyle Guerra de Macedo, director of PAHO, says that 75 per cent of those under one year old in the Americas are now immunized against polio; before the expanded programme started, the figure was 25 per cent. The new plan will concentrate on surveillance and on training health workers to implement vaccination schemes. While admitting that the goal will not be easily achieved, Guerra de Macedo pointed out that the Americas were the first region of the World Health Organization to succeed in eliminating smallpox. The polio virus, like the smallpox virus, cannot survive for long periods outside a host and there is no animal reservoir. Total world elimination of polio is therefore a realistic goal.

Tim Beardsley

Running down observatories

SIR — As a university astronomer who has had much to do with both the Science and Engineering Council (SERC) observatories at all levels, including serving on committees charged with looking into whether they could best be merged with each other, or elsewhere, I would like to comment on your leading article on "Running telescopes" (*Nature* 9 May, p.86) which is a mine of misinformation.

Before there is any talk of closing or merging anything, with all the personal and professional upset and delay that will certainly entail, the case has to be made, and indisputably made, that there are significant inefficiencies in the present system of two observatories at Herstmonceux and Edinburgh, sharing some of their tasks, computing for example, with the Rutherford Appleton Laboratory. To that extent I agree with *Nature* that the widest terms of reference must be given to the new committee, if there has to be one so shortly after the last, which I doubt, looking into the best way of running our overseas telescopes.

Building and operating a high technology instrument on an exceedingly remote and sometimes physically hostile foreign site, transporting shifts of UK university astronomers there, looking after them, educating them in the intricacies of the local equipment, which has to be maintained at the highest efficiency, providing them with the technical help to get first rate data, and later the sophisticated computer network required to reduce such data, must be a technical and management challenge of the same order as running an aircraft carrier full of Harriers at war in the Falklands. Mistakes are inevitable, and any number of committees can be set up to look into how the task can be better done, and of course the less they know about the practicalities the easier it will be for them to recommend sweeping changes.

The professional and the user, however, will only be convinced by a proper comparison, carried out largely by observationalists, of the existing UK set-up with its competent counterparts abroad such as the Cerro Tololo Inter-American Observatory (US) and the European Southern Observatory based in Munich. My own survey, for what it was worth, showed the UK observatories in a very favourable light. If the new committee fails to make a similar but much more careful comparison, co-opting the advice of astronomers abroad, and taking the time to do it properly, they will certainly have failed astronomers, like myself, out in the UK universities. At present we are lucky enough to have access to shared facilities which are remarkable not for their size, as your leading article implies, but for their sheer quality, something in which both observatories, and the Rutherford Appleton Laboratory, can take a just pride. Such quality arises not from lavish expenditure but from vision, high skill and dedication, as well as the very closest cooperation with the user community.

All this could be easily lost in the sort of reorganization or tidying up so beloved by (and alas in the United Kingdom indulged in by) second-rate administrators on their way up the civil service career ladder. One would have thought the amalgamations that led to British Leyland would have taught somebody up there a lesson.

The predictions are that the United States will be running short of astronomers in 2-3 years time. The demoralization that is now spreading through our UK observatories as a result of continual misinformed sniping by those who do not use them, and a never-ending series of superficial reviews, can in the end have only one result. And when the best SERC people have left on the plane, the rest of us, contemplating the resulting inevitable decline in the competitiveness of our own far from ambitious facilities (our colleagues abroad are going up to 10 or 15 metre class telescopes), will be studying the job columns too. To their credit, our observatories have played a major part recently in catapulting British optical and infrared astronomy from mediocrity into the front rank. In a society increasingly settling for the seedy and the second-rate perhaps that is their crime.

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Philistinism?

SIR — If the idea of exploring more unorthodox ways of funding science than using the increasingly tired arguments for an ever-increasing Science Vote is the role of a scientific philistine then I must accept that label. What a pity though that a once great industrial society should display cultural antipathy to modern commerce of the sort revealed in your leading article (25 April, p.657).

My purpose in trying to provoke a debate on these important issues (not incidentally the unwitting act on my part that you seem to think) is to get scientists to consider a little more, in these financially difficult times, their role in society and their justification for that role being supported from the country's tax base. How you can contend that it is valid to challenge the scientific community with such arguments but not ask individual scientists to accept their implications is beyond me.

Of course I realize that not all science is commercially exploitable. Much of my own research on viruses has been in that category. But I still claim that there is a need for scientists to think purposefully about whether commercial opportunity exists and how it can be harnessed. Though there are some of us who need no such urging, this is not an area in which we have demonstrated much skill in the past. We might all benefit if more of us thought it important and generated income for ourselves, our institutions and our country as a result.

A major point in my *Times* letter that you did not choose even to misquote was that such a strategy could well generate funds for basic science both directly and from government. It is surely worth giving our thinking a nudge in this direction, to counterbalance the traditional arguments so frequently put, even at the risk of incurring the wrath of the Editor of *Nature*

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Imunovir

SIR — Helen Wright's reply[1] fails to destroy Dimitri Viza's complaint[2] that the drug isoprinosine was hyped by excessive promotion, but rather confirms his implicit contention that the publicity for a product is proportional to the doubts about its activity. This contrasts with the case of an effective treatment for herpes; there has been no excessive publicity in the promotions of acyclovir, a drug whose virtues and limitations are well documented.

All but one of the references cited by Wright to prove the efficacy of Imunovir are to communications at meetings between 1973 and 1981, suggesting that none was followed up by a formal paper. She omits references to work suggesting the absence of effect[3,4].

There is a striking difference between Wright's arguments in defence of Imunovir — a 60 per cent cure of herpes bouts within a week, the praise of Prix Galien's jury for a drug arousing "new hopes for the treatment of infectious diseases, auto-immune diseases, allergy and — why not? — cancer" and the absence as yet of any convincing clinical trial. This is even more baffling if one considers that isoprinosine has been tested for more than 12 years.

I cannot therefore but agree that pharmaceutical companies would be well advised to avoid raising excessive hopes through lay media or otherwise. Since mediaeval times, people have been wary of panaceas. This is only one of the reasons why I am prepared to wager that in another five years isoprinosine will have fallen into oblivion. But Viza's proposal that there should be more scrutiny and responsibility in lay publications deserves to succeed.

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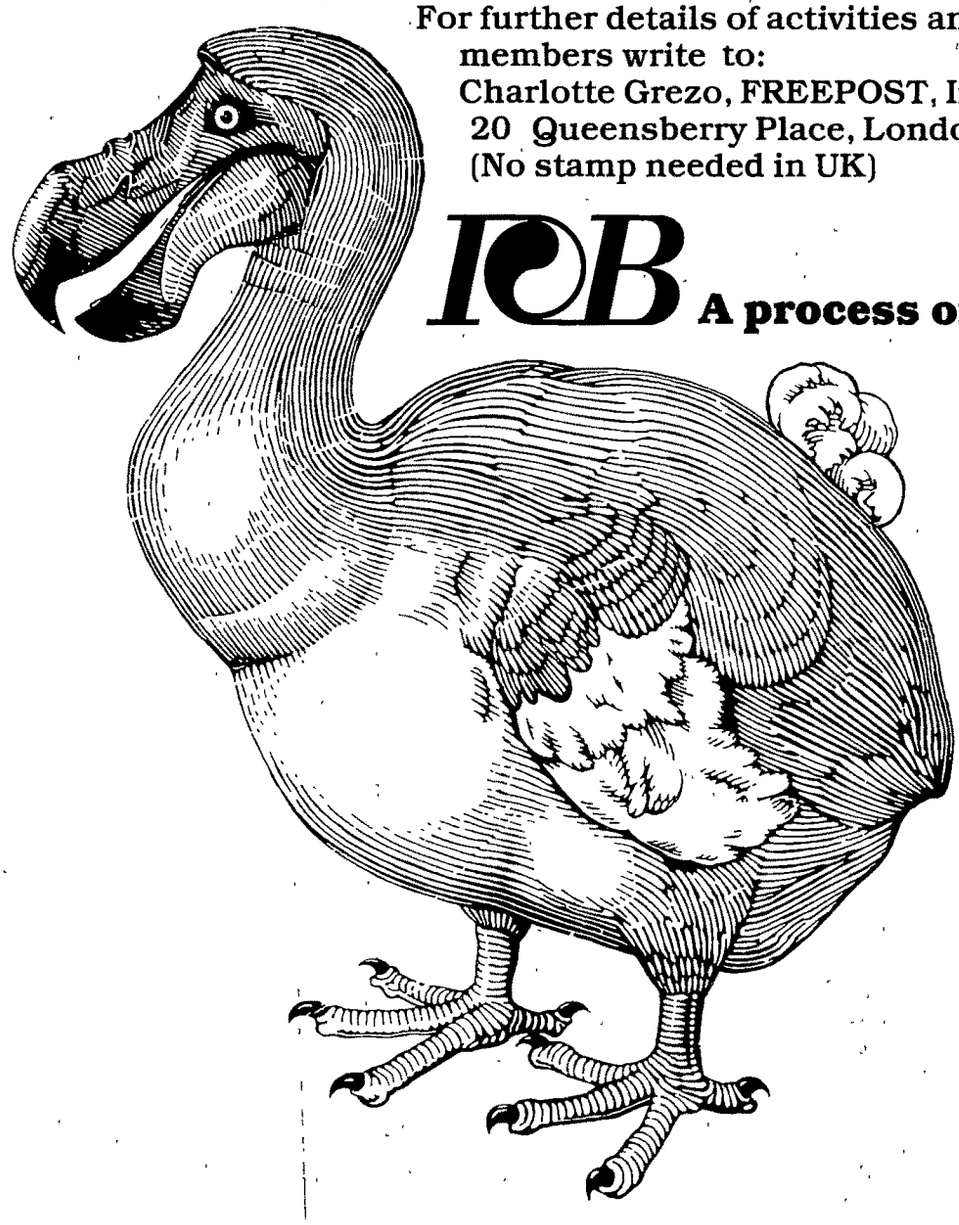
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Piecemeal ballistic missile defence

The US programme of research known as the Strategic Defense Initiative will come to fruition only in the next century (if then), but there may be tangible and awkward results much sooner.

EVER since President Reagan's announcement in March 1983 of the plan for a programme of research on ballistic missile defence, there has been a temptation to assume that the whole programme would come to fruition (or not) all at once. Then, we have assumed, it would fall to a Pentagon committee to recommend (or otherwise) the deployment of an integrated system of anti-missile defence. And then it would be for politicians to take up the question which they have promised must be explored before such a defensive system could be put in place, negotiations with the Soviet Union not least among them. Only then, the argument has gone, would it be decided what should be done with the products of many years of research.

A little thought will show that events cannot follow such a simple course. The objective of the system now being talked about in Washington, in its most flamboyant form, is first to detect hostile ballistic missiles early in their trajectory and then to destroy them by means of several layers of defence. If boost-phase missiles escape attack, there would ideally be means of destroying the components into which they separate, first "buses" containing several warheads and, later, individual reentry vehicles. Much of the argument of those working on the programme rests on the calculation that the most effective route to a missile defence will be an efficient way of destroying missiles while their booster motors are still burning, and are thus conspicuous targets for the detector satellites equipped with infrared sensors.

To say the least, each successful strike against a hostile missile in this boost stage would get rid of a great many warheads. But it is also generally agreed that the detection problem is also then much easier than during the intermediate stages (when decoys will be an obvious stratagem for the missile launchers to use). Indeed, detection becomes relatively simple again only towards the end of a trajectory, when warheads declare themselves by the heat they generate.

No secret knowledge is required to make reasonable guesses about the sequence in which the various components of this system will become available to the military. The technology for detecting missiles during the boost phase probably requires nothing radically new. Infrared detectors have indeed been used to spot rocket boosters rising through the atmosphere. There is no reason why detection should not be reasonably efficient at heights above the ground not very different from those at which people operate infrared telescopes, a few kilometres or so.

The practical task will be to incorporate an array of such detectors into a system of satellites and then handle the data that accumulate. Discrimination between real and imagined targets will obviously be a headache. Software designers will be fully stretched. But the first practical by-product of the research programme will be an early warning system of such sophistication that it might have been considered desirable in its own right.

The other almost certain outcome of the research programme is a better system of terminal defence than that planned by the United States in the late 1960s. The idea then was that a fast take-off missile (called "Sprint") would fire nuclear warheads at incoming warheads; now, it seems, people are persuaded that it would be better to send guided mechanical devices at incoming warheads, in the hope that they might be destroyed without killing the people whose defence is the object of the whole exercise.

So the research programme will almost certainly yield two military options for the United States — a much better (in the sense of much earlier) early warning system and a workable system of terminal defence. Whether the research programme will contribute anything else is still an open question.

The important article in last week's *Nature* (23 May, p.286) by Dr Richard Garwin is a generalized expression of the kind of scepticism with which the research programme will have to contend. Any system of satellites intended to be the bases ("battle stations" is the fashionable term) for the means by which targets will be destroyed will have to be much more elaborate than the enthusiasts have been saying. Even quite optimistic assumptions about the efficacy of lasers as means of shooting down hostile missiles imply much larger numbers of battle stations than people have been saying.

The need for a host of satellite battle stations is generally accepted. Because these machines, whatever their armoury (lasers, neutral-beam projectors or rail guns), will have to be in comparatively low orbits to get within striking distance (say 1,000 km) of a booster rising through the atmosphere, the chances are that any single one of them will be the wrong side of the Earth when needed. But the ballistic missile defence community has sought some comfort in the widely shared belief that the number of satellites required will be proportional only to the square root of the number of potential targets. Garwin disproves that cheerful assumption.

This is the technical nightmare of the whole project. Even though the mass-production cost of making, say, 1,000 battle stations will yield economies of scale, the efficient management of such a system would be a task of quite unprecedented character. Only a means of destroying boosters at much greater range than 1,000 km would bring drastic simplification. Because none of the potential guns has yet emerged as a possible leader, let alone been demonstrated, it seems a fair guess that this part of the programme will be much delayed. No Pentagon committee is going to be making a recommendation to deploy the system this side of the end of the decade. Whether that time will come this century is far from certain.

The consequences of this piecemeal fruition of the various components of the programme are themselves important. If, as seems likely, one early outcome is a feasible system of terminal defence, it seems likely that the United States will follow the Soviet Union in deploying such a system somewhere, presumably in the Minuteman missile fields where the MX missile is now to be deployed. (The Anti-Ballistic Missile Treaty originally offered each side the option of two such systems, later reduced to one.) An orbiting early-warning system, the other likely early product of the research programme, will be diplomatically more perplexing. Quite apart from the formidable technical difficulties of launching, operating and maintaining such a system, there seems very little doubt that it would violate the spirit if not the letter of the treaty.

The result is that, while the Strategic Defense Initiative will not yield tangible results for a decade or more ("if then", as the critics would add), there is every likelihood that some essential parts of the system will be ready for use long before that. So the United States may quite soon find itself having to keep to the promise extracted from the administration by Mrs Margaret Thatcher last December that deployment would depend on talks with the Soviet Union. Raising the questions now, at the arms control talks at Geneva, would be prudent.

John Maddox

Plant genetics

A plant joins the pantheon at last?

from Geoffrey North

As Keith Roberts has recently remarked in *Nature*[1], plants should be wonderful systems for the study of development. Why, then, has progress in understanding cell differentiation and morphogenesis in plants been so slow? One reason, surely, has been the lack of a plant equivalent of the fruitfly *Drosophila melanogaster*: a rapidly breeding species that is easy to keep in large numbers in the laboratory, and is ideal for intensive study by the combination of molecular and genetic techniques that has proved so successful in dissecting the regulation of development in *Drosophila*[2]. But it seems plant scientists need look no further, for the indications from a recent meeting* are that the small weed *Arabidopsis thaliana* (the common wall cress; see sketch) could prove to be just the right subject. And the hope that molecular biology will transform practical plant breeding must have been boosted by reports at the same meeting of the correctly regulated expression of exogenous genes introduced into plants using the natural vector system provided by the bacterium *Agrobacterium tumefaciens*.

Although the science of genetics began with Mendel's experiments with peas, on the whole plants are far from ideal subjects for molecular geneticists. They usually require a lot of space and special conditions for their upkeep, and, worse, as a rule undergo only one or at most a few breeding cycles in a year. Furthermore, the species on which plant geneticists have concentrated most of their efforts, such as maize, tend to have enormous genomes — in the region of two orders of magnitude larger than those of *Drosophila* or yeast, which is a considerable handicap if your aim is to isolate a specific gene. These considerations led Elliot Meyerowitz (California Institute of Technology) to the temperate weed *Arabidopsis thaliana*, a member of the Cruciferae (mustards) Although the first *Arabidopsis* mutations were reported in the 1940s and it has since been used extensively in the genetic analysis of plant biochemistry (C. Sommerville, Michigan State University; see ref 3 for a recent genetic map of *Arabidopsis*), its potential as a subject for intensive molecular genetic analysis seems hitherto to have largely been unexploited.

The features that attracted Meyerowitz to *Arabidopsis* are the ease with which it can be grown in the laboratory, its small size and extraordinarily short generation time (only 4-5 weeks), and its unusually small genome[4] On the basis of DNA reassociation kinetics Meyerowitz estimates that the *Arabidopsis* genome is only ~70,000 kilobases (kb)[5], the smallest yet for any plant and comparable to the yeast, nematode and *Drosophila* genomes. For comparison, the size of the human genome is ~2,000,000 kb, and that of rye is ~7,900,000 kb Furthermore, the DNA reassociation kinetics show that the *Arabidopsis* genome is remarkably low in repetitive sequences, which should facilitate the application of the 'chromosome-walking' technique for isolating genes that made possible the cloning of the bithorax complex of *Drosophila*. Meyerowitz hopes that the small genome of *Arabidopsis* will rapidly be covered with sites of restriction-fragment length polymorphisms — his group have already detected a few by simply using randomly selected clones from an *Arabidopsis* genome library. These can be used to map new mutations and as starting points of chromosome-walks to clone the sites of the mutations

The economy of the *Arabidopsis* genome is also reflected at the level of specific genes; for example, where most plants have multiple copies of the genes for seed proteins, *Arabidopsis* has, according to Meyerowitz, only one. And using their cloned *Arabidopsis* seed protein gene as a probe, Meyerowitz's group have carried out the first analysis of gene expression in plants by hybridization *in situ*. In sections of seeds, they can detect seed protein messenger RNA only in embryo cells, which are known to be the sites of seed protein synthesis in *Arabidopsis*. The result in itself is to be expected; what is important is that it shows the powerful technique of hybridization *in situ* can be applied in *Arabidopsis*. It is not always easy to make it work and this has, for example, been the principal barrier to progress in research on homoeotic gene expression in vertebrates.

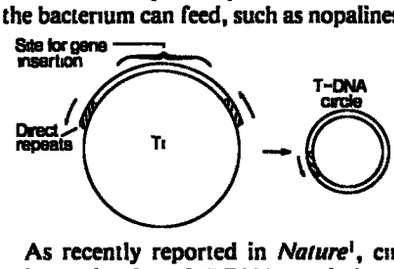
Arabidopsis seems to be as amenable to the techniques of cell culture, plant regeneration and *Agrobacterium tumefaciens*-mediated transformation[6] as plants such as tobacco (see below and box). One of the problems that has frustrated plant scientists in recent years has been the lack of any one species that is both amenable to such techniques and has been well-studied genetically. And although I. Potrykus (Friedrich-Miescher Institute) and E. Howard (CSIRO) both reported that they have been able directly to introduce DNA into protoplasts of monocotyledenous plants, the difficulties in regenerating such plants from cells in culture make it likely that *Arabidopsis* will be the first genetically well-defined plant into which exogenous genes can be introduced readily.

The most exciting prospect offered by *Arabidopsis* is in its potential for the identification, genetic mapping and cloning of those genes which, on the basis of the effects of their mutations, seem likely to be very closely involved in the regulation of

*The 1985 UCLA symposium on plant genetics was held at Keystone, Colorado, USA from April 13 19 1985

Plant transformation

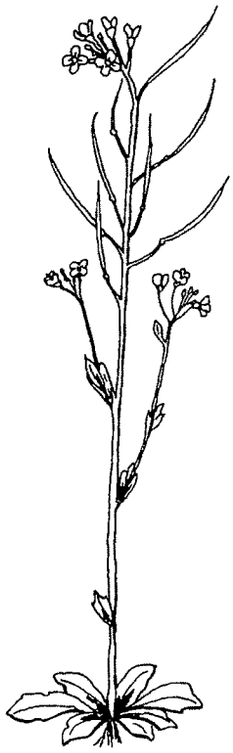
To date, the success of plant geneticists in introducing new genes into plants has depended on the exploitation of a remarkable natural system of interspecific gene transfer. The bacterium *Agrobacterium tumefaciens* can induce the formation of tumours on many plant species. This is known to involve the transfer of a fragment of DNA (the T-DNA), carried on the bacterium's tumour-inducing (Ti) plasmid, to the genome of the infected plant cell. It is the genes carried by the T-DNA that cause the transformed cells to divide to form tumours and that pervert the metabolism of the tumour cells so that they make products on which the bacterium can feed, such as nopalines



As recently reported in *Nature*[1], circular molecules of T-DNA are induced within the bacterial cells in response to some plant-derived signal. It is presumed that these molecules repesent intermediates in the process of gene transfer — though as yet no T-DNA circles have been detected in plant cells. The junction of the T-DNA circles occurs at a precise point within the short (25 base pair) direct repeats that flank the T-DNA on the Ti plasmid, and simply by inserting a piece of DNA between these repeats it can be transferred to the genome of a plant cell. So that whole plants can be regenerated from the transformed cells, 'oncogenic' genes of the T-DNA are usually removed and replaced by a selectable marker, such as an antibiotic-resistance gene.

Unfortunately, it has so far not been possible to use this system to transform monocotyledenous plants, despite the indications that they can be infected by *Agrobacterium tumefaciens*[2,3]. One alternative approach that is being pursued at present is to see whether transposable elements could be used to introduce genes into plants, much as P-elements are used to transform *Drosophila*. As a start in this direction, R.B. Simpson (ARCO, Dublin, California) reported at the plant genetics symposium that his group has begun to explore the potential of the maize *Mu* (Robertson's *mutator*) transposable elements as vehicles for transforming monocots **Geoffrey North**

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ARABIDOPSIS THALIANA

Redrawn from *The New Concise British Flora* by W. Keble Martin (Ebury Press and Michael Joseph, London, 1982)

development. Several such mutations were described in Colorado, for example, by D. Meinke (Oklahoma State University), who explained that he and his colleagues have identified a number of embryonic-lethal mutations that cause arrest at different stages of development by exposing *Arabidopsis* plants to the mutagen EMS and then screening the seed pods for aborted seeds. Meyerowitz's group have reanalysed a mutation termed *apetala-1*, classically described as causing a failure of petal development; they find that occasionally petals are replaced by entire new buds.

The *apetala-1* mutation is somewhat reminiscent of the homeotic mutations of *Drosophila*, in which one body part may be transformed into another. Perhaps more closely comparable, at least at the phenotypic level, are the maize mutations *Corngrass* and *Teopod 1* and *2*, described by S. Poethig (University of Pennsylvania). The repetitive structure of a maize shoot, with units (termed phytomers) comprising a leaf, a node and a portion of stem, is at least superficially similar to segmentation in animals, the pattern of which can be affected by homoeotic mutations. In maize, at fairly precise positions up the plant, the character of the phytomers changes: from vegetative at the bottom through female flower (ear)-forming to male flower (tassel)-forming at the top. The effects of *Corngrass* and *Teopod* mutations can be interpreted as transforming upper phytomers into lower phytomers, as shown by leaf-shape and the replacement of flower parts by vegetative structures.

Another maize gene worth mentioning in this context is the *R*-locus, which is not only intriguing in its own right but illustrates another strategy for the cloning of plant genes. The interest in the *R*-locus lies in the observation that mutations in different regions of the locus specifically affect the coloration of different parts of the plant so, as J. Kermicle (University of Wisconsin) explained, it seems likely that the *R*-locus is somehow involved in regulating the tissue-specific pattern of expression of the enzymes of anthocyanin synthesis in maize. S. Dellaporta (Cold Spring Harbor Laboratory) reported that the insertion of a transposable *Ac* (*Activator*) element into the *R*-locus of mutant maize has allowed the cloning of the locus — the *Ac* element had already been cloned, so could be used as a stepping-stone to clone the adjacent *R* DNA.

If the *R*-locus really is a 'master' regulatory gene, then its most likely targets would be DNA sequences that determine the pattern of expression of the genes for the enzymes of anthocyanin synthesis. The technique of introducing defined fragments of exogenous DNA into plants by *Agrobacterium tumefaciens*-mediated transformation, alluded to briefly above, offers a direct route to the identification of such sequences, and the power of the approach was well illustrated at the meeting. R. Goldberg (UCLA) reported that when fragments of DNA including either the trypsin inhibitor or seed lectin genes of soya bean are introduced into tobacco cells and whole plants are regenerated from the transformed cells, the exogenous genes are developmentally regulated just as they are in soya-bean plants. RNA transcripts can be detected in developing embryos at the correct times during embryogenesis, and in other tissues either no transcripts or the same low levels that occur in the equivalent tissues of soya bean are found. And when a large fragment of soya-bean genomic DNA, containing the seed-lectin gene and several neighbouring genes with different development profiles of expression, is incorporated into tobacco plants each gene is differentially regulated in the transformed plants in precisely the same way as in the soya bean. Similarly, Sengupta-Gopalan *et al.*[7] have found that when the gene for the major seed storage glycoprotein (β-phaseolin) of French bean is introduced into tobacco plants, on a fragment of DNA that includes the β-phaseolin coding-region with about a kilobase of 5′ flanking sequence, it retains its normal pattern of expression: β-phaseolin can first be detected in the seeds of the transformed plants at the time it normally would appear in developing French bean seeds, several days after the tobacco plants' own storage proteins have started to accumulate.

The implication of these results is that the fragments of soya-bean and French-bean genomes introduced into the tobacco plants include the information to specify in which tissue the genes should be expressed. The definition of the sequences that comprise this information should be relatively straightforward; but to know how to direct the expression of any gene to seed-tissue should be an exciting prospect for those who wish to use molecular genetic techniques to improve crop plants. □

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Geoffrey North is Deputy Biology Editor of Nature.

Planetary science

More news from Mars

from I.P. Wright and M M. Grady

SAMPLES of Mars have been delivered to the Earth as meteorites, but this has been appreciated only in the past few years, following, among other things, the discovery of meteorites from Antartica that have a demonstrable lunar origin. The stimulus this has provided for the discipline of Solar System geochemistry was clearly evident at a recent conference on lunar and planetary science*. Four shergottites (S), three nakhlites (N) and one chassignite (C) together make up the eight so-called SNC meteorites thought to originate from Mars. Detailed studies of these, and in particular the results of a consortium investigation of the Shergotty meteorite (organized by J.C. Laul, Battelle Pacific North West Laboratory, Washington), have thrown new light on martian history as well as the processes by which the SNC meteorites themselves were delivered to Earth. The SNC meteorites provide a bridge between laboratory-based analyses aimed at elucidating the origins and evolution of the Earth, Moon and meteorites, and planetary geology.

Research groups at the Battelle Pacific North West Laboratory, Max-Planck-Institut fur Chemie, Mainz and Purdue Universities (headed by J.C. Laul, H. Wanke and M.E. Lipschutz, respectively) have made detailed chemical analyses of the Shergotty meteorite, which provide constraints on the bulk composition of the parent body. Their deductions of the composition of the parent body comply with that inferred from geophysical data (density, moment-of-inertia factors, etc.) concerning the core/mantle structure of Mars. There are subtle variations in chemical composition that demonstrate that Shergotty is heterogeneous on a centimetre scale; for example, there are large variations in concentrations of volatile elements such as cadmium, tellurium, thallium and bismuth. The separated minerals contain widely differing proportions of rare-earth elements (REE), with phosphates such as apatite and whitlockite containing 500 times the proportions found in chondrites. In addition,

*The 16th lunar and planetary science conference was held in Houston, Texas, 11-15 March, 1985

the light REE seem to be concentrated in the iron-rich rims of clinopyroxene crystals. It is clear from the trace-element data that Shergotty and EETA 79001 (an Antarctic shergottite) are not related to the same parent magma. Further chemical studies show a high abundance of tungsten in Shergotty (which has a molybdenum/tungsten ratio of 1.0, compared with 5.9 for the terrestrial mantle); this seems to accord with various processes thought to operate during core formation and is consistent with the view that Mars was formed by homogeneous accretion.

Previous oxygen isotope measurements of whole-rock samples have demonstrated that SNC meteorites form a distinct group that probably originated on a single parent body distinct from the Earth-Moon system. The oxygen isotope composition of mineral separates of Shergotty has been determined by R.N. Clayton and T.K. Mayeda (University of Chicago). Data from mineral pairs give equilibration temperatures of 1,100 ± 100°C; the degree of isotope fractionation between the mineral pairs is lower than that often seen in equivalent terrestrial materials and demonstrates that little, if any, secondary exchange with water has taken place following crystallization. Not all the minerals analysed conform to this interpretation. Indeed, a maskelynite (shocked plagioclase) separate from Shergotty has an extremely anomalous oxygen isotope composition, although this cannot be due to the shock process itself because maskelynite from other shergottites shows no unusual isotope composition. The 0.4 per cent enrichment of ^{18}O in the maskelynite from Shergotty remains an intriguing and unexplained result, possibly reflecting low-temperature alteration.

Nitrogen in shocked glass from EETA 79001 has previously been shown to have an isotope composition enriched in ^{15}N, consistent with an origin from the martian atmosphere. A portion of the nitrogen in Shergotty also appears to be of atmospheric origin. Two groups, R.H. Becker and R.O. Pepin (University of Minnesota) and R.H. Carr, I.P. Wright and C.T. Pillinger (Open University), have found evidence for a nitrogen component in Shergotty that shows a 6 per cent enrichment of ^{15}N. It is thus considered that the shergottites probably contain microscopic and heterogeneously distributed shock-produced glasses that in turn contain samples of the martian atmosphere. J. Yang and S. Epstein (California Institute of Techology) and Carr *et al.* find that some of the carbon in Shergotty is enriched in ^{13}C, consistent with both isotope measurements of CO_2 in the shock-produced glass in EETA 79001 and by *in situ* measurements of the martian atmosphere by the Viking spacecraft. Thus, some of the CO_2 in Shergotty also seems to be of martian atmospheric origin, although the picture is somewhat confused by the presence of a number of indigenous carbon-bearing components that need to be further resolved before the carbon chemistry of the SNC meteorites is completely understood.

The glass fraction of EETA 79001 has been subjected to detailed optical examination by D.W. Muenow and J.L. Gooding (University of Hawaii; NASA Johnson Space Center, Houston). Using scanning electron microscopy, they have recently identified two distinct components in the glass fraction — a smooth glass and a 'ribbed' glass, which is thought to be quench-textured augite. Vesicles have also been identified in the glasses, as have sulphur-rich aluminosilicate crystals that are finely disseminated throughout the samples. It is possible that these secondary minerals are products of Antarctic weathering, although they may have originated on another volatile-rich planet, such as Mars.

J.H. Jones (University of Arizona) stirred up controversy by proposing that the previously accepted chronology of the shergottites should be called into question. Briefly, the current dogma is that the shergottites have an igneous origin at 1,300 Myr, then underwent impact metamorphism and subsequent ejection from the martian surface at 180 Myr. The new hypothesis, based on a reappraisal of existing trace element and isotope data, suggests that the shergottites were actually formed in an igneous event about 180 Myr ago. Furthermore, the cosmic-ray exposure age of shergottites (2.5 Myr), previously thought by most people to represent the time the meteorite fragmented in space, may actually approximate to the time the samples were ejected from Mars. The shorter exposure age of EETA 79001 would, however, require two separate ejection events for the shergottite meteorites, which poses problems as it demands high cratering rates on Mars over the past few million years.

The results obtained from studies of SNC meteorites have widespread implications for theories of Solar System formation, planetary accretion, thermal histories of planets and petrogenesis on planetary bodies. Chemical and isotope analyses of SNC meteorites have given a clearer appreciation of the composition of the martian surface and atmosphere. There is a strong feeling in the community of lunar and planetary investigators that a Mars sample-return mission should be given high priority in space exploration programmes in the years ahead. □

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Developmental neurobiology

What guides growing axons?

from Dennis Summerbell and R. Victoria Stirling

DURING normal development, neurones form processes that grow long distances to connect with their targets. They produce a precise and repeatable pattern that is essential for normal functioning of the nervous system. There are numerous theories about the mechanisms involved in forming this pattern and three papers in this issue illustrate different ways of looking at the development of these connections, in tissue culture, in explants and *in vitro*. None of the papers present new explanations but all are important because they involve observing events as they happen, rather than at a fixed end point.

By removing nerve cells into tissue culture, one can control most carefully for critical single factors. We already know that axons prefer to grow along particular substrates, for example, laminin, but can one demonstrate thay they have specific preferences? On page 409, Bonhoeffer and Huf describe a Y-maze in which embryonic chick retinal axons from one edge of the eye are given the choice of growing along axons from the same position or axons from the opposite edge 180° away. Axons from one particular location (temporal retina) clearly prefer to grow along their neighbours, whereas axons from the opposite (nasal) side show no such preference. Here, in a dish, we can watch axons exhibiting the kind of behaviour suggested from static observations of their trajectories in fixed amphibian brains, in which temporal axons are found in a tight bundle in the optic tract while those from nasal retina, 180° away, are distributed across its entire width. The development of the highly ordered projection from the eye to the brain may depend on such differences, using them, for example, to set up the orientation of the map, and this kind of experiment has the potential for analysing the molecular basis for the differences.

But such fundamental yes/no decisions are only part of the story; a second approach is systematically to remove putative guidance cues and observe the resulting pattern of axon growth. The developing insect wing provides a culture system for the study of nerve growth that is less abstracted from the whole animal. The wing primordium starts life as a hollow flattened disk (the imaginal disk), rather like a pitta bread, in which the guidance of axons from peripheral sensory cells can be followed. Blair, Murray and Palka (on page 406) have adopted this model to look at the role of the substrate in axonal navigation. Axons from the sensory cells in the wing follow stereotyped pathways towards the central nervous system. Among the possible cues they might use are the channels between the dorsal and ventral surfaces of the wing, but when the disk is split into

separate dorsal and ventral halves, the dorsal axons are still able to find their way despite the absence of channels to guide them. Another possible cue is the nearby neurones, which in whole embryos appear to act as 'stepping stones' or 'guideposts'. Laser destruction of these guideposts leads to disoriented growth of specific axons (Raper, J.A., Bastiani, M.J. and Goodman, C.S. *J. Neurosci.* **4**, 2329; 1984). In the present experiments, the removal of the proximal guidepost neurone did not prevent the axons of more distal cells from moving initally in the right direction.

In both these experimental systems, molecular dissection could be contemplated, but one must remember that cells in culture are not in their natural environment. Some of the important cues may not be expressed, whereas others may not be relevant *in vivo*. The finer intricacies of connections depend on the interaction of axons with their continuously changing environment, so that the ultimate level of observation must be the intact, living animal. Recent advances in techniques for intracellular dye injection coupled with video-image enhancement have been harnessed brilliantly by Purves and Hadley on page 404 of this issue. The superior cervical ganglion in the neck of the rat is an accessible cluster of cells nicely suited to this technique; by using image intensification, the cell can be photographed without obviously harming it. They first expose the ganglion, fill a particular cell with fluorescent dye and photograph its branching pattern. Several days or even a month later they are able to find the same cell again and repeat the performance. They observe that the branches of the neurone change their shapes progressively over time: nerve cells are not static but are continually adapting, even in the adult animal.

It is precisely in these types of developmental systems that molecular techniques need to be applied. Increasingly, we will identify the appearance of cell surface and extracellular matrix constituents by the use of monoclonal antibodies and discover unique, regionally-localized transcription by *in situ* hybridization. Such results must be correlated with the kind of developmentally interesting events described in these papers. For example, the appearance of cell-adhesion molecules provides a powerful tool to study development both at the level of cells in the organism and at the level of regulation of gene expression in the cell (Edelman, G.M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1460; 1984).

It is becoming increasingly apparent that no single factor is responsible for the development of specific neural connections. In the intact animal, there seems to be a redundancy of guidance cues. These papers show that the intact organism must not be forgotten in the quest for molecular answers to developmental questions. □

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Vision

Disparities in depth perception

from C. Longuet-Higgins

ONE might be surprised that 150 years after the invention of the stereoscope anything remains to be discovered about stereopsis, but on page 402 of this issue Graeme Mitchison and Suzanne McKee describe a novel effect called 'interpolation', which they observe when looking at a particular kind of stereogram. In the past few years stereopsis has had an injection of new interest from two developments in particular: the demonstration by B. Julesz and J. Chang. (*Biol. Cybernet.* **33**, 107; 1976) that pattern perception is not a prerequisite for stereoscopic fusion; and the so-called 'computational paradigm' — the recognition that vision is the outcome of an elaborate neuronal computation for which our eyes supply only the input.

The foremost exponent of the computational paradigm was the late David Marr, whose book, *Vision* (Freeman, San Francisco, 1982), dealt in some detail with stereopsis. With Tomaso Poggio he produced, in succession, two alternative theories for the mechanism of stereopsis. Though the details of the process are still largely obscure, few visual psychologists would deny Marr's premise that an important preliminary to stereoscopic fusion is the matching of corresponding elements in the two retinal images — the well known 'correspondence problem' of stereopsis. Once the elements have been matched, the visual system can assess the disparities between their retinal positions and draw conclusions about the relative distances of the visible features of the scene.

The observations of Mitchison and McKee bear most directly on the question of how the visual system carries out the matching process. Most of their stereograms, presented for a time too short for the observer to scan the display, were interpreted exactly as predicted by standard stereoscopic theory. The apparent difference in depth between a pair of dots in the stereogram was simply proportional to their horizontal disparity, that is, the difference between their horizontal distances apart in the left and right retinal images. This presupposes that the elements of the two images have been successfully matched; otherwise their disparities are ill-defined.

With the stereograms that gave predictable impressions of depth, the only uncertainty in the outcome arose from alternative ways of matching the images; the choice between such alternatives seems to be broadly consonant with what is known about the 'disparity gradients' associated with matched pairs of visible elements (Burt, P. and Julesz, B. *Science* **208**, 615; 1980). But the stereograms that Mitchison and McKee find most interesting are those containing regular grids of very closely spaced points, the critical spacing being about 6′ of arc — roughly the angle subtended by adjacent grooves of a nail file held in the hand. At this spacing the visual system seems to 'lose count' of the total number of points in each image, and to estimate the position of the grid by the horizontal disparity between its two edges, ignoring any differences in the numbers of intervening points in the two images; the whole grid is seen as roughly coplanar.

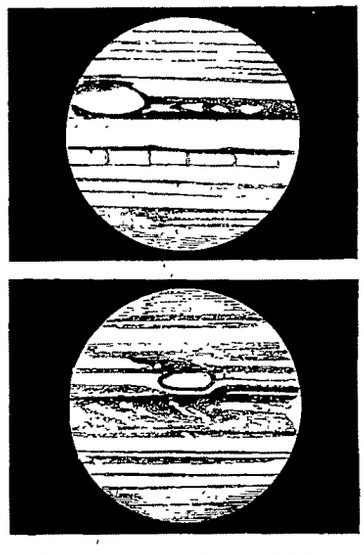
Mitchison and McKee point out that their observations cast doubt on Marr and Poggio's concept of a 'coarse-to-fine vergence strategy', in which the correspondence problem is solved by progressively finer adjustments in the directions of gaze of the two eyes. It will be interesting to see whether they can develop a computational theory of the matching process with a predictive power equal to that of the theories they challenge. □

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Erratum

Due to an editorial mistake in the article by A.R. Gardner-Medwin 'Potential challenge from glia' (*Nature* 16 May, page 181), it was implied that several long-established properties of glial cells were new findings reported in recent papers. The lines "including . . . . fascination" should be removed from the penultimate sentence of the first paragraph; instead they should complete paragraph 2, which will then read " . . biological cells, high permeability . .".

100 years ago



Probably recurrent markings on Jupiter. Top, 27 November 1857 (Dawes); bottom, 12 h 50 min on 25 February 1885 (Denning).
From *Nature* **32** 33, 14 May 1885.

Geophysics

Electrical earthquake prediction

from Paul W. Burton

Two thousand years ago, Aristotle proposed that earthquakes were caused by subterranean winds. The Greeks are still active in the often contentious business of earthquake prediction and the team of P. Varotsos, K. Alexopoulos and K. Nomicos are well aware of controversy surrounding their work. In two papers which appeared recently in *Tectonophysics*[1,2], Varotsos and Alexopoulos have put forward their observations and ideas on variations in the Earth's electric field preceding earthquakes. They now claim to be able to forecast the magnitude and epicentre of earthquakes in Greece; to prove this they have taken the unusual step of issuing and publishing registered telegrams of their predictions, to establish a check on their work.

Phenomenological earthquake prediction is essentially the study of precursory phenomena — those events that precede an earthquake. These range from peculiarities in animal behaviour and lights in the sky to the more geophysically obvious and comprehensible precursors of increased minor seismicity. The work of Varotsos and Alexopoulos is confined to electrotelluric currents in the Earth. These naturally occuring electrical currents, which are not fully understood, are measured by probing the surface at two or more points, approximately 30 metres or more apart. They might be 'channelled' along conductors within rocks, such as fluid-filled cracks; because earthquakes involve changes in the stresses in the ground and ultimately movement along fractures, it is plausible that changes in the electrotelluric regime could indicate imminent earthquakes.

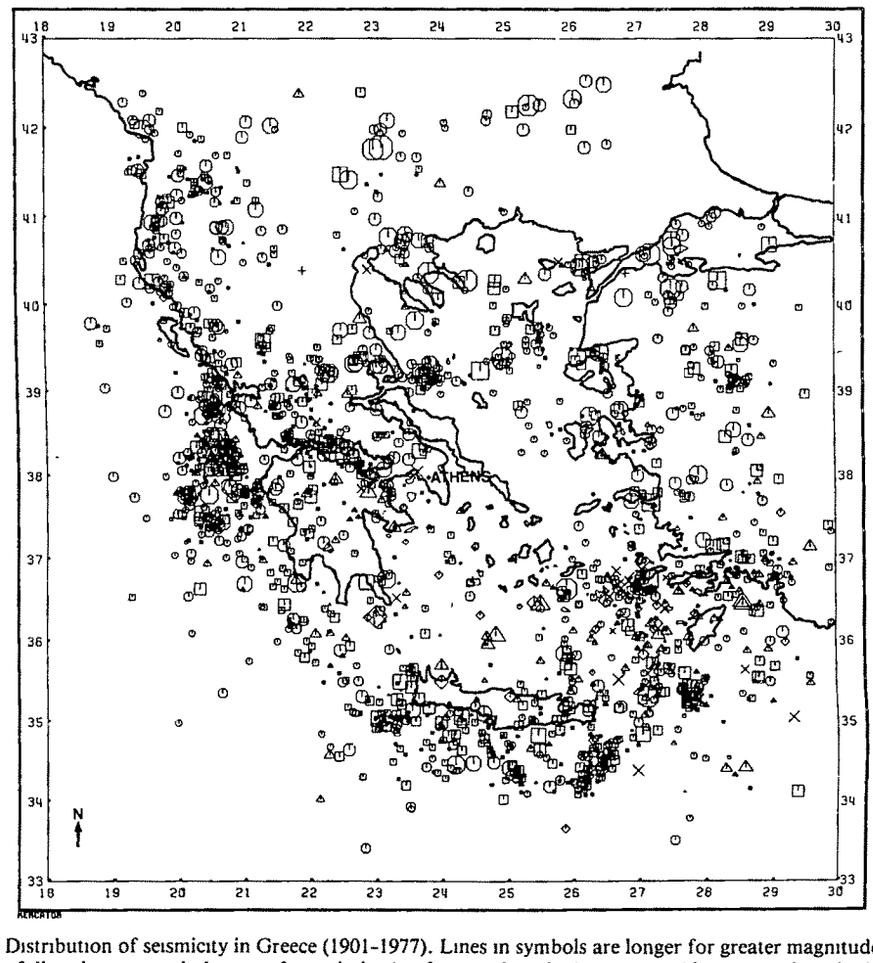
This team has monitored the telluric field at several sites in Greece since March 1981 and searched for precursory seismic electric signals (SES). Their most important conclusion is "that every sizeable earthquake is preceded by an SES and inversely every SES is always followed by an earthquake, the magnitude and the epicentre of which can be reliably predicted". These seismic electric signals are observed 6-115 hours before an earthquake, although there are distinct groupings within this range. Varotsos and Alexopoulos find no correlation between either the SES lead time or duration and the magnitude of the ensuing earthquake. However, SES from several earthquakes at different distances from a station show changes in electrical potential that are inversely proportional to distance; the SES amplitude from one seismic region recorded at one station shows a simple log-linear relationship to magnitude. Comparison of information from several stations enables calibration of earthquake signals and prediction.

Specialists and non-specialists will rake through these papers to judge how far the work is advanced and how justified are the publicized conclusions. The reproduced charts showing the SES variation with time are often confusing at first sight and do not present their information clearly. But they are reminiscent of an era when seismograms themselves looked primitive to the uninitiated.

Calibration of one station in western Greece involved collecting 20 SES measurements over four months from earthquakes exceeding magnitude 4 in the Kefalinia (Cephalonia) region and comparing them with magnitudes determined by the National Observatory of Athens. A few hundred earthquakes above this magnitude were probably recorded within 100 kilometres of Cephalonia during this time and one wonders how Varotsos and Alexopoulos dealt with all these other SESs, which they presumably also recorded. Consequently, it seems odd that there is little comment on the overall seismicity of the region. Greece is the most seismically active area in Europe (see figure), and there are clear dangers in invoking correlations between frequently occurring events. When Varotsos and Alexopoulos put forward their case, backed by registered telegrams, for having forecast 21 out of 23 earthquakes of magnitude 5 or larger that occurred near their network during eight months in 1983, they do not emphasize that one of these was forecast with magnitude 4. Taking this lower threshold, there were probably a few hundred earthquakes within a radial distance of about 1.5 degrees of the station at Pirgos in the western Peloponnese in the same period. With so many earthquakes, many will seek further verification of the correlation between particular earthquake, SES and telegram, and it would also be interesting to know how many telegrams contained information which was found subsequently to be incorrect or fortuitously correlated.

Nevertheless, many people are hopeful that a significant breakthrough has been made. This prompts consideration of the general problems raised by earthquake prediction. There will doubtless be engineering seismologists, earthquake engineers, land-use planners and government decision-makers who would hope that attention and funds are not diverted from other more practical aspects such as continued programmes of house strengthening or better building codes in earthquake-prone zones.

There is also a degree of *a posteriori* demonstration involved, which raises the



Distribution of seismicity in Greece (1901-1977). Lines in symbols are longer for greater magnitude of disturbance; symbols range from circles (surface earthquakes) to crosses (deepest earthquakes).

very real consideration of what to do when a prediction concerns an earthquake that might have substantial consequences. The Seismological Society of America has issued guidelines[3] which state that "a scientist has fulfilled his or her obligations by informing colleagues, employer and the appropriate public agencies of the prediction hypothesis, its basis, and any critiques, concurrencies or nonconcurrencies based on independent reviews". The United States also has a formal mechanism for dealing with earthquake predictions, in which the responsibility for informing both the public and public officials of vetted earthquake predictions is vested in the Director of the US Geological Survey. Community preparedness for earthquakes would also seem to be a prerequisite. In Japan, the Earthquake Assessment Committee is backed up by the Large-Scale Earthquake Countermeasures Act[4]. Greece has recently followed these leads and established, by Act of Parliament, the Organization for Aseismic Protection But when the lead time for an electrotelluric prediction is only 6–115 hours and it may relate to any part of Greece, it is hard to see what might be expected of such an organization.

So, inevitably, what should be done next? There is obviously a case for continued investment in seismological studies and earthquake source physics, but such investigations alone do not allow people to sleep well in their beds at night. In the long term, the less glamorous role of the earthquake engineer and the careful implementation of building codes and environmental hazard maps may perhaps be more substantial, if unsung, life-savers. □

1 Varotsos, P & Alexopoulos, K *Tectonophysics* **110**, 73 (1984)
2 Varotsos, P & Alexopoulos, K *Tectonophysics* **110**, 99 (1984)
3 Guidelines for Earthquake Predictors in *Bull seism Soc Am* **73**, 1955 (1983)
4 Earthquake Prediction and Preparedness in Japan in *Earthquake Inf Bull* (U S Dep Int Geol Sur) **15**, 142 (1983)

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Neurochemistry

New light on synapsin I

from Richard Rodnight

MANY crucial processes in the nervous system, such as synaptic transmission, involve complex interactions between the neuronal plasma membrane and components of the neuronal cytosol In the search for the molecular mechanisms of such processes, the identification and characterization of nerve-specific proteins is an essential step. There have been few successes in this area, but one that stands out is the discovery over a decade ago by P. Greengard and colleagues[1] of a major phosphorylated protein in brain, originally termed protein I, but now called synapsin I. Subsequent work by Greengard's group elegantly demonstrated that synapsin I occurs exclusively in the nerve terminals, where it is bound to the surface membrane of the synaptic vesicles, the storage sites for neurotransmitters[2]. This highly specific location clearly pointed to an important role for the molecule in some aspect of synaptic transmission (for example, the still enigmatic mechanism for transmitter release) but no precise evidence for its function emerged. Now, A.J Baines and V. Bennett, on page 410 of this issue[3], present evidence to show that synapsin I is very similar, if not identical, to an important spectrin-binding cytoskeletal protein termed brain 4.1.

The significance of this exciting discovery may reside in the fact that the analogous protein in erythrocytes, erythroid 4.1, can form stable ternary complexes with spectrin and the contractile protein actin. In the erythrocyte, these complexes constitute a stable supportive lattice at the inner face of the cell membrane and are therefore important in maintaining the shape of the cell; in nerve terminals (where spectrin and actin also occur) they may play a more dynamic role in modulating the interaction between synaptic vesicles and the terminal membrane.

To appreciate the significance of this development, we must take a closer look at both the cytoskeletal proteins and synapsin I. In the neurone a key molecule is brain spectrin[4] (also called fodrin or calspectrin), a tetramer formed from two subunits of approximate relative molecular mass (M_r) 260,000 and 265,000, antigenically related to erythrocyte spectrin (for further comment on spectrins as a family of proteins, see ref. 5). Similar to its red-cell analogue, brain spectrin binds both actin and protein 4 1 (or synapsin) and is in turn anchored through its smaller subunit to the inner surface of the neuronal membrane by yet another cytoskeletal protein, ankyrin[4]. Synapsin I consists of two subunits of approximate M_r 80,000 and 85,000 and, as noted by Baines and Bennett[3], has been mainly studied as a substrate for cyclic AMP-dependent and calcium/calmodulin-dependent protein kinases[6]. Each subunit comprises a globular head and a highly basic tail with phosphorylation sites on both domains. The basic tail of the molecule is bound to the external surface of the synaptic vesicle membrane by ionic forces that are weaker when the tail is phosphorylated by the calcium/calmodulin-dependent protein kinase[1]

It is particularly interesting that changes in the phosphorylation state of synapsin induced by electrical stimulation of nerves or by drugs that stimulate by depolarizing the neuronal membrane seem to involve mainly the calcium/calmodulin-dependent phosphorylation. This makes it possible that a consequence of such stimulation (which may be the prelude to neurotransmitter release) is to break the bond between synapsin I and the vesicle membrane Previously, the significance of this reversible binding of synapsin I to vesicles was obscure, but now that Baines and Bennett have shown that the molecule also binds to the neuronal cytoskeleton through spectrin, intriguing possibilities open up. For example, by analogy with the interaction of protein 4.1 and spectrin in erythrocytes, the binding of vesicle-associated synapsin I to brain spectrin may greatly enhance the capacity of brain spectrin to crosslink actin filaments, thereby inducing a change in the shape of the submembranous cytoskeleton and re-orientating the vesicle in relation to the terminal membrane. Further changes in vesicle orientation might follow disruption of the synapsin I – vesicle bond as a consequence of the calcium/calmodulin-dependent phosphorylation of synapsin I induced by calcium-ion fluxes in the nerve terminal. It may be significant that spectrin has binding sites for calmodulin.

Many questions need to be answered however, before precise roles for the synapsin-spectrin interaction in synaptic transmisison can be formulated. First, it should be pointed out that Bennett's group have so far been unable to demonstrate an effect of synapsin I on the crosslinking of actin by spectrin, although other workers have reported such an effect with erythrocyte protein 4.1 (ref.7). Perhaps the explanation of the discrepancy lies in differences in the function of the spectrin-actin-4.1 complex in the red cell and nerve terminal — in the former the need for a stable supporting structure is evident, whereas in the latter a more dynamic function may be envisaged, possibly involving transient states that would be difficult to detect in the test tube. Second, the precise intraneuronal distribution of brain spectrin is far from clear. As Baines and Bennett point out, spectrin is axonally transported and spectrin-like molecules can associate with intracellular organelles[8]. Moreover, the two subunits of spectrin do not always occur together and the larger subunit has a wider distribution[9], although both are needed to crosslink actin filaments. Nevertheless, there is enough evidence to suggest that spectrin, perhaps together with tubulin, could form part of a much more extensive cytoskeletal network than a purely membrane-associated structure Such a network might serve to regulate the disposition and movement of synaptic vesicles in the nerve terminals by binding vesicle-associated synapsin I

A final conundrum concerns the fact that both spectrin-like molecules and proteins immunologically related to erythrocyte 4 1 have a wide distribution in cells; indeed

Goodman *et al.*[10] present evidence indicating that, at the level of the light microscope, the distribution of spectrin and 4.1 (as detected by an antibody to erythrocyte 4.1) in the cerebellum are identical and extend to neuroglia as well as neurones. The latter contrasts with the virtually exclusive localization of synapsin I in the nerve terminal[1]. These results cannot be explained purely on the basis of the very high concentration of synapsin I in the nerve terminal (where it constitutes about 6 per cent of the vesicle protein), because immunocytochemistry using anti-synapsin immunoglobulin confirms the restricted location. Therefore, it seems likely that synapsin I is a highly specialized form of 4.1 found only in neurones. This conclusion confirms the overwhelming impression that synapsin I must play a unique role in some aspect of synaptic transmission. □

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3 Baines, A J & & Bennett, V *Nature* **315**, 410 (1985)
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5 Baines, A J *Nature News and Views* **312**, 310 (1984)
6 Nestler, E & Greengard, P *Nature* **303**, 583 (1983)
7 Burns, N.R., Ohanion, V & Gratzer, W B *FEBS Lett* **153**, 165 (1983)
8 Aunis, D & Perrin, D *J Neurochem* **42**, 1558 (1984)
9 Cumming, R & Burgoyne, R D *Biosci Rep* **3**, 997 (1983)
10 Goodman, S R , Casoria, L A , Coleman, D B & Zagon, I S *Science* **224**, 1433 (1984)

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Solid-state physics

How superconductivity fades near metal–insulator transitions

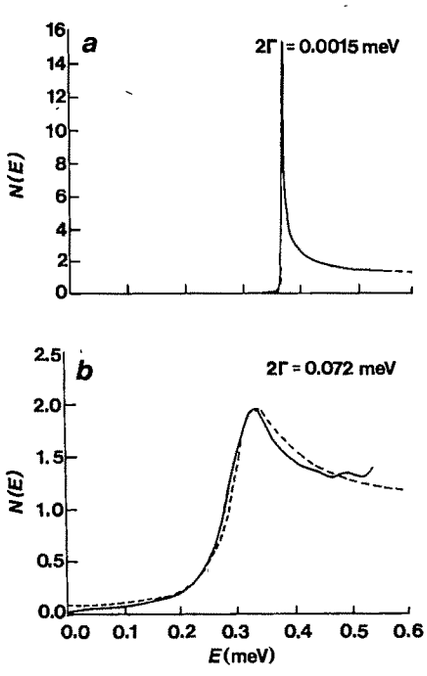
from W.L McLean

ALTHOUGH the importance of quantum mechanics in solid-state physics has been well established for many decades, it came as a rude surprise about five years ago that startling quantum effects in metallic conduction had been overlooked. Unusual behaviour and bizarre predictions near the metal-insulator transition have recently come to be expected. Further challenging questions arise when the system can also be superconducting. One of these that is less innocent than it first seems is whether superconductivity can persist into the insulating side of the transition. If not, does it die out right at the transition or while the system is clearly still on the metallic side? R.C. Dynes, J.P. Garno, G.B. Hertel and T.P. Orlando have recently shed new light on this area by measuring electron tunnelling in the granular aluminium system (*Phys. Rev. Lett.* **53**, 2437; 1984).

Granular aluminium, which is a mixture of grains of metallic aluminium and amorphous aluminium oxide, is one of a number of systems from which a series of specimens, ranging from pure metallic to pure insulating, can be prepared by varying the metal volume fraction. The granularity, which can be seen by transmission electron microscopy, is clearly marked in the middle range of concentrations, where the metal-insulator transition occurs. Many theoretical models that attempt to explain properties of granular metals do explicitly take this granularity into account.

A very different interpretation that has been used by Dynes *et al.*, among others, is that the properties of granular aluminium can be interpreted as if the system were an amorphous metal in which the disorder is on an atomic scale. This outlook is commonly justified by the assumption that all relevant length scales are larger than the grain size, and it is in this framework that the importance of the new tunnelling measurements becomes apparent.

The experiments use the technique of electron tunnelling at low temperatures of approximately 0.06 K, which is much less than the superconducting transition temperature, to measure the density of states of the elementary excitations (quasi-particles) of the superconductor. In low resistivity specimens it is not surprising that



The density of states, $N(E)$ deconvoluted from the experimental data. The dashed line is a BCS density of states broadened by the value of Γ shown in each figure. The 'lifetime' of a quasi-particle is $\hbar / \Gamma$. *a*, Specimen with small broadened effects; *b*, specimen very close to metal-insulator transition (modified from Dynes, R.C. *et al Phys. Rev. Lett.* **53**, 2437; 1984).

granular aluminium behaves exactly as an ordinary superconductor with a sharp asymmetrical peak in the density of states clearly defining the edge of an energy gap, in good agreement with the Bardeen, Cooper, Schrieffer (BCS) theory of superconductivity. At higher resistivities very close to the metal-insulator transition, however, the density of states is no longer zero at low excitation energies and the discontinuity is replaced by a broad smooth maximum.

Dynes *et al.* have successfully fitted their data with a BCS density of states modified to take into account broadening caused by a finite lifetime of the quasi-particles in the granular superconductor. In this case the lifetime is limited by inelastic scattering processes, which have been of great interest recently in connection with the tendency of electron states to become localized as the metal-insulator transition is approached. The values of the inelastic scattering rates deduced from the tunnelling measurements are consistent with those deduced from a different experimental approach, measurement of the magnetoresistance.

Thus, superconductivity seems to die away near the metal-insulator transition by a gradual lifetime broadening of the density of states concomitant with the appearance of quasi-particle states in the region where there is an energy-gap in lower resistivity specimens. A particular oddity of these results is that they imply that moderately strong indications of superconductivity still appear close to the energy at which the sharp peak occurred in the low resistivity specimens. This may be connected with the long tails reported by Dynes *et al.* in the resistive transitions of the highest resistivity specimens.

Further intriguing questions are: first, what happens at much lower temperatures when the lifetimes of the quasi-particles should become longer? Does the full BCS gap reappear? The implication is that the closer one approaches the metal-insulator transition, the lower must the temperature be in order to see the full superconductivity. Second, does some inelastic scattering mechanism peculiar to the granular structure come into play or are the mechanisms found in amorphous metals still operating in the granular system?

Although the new results seem to provide firm support for the quasi-homogeneous medium approach, it still remains to reconcile this point of view with approaches that do assume the granularity to be relevant. These include those workers who seek to explain the properties of granular metals in terms of percolation through an assembly of randomly linked grains, and those focusing on the consequences of electrostatic charging of the grains on the stability of the superconductivity. □

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Explanation of the inward displacement of Io's hot plasma torus and consequences for sputtering sources

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Radial profiles of the ion density and flux-tube content in the Io torus have peak values inside Io's orbit, even though Io is the effective source of these ions. Formation of an inward peak constrains either the velocity distributions or source regions of sputtered neutrals. A further constraint is that the ionization of neutrals on trapped trajectories that return to Io's surface must be limited. A dominant sulphur source is most easily reconciled with these constraints.

Io, the innermost of the four Galilean satellites, has a dense torus of plasma associated with it. This torus, perhaps the most intriguing feature of Jupiter's magnetosphere, has been the subject of intensive research and debate. Recent ground-based measurements of the torus[1], as well as measurements from the Voyager spacecraft[2,3], have shown that both the ion density (Fig. 1*a*, *b*) and NL^2, the flux-tube content (Fig. 1*c*), attain their maximum values as functions of radial distance from Jupiter well inward of Io's orbit, with NL^2 falling off sharply inside ~5.6 jovian radii (R_J).

Many features of the radial profiles can be understood in terms of radial diffusive transport[4-7]. Because diffusion driven by macroscopic processes in planetary magnetospheres is accomplished by the interchange of magnetic flux tubes, NL^2, proportional to the number of ions per unit magnetic flux, is the diffusing quantity[4]. (The parameter L labels a field line and, in a dipole field, L is the distance in planetary radii from the centre of the planet to the equatorial point on the field line. N is the number of ions per unit L.) The radial profile of NL^2 is consistent with diffusive transport of ions only if their source attains a maximum value near 5.6-5.7 R_J. Centrifugal forces drive rapid outward diffusion, consistent with the relatively slow outward decrease of NL^2 beyond 5.7 R_J. Slow inward diffusion driven by atmospheric winds explains the sharp decline inward from 5.6 R_J, but the heavy ions that form the torus must originate from matter on Io itself. The main purpose of this paper is to explain why, although Io is the actual source of particles, the peak in NL^2 occurs inside its orbit. Specifically, we have calculated the trajectories of neutral particles ejected from Io and have examined the conditions under which they tend to be ionized inside Io's orbit. We find that the requirement of a localized peak constrains either the spectrum of sputtered neutrals or their source locations on Io, or both. A further constraint is placed on the ionization of neutrals on trapped (non-escape) trajectories.

Calculations and modelling

It is generally agreed that the ions in the torus originally are ejected from Io as neutrals forming neutral clouds extending far from Io. These neutrals are ionized eventually to form the torus[8-10]. This sequence suggests a possible explanation for the peak in NL^2 inwards of Io's orbit; perhaps the trajectories of neutrals are such that they tend to be inward of Io's orbit at the time they are ionized[1]. We have investigated this possibility using simple models for the production of ions from the neutrals. We have assumed that neutral particles are ejected from Io's equator with initial positions spaced uniformly in longitude and separated by 5° (see Fig. 2*a*). At each initial position, 100 neutral particles are 'launched' radially, with specified initial velocities, each set of particles having a distribution of lifetimes of the form (Fig. 2*b*)

$$P(t)\,dt = (dt/\tau)\exp(-t/\tau) \quad (1)$$

We ignore the consequences of misalignment of magnetic and rotational poles. Thus, the position of a neutral at the end of its life defines the radial coordinate of a newly created ion. To be physically meaningful, it is important that a distribution of lifetimes be used for the model, because if only the average lifetime were used there would be no way for the particles from a given source position with given velocity to spread in radial-position.

At each initial position, trajectories were calculated for velocities in the range 1.0-4.0 km s^{-1} in increments of 0.1 km s^{-1}. We used an orbit calculation program (OBCAL) that integrates the equations of motion for the particles, taking into account the gravitational forces of Jupiter and Io, the other three Galilean satellites and the J_2 term in Jupiter's gravitational potential. (The Galilean satellite trajectories are modelled as slightly inclined ellipses.) Ephemeris data for the Galilean satellites were provided by the Jet Propulsion Laboratory (JPL). The program was tested by comparing calculations of the positions of both Io and the Galileo spacecraft with calculations performed by JPL; discrepancies were $<5\times10^{-3}$ R_J.

Sputtering, in which charged particles collide with neutral matter and neutral atoms and molecules are emitted, is believed to be the dominant process responsible for ejecting matter from Io[10]. To calculate the number of particles at a given velocity, we have assumed velocity distributions expected for sputtering. The particles are assumed to come from the surface of Io; however, this should not be construed as eliminating the possibility that the particles could originate from the atmosphere or exobase. As any assumed surface source distribution maps to a shifted velocity distribution at the atmosphere or exobase, our solutions also apply to the mapped sources at these altitudes. Note that all our sources are located at Io's equator and Io is assumed to lie in the magnetic equatorial plane; these assumptions serve to make the calculation manageable. High-latitude particles emitted radially would have similar trajectories except that their orbits would be inclined with respect to the spin equator. Because energy is used to incline the trajectory rather than to change the apsides of the trajectory, high-latitude particles in general need higher initial velocities to escape and to achieve the same eccentricity as equatorial particles. Therefore, the trajectories of high-latitude particles should be similar to those of equatorial particles, except that the orbits will be inclined and higher initial velocities will be necessary to achieve the same displacement from Io's orbit. By neglecting the tilt of Jupiter's dipole, we do not take into account that the L shell of

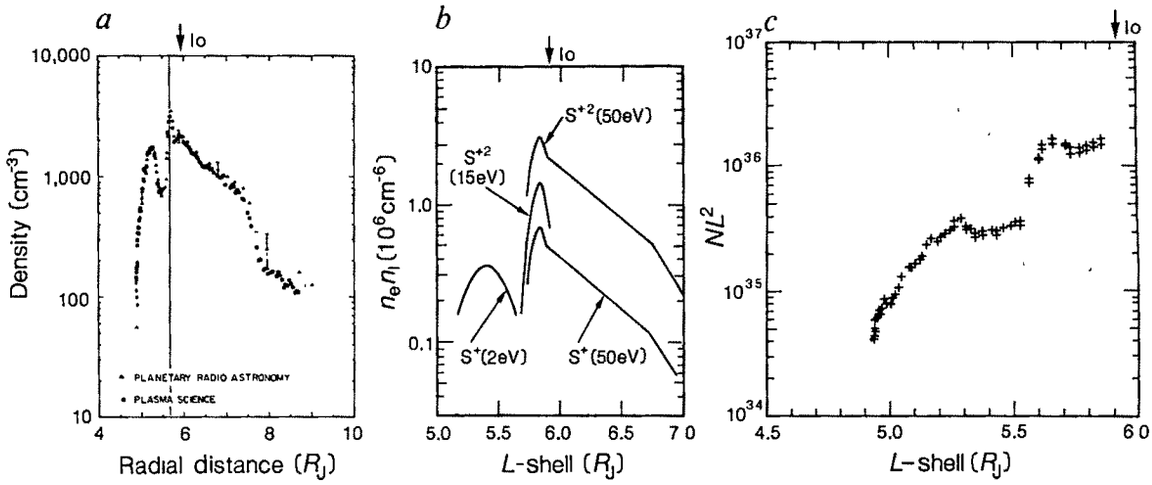


Fig. 1 *a*, Radial profile of *in situ* measurements from the Voyager spacecraft of charge density in the Io torus. The peak occurs well inside Io's orbit, at ~5.7 R_J (adapted from ref. 3). *b*, Radial profiles of ground-based measurements of sulphur-ion densities. Again the peak occurs inside of Io's orbit (adapted from ref. 1) *c*, Radial profile of NL^2, proportional to the total number of ions per unit magnetic flux, based on measurements from the Voyager spacecraft. This function, conserved in adiabatic transport, enters the canonical form of the equation for radial diffusion. The peak in NL^2 occurs at 5.6–5.7 R_J and thus requires a source of ions in this region. There also seems to be a 'plateau' at 5.2–5.3 R_J in the cold torus (this also appears as a small peak in *a* and *b*; adapted from ref. 2).

a newly created ion is necessarily the same or larger than the radial distance at which ionization occurs. However, because the electron density (and hence the ionization rate) is much higher in the centrifugal equator[11], neutrals will tend to be ionized when they are in the centrifugal equator. The change in L shell is then very slight (<0.02 R_J) and should not have any significant effect on the profiles shown here.

Results

Unless otherwise noted, our calculations assume a spatially uniform probability of ionization. First, we consider a mean lifetime of 40 h, appropriate for sulphur atoms being ionized by electron impact near Io. This is the lifetime that results if we use commonly accepted rate coefficients[12–14] and an electron density of ~2,000 cm^{-3}. Figure 3*a* shows the radial distribution of new ions from neutral particle sources distributed from 0 to 90° in longitude. Neutral velocities are assumed to obey what is called a thermal spike velocity distribution[15]

$$f(v)\,dv \propto v^3 \exp(-mv^2/kT)\,dv \qquad (2)$$

and only neutrals above the escape velocity ($v > 2.3$ km s^{-1}) are considered. (Here T, an arbitrary parameter characterizing the steepness of the distribution and not related directly to the actual temperature of the source, was chosen as 4,000 K. The thermal spike velocity distribution is only an approximation to observed sputtering spectra. For example, values of 2,000 3,600 and 5,200 K have been used to represent spectra for sputtering of various materials[16].) Note that a peak in density occurs between 5.6 and 5.7 R_J as required by the data. One should also recognize that the plotted profiles in Figs 3–5 are proportional to the source rate in a diffusion equation for NL^2.

Figure 3*a* shows that sputtered neutrals above the escape velocity with a plausible velocity distribution produce the spatial localization needed for a source of Io torus ions. The neutrals that tend to contribute most to the ionization peak between 5.6 and 5.7 R_J are those with a velocity slightly above the escape velocity (2.3–2.5 km s^{-1}). This can be understood as follows: particles that barely escape from Io, once away from Io, are on nearly circular trajectories around Jupiter and thus tend to be ionized at roughly the same distance independent of the lifetime of the neutral. Those neutrals that are injected inward thus tend to be ionized inwards of Io's orbit in a fairly narrow range of radial position. For higher injection velocities, neutrals tend to be on more elliptical trajectories and are thus ionized with a wider spread in radial position. As the thermal spike velocity distribution heavily favours injection of neutrals at lower velocities, neutrals with injection velocities favourable for formation of the inward peak tend to dominate the escaping particles. The 0–90° region of longitude is also favourable for formation of the desired peak because neutrals ejected from this region proceed on trajectories inwards of Io's orbit. However, as will be shown later, a source of neutral particles spread uniformly in longitude can also form the required inward peak. Our parameters are chosen to describe sulphur; however, because the results are relatively insensitive to lifetime they probably apply to oxygen as well.

Note that when all important gravitational forces are considered, particles with velocities as low as 2.3 km s^{-1} can escape Io even though the escape velocity from Io as an isolated body is almost 2.6 km s^{-1}. The number of neutrals ejected above the escape velocity gives the only constraint on the T parameter in the thermal spike velocity distribution. With $T = 4{,}000$ K, as in this calculation, ~4% of the neutrals escape. As will be shown below, this gives an adequate supply rate of neutrals.

Thus far, we have considered only neutral particles on trajectories that allow them to escape from Io. However, there is nothing in our model to prevent the ionization of neutrals on trapped trajectories (particles that, if not ionized, ultimately fall back to the surface of Io). Because the sputtering velocity distributions favour low velocities, a large fraction of neutrals will be on these non-escape trajectories. If we consider the same velocity distribution as in Fig. 3*a* with the same longitudinal region of sources, but include the ionization of neutral particles below the escape velocity, the radial distribution of newly created ions shown in Fig. 3*b* is formed. A small peak forms between 5.6 and 5.7 R_J, but contrary to the observations, a sharp peak forms at 5.9 R_J. This sharp peak forms because many more neutral particles are on trapped trajectories than on escape trajectories. Because of Jupiter's gravitational force, these low-velocity particles get much farther away from Io and take much longer to fall back to Io's surface than if only Io's gravitational force were considered. We are presently working on calculations of the neutral density near Io and find that the requirement of an adequate source rate of neutrals above the escape velocity implies that a dense neutral cloud (~10^5–10^6 cm^{-3}) forms within 3 Io radii of the surface of Io. For the case shown in Fig. 3*b*, a source rate of 3×10^{28} ions s^{-1} into the torus implies that $>10^2$ ionizations s^{-1} occur near Io, greater by an order of magnitud

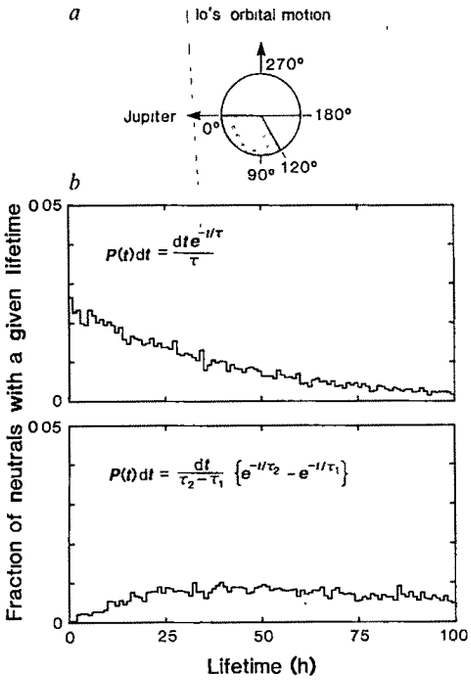


Fig. 2 *a*, Coordinate system for neutrals ejected from Io. The circle represents Io seen from above Io's orbit plane. For the calculations, particles were ejected uniformly in longitude with a separation of 5° between each position. The shaded area of the diagram shows the region where sulphur volcanoes are observed on Io[25]. Note that the coordinate system is right-handed, opposite to the one used by ref. 25. *b*, Histogram of a typical distribution of lifetimes, generated for a mean lifetime against ionization of 40 h for a spatially uniform probability of ionization. The distribution gives the probability that an atom survives a time t and is ionized between t and $t+dt$ The radial position of a neutral at the end of its life defines the radial coordinate of the newly created ion c, As *b* for the case of a molecule that is first dissociated and the products subsequently ionized: τ_1, mean lifetime for dissociation of S_2, was assumed to be 46 h (see text); τ_2, the mean lifetime for ionization of S, is 40 h. A spatially uniform probability of electron impact is assumed. As the probability of ionization in the first few hours is small, the number of ions formed from neutrals on trapped trajectories is relatively insignificant. Because any S_2^+ ions formed are lost quickly by dissociative recombination, only S_2 molecules that are first dissociated contribute significant numbers of ions to the torus.

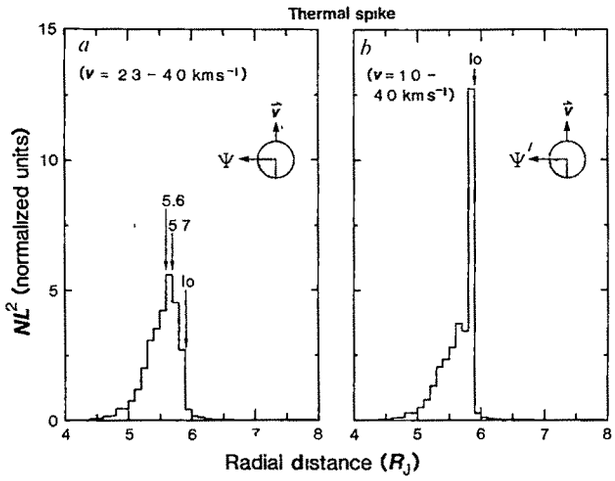


Fig. 3 *a*, Radial profile of NL^2 divided by the total number of particles, calculated for neutrals ejected from longitudes between 0 and 90° on the surface of Io with a thermal spike distribution of velocities, considering only velocities above the escape velocity A distribution of lifetimes as shown in Fig. 2*b* with a mean lifetime of 40 h was used for the neutrals. The peak at 5.6–5.7 R_J is the major feature required of a source of Io torus ions. For neutrals above the escape velocity, any velocity distribution of ejected neutrals that falls off rapidly enough with increasing velocity will cause the inward peak in NL^2 to form. A rapid fall off with increasing velocity is in fact a characteristic of velocity distributions expected for sputtering. This figure and all succeeding figures show possible source profiles of Io torus ions. The profile of the total ion density would be modified by the effects of diffusive transport, but the peak would remain in the same location. *b*, As *a* except that neutrals with velocities below the escape velocity are included A sharp peak forms at 5.9 R_J, in contrast with the observations. If we normalize the number of neutrals above the escape velocity to be 2×10^{28} s^{-1}, an adequate source rate[20] of 3×10^{28} ionizations s^{-1} in the torus is obtained, but the profile shown here would require 10^{28} ionizations s^{-1} within 3 Io radii of Io's surface. Observations place a limit of 10^{27} ionizations s^{-1} of sulphur and oxygen atoms near Io. Thus, for consistency with observations, some process must limit ionization of sulphur and oxygen atoms on trapped trajectories near Io Injection of S_2 neutrals as the dominant source of torus ions is consistent with this requirement. The profiles shown can be regarded as the source rate in a diffusion equation governing NL^2.

than the limit placed on ionization of sulphur and oxygen atoms in the vicinity of Io by observation[17].

As the Io torus requires an injection rate of $2\text{–}7\times10^{28}$ ions s^{-1} (refs 18–20) and the sputtering velocity distributions greatly favour the production of particles on trapped trajectories, some process must limit the ionization of atoms on these non-escape trajectories. The ionization limit is required both to explain the formation of an inward peak and to be consistent with the observations of limited ionization near Io. One way that the ionization of atoms near Io could be limited is if the predominant sputtering products are molecules. If an electron impact is required to first dissociate the molecule, and the products are ionized by subsequent electron impacts, then neutrals on trapped trajectories would fall back to the surface of Io before they could become ionized.

For estimates of the importance of this two-step process we consider the sputtering of sulphur. Sulphur sputtered from the surface of Io has often been ignored as a source of torus particles because some estimates[10,15] indicated that sputtering yields for this element would be small compared with those for SO_2. These sputtering yield estimates were based on the assumption that the binding energy is much higher for sulphur than for SO_2 (ref. 10). However, recent experiments[21] have shown that sulphur gives anomalously high sputtering yields of the order of 10^4–10^6 per incident ion when bombarded with 30–300 keV He^+ ions, a yield as much as five orders of magnitude greater than estimates for the sputtering yield of SO_2 (ref. 10). The experiments indicate that the predominant product of sputtering of sulphur is S_2, with some larger sulphur molecules present as well. S_2 would dissociate and ionize as follows (the mean lifetime, τ, is shown for each process); because lifetimes for S_2 ionization and dissociation were not available, lifetimes for O_2 were substituted[22]

$$S_2 + e^- \rightarrow S + S + e^- \quad (\tau_1 = 46\text{ h}) \tag{3}$$

$$S + e^- \rightarrow S^+ + 2e^- \quad (\tau_2 = 40\text{ h}) \tag{4}$$

On the other hand, the S_2 molecule itself can be ionized

$$S_2 + e^- \rightarrow S_2^+ + 2e^- \quad (\tau_3 = 32\text{ h}) \tag{5}$$

There is no observational limit on the ionization per second of S_2, as the observations near Io constrained only the ionization rates of atomic sulphur and oxygen. However, experiments with O_2^+ and NO^+ show that dissociative recombination occurs rapidly for these molecules for electron temperatures in the range 0.045–4 eV (ref. 23). Extrapolation of the dissociative recombination rates to 5 eV gives a lifetime in the range 4–9 h (for an electron density of 2,000 cm^{-3}), which is small compared with the ion diffusion time (30–100 days). Thus, the reaction

$$S_2^+ + e^- \rightarrow 2S \quad (\tau_4 = 4\text{–}9\text{ h}) \tag{6}$$

proceeds sufficiently rapidly that any S_2^+ ions formed will be converted into neutrals. The neutrals will then have the corotation velocity and will quickly leave the torus. Therefore, S_2^+ ions

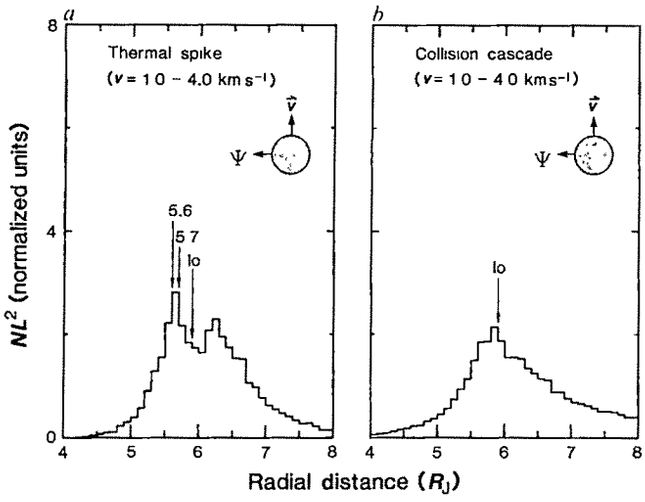


Fig. 4 *a*, Radial profile of NL^2 divided by the total number of particles. For this and all succeeding figures, the distribution of lifetimes described in Fig. 2*c* was used. Neutrals were ejected from all longitudes of Io, with a thermal spike distribution of velocities (see text). The range of velocities was 1.0–4.0 km s^{-1}. The required inward peak forms at 5.6–5.7 R_J. This is true for any velocity distribution that peaks below the escape velocity and falls off rapidly with increasing velocity. *b*, As *a* but for the limiting case of a collision cascade distribution (see text). A peak at Io is formed that is inconsistent with the required source This velocity distribution does not fall off rapidly enough to form the inward peak, but does provide a direct source of neutrals over a broad radial range.

will not add significantly to the NL^2 profiles, and we need consider only those neutrals that are first dissociated and then ionized.

For a spatially uniform probability of electron impact, the probability of a neutral's being ionized between time t and $t+dt$ following an earlier dissociation is of the form:

$$P(t)\,dt = \frac{dt}{\tau_2 - \tau_1}\left(e^{-t/\tau_2} - e^{-t/\tau_1}\right) \qquad (7)$$

If we generate neutral lifetimes using this probability distribution (Fig. 2*c*) and we use the thermal spike velocity distribution that we used in Fig. 3, but with neutral sources at all Io longitudes, we obtain the radial distribution of newly created ions shown in Fig. 4*a*. As the dissociation of the molecules must occur before the individual atoms are ionized, only a fraction of particles on trapped trajectories are ionized and the peak at 5.9 R_J is reduced. A clear peak of ion density forms at 5.6–5.7 R_J, as required by the data.

For the results shown in Fig. 4*a*, we have looked at a specific mechanism that can limit the ionization rate near Io if the source is rich in S_2. However, other processes can limit the ionization rate near Io independently of the neutral species. Because the neutral cloud within 3 Io radii of Io's surface has neutral densities orders of magnitude higher than the electron density, the physics must differ greatly in the neutral cloud from elsewhere in the torus. Other, possibly more important, mechanisms may limit ionization rates for neutrals in the dense cloud, and thus decrease the ionization probability for particles on trapped trajectories. Based on the spectroscopic observations, such a limitation certainly occurs. With a limitation on ionization near Io, any neutral species with a velocity distribution that peaks below the escape velocity and falls off rapidly enough with increasing velocity will form the inward peak.

Some workers[15,16] believe that the thermal spike distribution we have assumed is accurate only for high energy (keV–MeV) sputtering ions. If neutrals are sputtered by corotating ions, a collision cascade velocity distribution of the form

$$f(v)\,dv \propto v^3\,dv/(v^2+v_0^2)^3 \qquad (8)$$

may give a better representation. Here v_0 is the velocity corresponding to the binding energy. A relatively flat velocity distribution for neutrals does not form an inward peak if sources are distributed uniformly around Io's equator. The steepest velocity profile allowed by equation (8) corresponds to $v_0 = 0$; in Fig. 4*b* we have plotted the ion distribution obtained for this limiting case. As can be seen in Fig. 4*b*, a small peak at Io is formed. Even in the limiting case, a collision cascade distribution does not fall off rapidly enough to produce the required inward peak.

The results shown in Fig. 4 were calculated for sources independent of Io longitude, but there are reasons why ejection of particles from Io may be asymmetrical in longitude. For example, the nature of the plasma flow around Io may lead to preferential regions of access for the sputtering ions[24]. This asymmetry would be highly dependent on the character of the interaction between Io and the torus plasma, which at this time is not well understood. An asymmetry in the source locations of sputtered particles could also occur if the dominant source of the neutrals on the surface were localized. SO_2 is found at roughly all longitudes; however, sulphur is confined to the region from 0 to 120° as illustrated in Fig. 2*a* (ref. 25). As mentioned previously, recent experiments[21] have shown that sulphur gives very high sputtering yields, orders of magnitude greater than the sputtering yields estimated for SO_2. Such large sputtering yields suggest that sulphur may be the dominant neutral injected into the torus. Furthermore, some calculations[26] suggest that a 4:1 injection ratio of sulphur to oxygen is consistent with the observed ion partitioning. If we consider sulphur neutrals with the thermal spike velocity distribution coming from the sulphur-rich regions on Io, a very sharp peak is formed (see Fig. 5*a*). Even for a collision cascade velocity distribution, the required peak is still formed if the sources are localized in the sulphur-rich regions (Fig. 5*b*).

Discussion

Our results show that the requirement that neutrals sputtered from Io must ultimately form a distribution of ions with a peak in NL^2 near 5.7 R_J, substantially constrains models of the source. Ionization of neutrals on trapped trajectories must not contribute significantly to the ion population. The velocity distribution of ejected neutrals must fall off sufficiently rapidly with increasing velocity so as to favour heavily those particles near the escape velocity, or the source must be localized in longitude, or both. These requirements are easily reconciled with sulphur being the dominant neutral injected into the torus. S_2 sputtered from the surface of Io must first be dissociated before it is ionized, limiting the ionization of atomic S near Io. S_2^+ ions that are formed will be lost rapidly by dissociative recombination. The sulphur-rich volcanoes are, in fact, confined to longitudes favourable for producing the observed peak[25].

The high sputtering yields for sulphur[21] allow a velocity distribution that falls off rapidly with increasing velocity and yet still gives an adequate source rate for maintaining the torus. For example, for a 3% escape fraction, 20% surface coverage and incident H^+ ions with energies >220 keV, it has been estimated previously[10] that a sputtering yield of one would give 10^{22} neutrals per second injected into the torus. A 4% escape fraction is obtained for the thermal spike velocity distribution of equation (2) with $T = 4{,}000$ K. The measured sputtering yield of 10^6 for sulphur would then imply an injection rate of $>10^{28}$ s^{-1}, approximately the rate required from other arguments[10,18,20]. This rate estimate may be low, as bombardment by heavier ions could lead to even higher sputtering yields, and lower energy ions (corotation energy to 220 keV) will undoubtedly contribute significant additional sputtering. Because flux-tube interchange diffusion produces strong outward mixing, the proposed sulphur source would produce a hot outer torus dominated by sulphur ions. It is not known whether sputtering of sulphur at Io will be as efficient as in the laboratory. In particular, the sputtering efficiency seems to increase with increasing source temperature[21]. In this regard, the 'hotspots' seen on Io[27] may be areas of more efficient sputtering. The

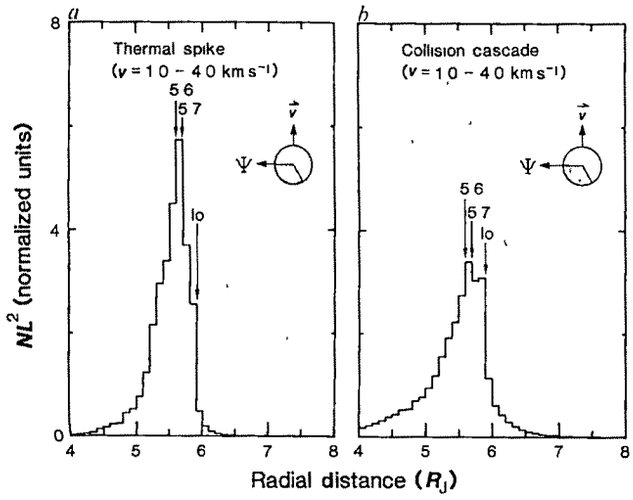


Fig. 5 *a*, As Fig. 4*a* except that the neutrals were ejected only from the sulphur-rich regions of Io[25] shown in Fig 2*b*. A sharper inward peak is formed. As recent experiments[21] indicate that sulphur gives anomalously high sputtering yields, the sulphur-rich regions may be the predominant source of Io torus ions. Ionization occurring in a narrow range of radial location near 5.7 R_J as shown here could explain the narrow, 'ribbon-like' structure seen in ground-based observations of sulphur ions[1]. *b*, As *a*, except that a collision cascade velocity distribution with a binding energy of 0.1 eV was used. An adequate inward peak is still formed, even though the fall off of the velocity distribution is much more gradual. Recent experiments[21] on the sputtering of sulphur have shown that for sputtering by 3-9 keV Ar^+ ions, an effective surface binding energy of 0.1–0.3 eV is appropriate for sulphur.

longitudinal locations of the hotspots are also favourable for formation of the inward peak.

It is more difficult to reconcile our results with SO_2 as the dominant source. The requirement that the velocity distribution fall off rapidly with velocity, coupled with the much lower sputtering yields for SO_2, make it unlikely that a dominant SO_2 source on the surface of Io could provide the necessary escape rates to populate the torus. Although our results do not eliminate sputtering from a thick SO_2 (or sulphur) atmosphere as the dominant source, they do place constraints on this possibility. It has been noted[10,28] that sputtering from such an atmosphere can easily maintain the required total source rate of neutrals. However, the velocity distribution expected for this source[28] (similar to a collision cascade distribution) does not fall off rapidly enough for the neutrals to form the inward peak unless the source is longitudinally asymmetrical. The primary sputtering product from a thick SO_2 atmosphere (density of $\sim 10^{10}$ cm^{-3} at the surface) would be atomic oxygen[10,20], so again a mechanism that limits the ionization of neutrals near Io would be necessary. If the source is a thick SO_2 atmosphere, sputtering models predict more than adequate oxygen escape rates but they encounter problems in obtaining even the minimum required sulphur escape rates[10,20]. A thick atmosphere on the dayside of Io coupled with a thin atmosphere on the nightside that allows sputtering of sulphur from the surface of Io could resolve this dilemma.

In our calculations, assumptions regarding lifetimes for neutrals ignore any variation of ionization probability with position. Thus, we have effectively assumed a uniform electron density (n_e) and temperature (T_e) distribution throughout the torus. In actuality, n_e and T_e drop sharply inside 5.7 R_J, making ionization in the region of the observed peak more likely. By using a model with uniform n_e and T_e, we are able to investigate the question of whether or not the peak can form inward of Io solely because neutral particles tend to spend most of their time in this region. As our calculations have shown, there are physically plausible scenarios in which neutral particles will tend to be ionized to form a peak inwards of Io, even for uniform distributions of n_e and T_e. This then explains how the peak in the ion density could form inwards of Io in the first place. Heating of electrons by collisions with pickup ions would then, in turn, produce the observed radial variation of T_e (ref. 29). The T_e variation would increase the probability of ionization near the peaks of the derived profiles of newly ionized neutrals. Thus, in a self-consistent calculation, the locations of the peaks we have obtained would not change, but their steepness would be enhanced. We believe that 'the ribbon' of S(II) emission observed[1] near 5.7 R_J may be explained in this way. We have not taken into account the possible effects of collisions on the trajectories in our calculations. Although collisional processes could introduce some spread into the trajectories of the neutrals, our basic result, that the inward peak forms if the distribution of neutral velocities falls off rapidly with increasing velocity, should remain unaffected.

By assuming that the dominant neutral source of torus ions is S_2, we showed that only small numbers of ions are created near Io as necessary for consistency with observations. The limitation to the ionization rate near Io can be achieved in other ways. For example, the presence of the neutral cloud near Io can limit the amount of ionization. Within 3 Io radii of Io's surface, the density of sputtered neutrals will be much greater than the electron density, so it is possible that ionization near Io is limited by the availability of the electrons or by electron cooling resulting from interaction with the neutral cloud. A cooler electron temperature in the Io flux tube has in fact been observed[30]. If the presence of the neutral cloud does in fact limit ionization and other neutral species meet the constraints on the velocity distribution and/or source locations for formation of the inward peak, then S_2 need not be the dominant neutral injected into the torus.

Similar trajectory calculations have been performed previously for sodium[31,32] and a model, including the electron density and temperature profiles, has been published for oxygen[33]. The latter calculation provides useful insight into the supply of ions to the torus, but it does not explain why the radial profiles of ion density and NL^2 peak inwards of Io's orbit. In the cited calculation[33], the measured n_e and T_e profiles were assumed and then the position where the particles were ionized was determined. The calculated radial profile of newly created ions obtained in ref. 33 peaks roughly at Io's orbit (Figure 8 of ref. 33), farther out than the peak in the ion-density data. As electron and ion number densities must peak in the same location, the electron distribution assumed for the calculation in ref. 33 would not be maintained in a steady state. Furthermore, because the newly created pickup ions are responsible for the radial variations of n_e and T_e, a calculation that assumes observed radial profiles for these quantities does not demonstrate how the peaks developed inwards of Io in the first place. In addition, we note that the calculated[33] peak in the ion-creation rate was obtained for particles launched from the exobase (at 2,600 km) with a velocity of 2.6 km s^{-1}. This velocity maps roughly to a velocity of 3.0 km s^{-1} for a particle leaving the surface of Io. As seen in Fig. 4*b*, when velocities much larger than the escape velocity become important, the inward peak does not form for sources distributed uniformly in longitude. In fact, for a velocity of 3.0 km s^{-1}, even a source restricted in longitude will not form the inward peak. Thus it seems that the neutral velocity chosen for the calculations of ref. 33 is too high to form a peak much inside Io's orbit. Finally, we note that because neutrals with velocities below the escape velocity were not considered in ref. 33, the important effects of ionization of neutrals on trapped trajectories were ignored in their calculations.

Conclusions

We have investigated models for the formation of the peak in ion density and NL^2 inwards of Io's orbit. We summarize our findings as follows: (1) Neutral particles with velocities above the escape velocity sputtered from sources independent of Io longitude will form a peak inward of Io between 5.6 and 5.7

R_J, provided that the velocity distribution drops off sufficiently rapidly. We have shown that a thermal spike velocity distribution (equation (2)) produces such a peak whereas distributions that fall off as v^{-3} or less do not create the required peak. (2) If the sputtering sources are confined to longitudinal regions on Io where sulphur predominates[25], the inward peak is obtained even for less steeply decreasing velocity distributions (such as collision cascade). If a thermal spike velocity distribution is used, a sharper inward peak is formed. (3) Velocity distributions predicted for sputtering of neutrals place many more particles on trapped (non-escape) trajectories than on escape trajectories, so an adequate source rate[20] of ions in the torus ($2\text{–}7\times10^{28}$ s^{-1}) implies that a dense neutral cloud (10^5–10^6 cm^{-3}) will form within 3 Io radii of Io's surface. Ionization of atomic sulphur and oxygen in this cloud must be limited by some process to satisfy observational constraints on ionization[17] and for formation of the inward peak. (4) The above results are more readily reconciled with S_2 being the dominant neutral injected into the torus rather than SO_2 or O. In particular, the large sputtering yields recently demonstrated for sulphur[21] would allow sputtered sulphur to have a steeply decreasing velocity distribution and still give an adequate source rate of neutrals into the torus. In addition, the sulphur-rich volcanoes on Io are confined to a longitudinal region favourable for producing the inward peak[25]. A dominant SO_2 source on the surface is unlikely, because if SO_2 were sputtered with the required rapidly decreasing velocity distribution, the source rate of neutrals into the torus would seem to be inadequate. If the presence of the dense neutral cloud near Io limits ionization of all neutral species, a thick SO_2 atmosphere cannot be eliminated; however, velocity distributions previously estimated for this possibility[28] do not fall off rapidly enough with increasing velocity to produce the inward peak without invoking a source that is asymmetrical in longitude. (5) Although the above arguments suggest that the dominant source of ions in the hot torus is neutral sulphur, consistent with previous evidence from ion partitioning[26], an SO_2 or O source with a collision cascade velocity distribution would provide ions more widely distributed in radial distance and these ions may dominate the inner cool torus, whose composition need not be the same as that of the hot torus.

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Speed of sound in the solar interior

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Frequencies of solar 5-min oscillations can be used to determine directly the sound speed of the solar interior. The determination described here does not depend on a solar model, but relies only on a simple asymptotic description of the oscillations in terms of trapped acoustic waves.

SOLAR 5-min oscillations are acoustic waves that propagate in a waveguide beneath the surface of the Sun. For any particular wave, the guide is a spherical shell whose boundaries depend on the frequency and wavenumber of the wave. Upward directed waves are reflected just benath the photosphere, where the density scale height is comparable with the wavelength. Refraction of a wave initially propagating downwards deflects the path back towards the surface, at a point where the local horizontal component of the speed of the wave is equal to the sound speed.

The frequency of a wave that resonates in the waveguide depends on the variation of the sound speed with depth. The latter determines both the locations of the boundaries of the cavity within which the wave can propagate and the time it takes for the wave to traverse that cavity. By considering successively the frequencies of a sequence of modes that penetrate deeper and deeper, it is possible, at least in principle, to measure how the sound speed varies with depth.

Using an approximation to the observed dispersion relation presented in ref. 1 for modes with degrees in excess of 100, it has already been possible to estimate the sound speed in the upper half of the convection zone[2]. More recent observations[3,4] have isolated modes with degrees as low as unity, which sample almost the entire volume of the Sun. Therefore, we should now have sufficient data to infer the sound speed almost everywhere

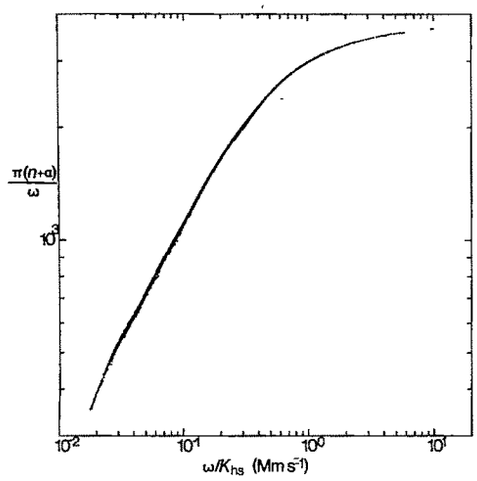


Fig. 1 Observed modes of solar 5-min oscillations collapsed according to the dispersion relation (1) with $\alpha = 1.58$. Units of ω and k_{hs} are s^{-1} and Mm^{-1}, respectively.

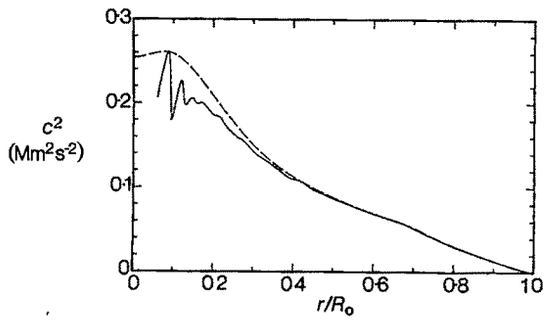


Fig. 2 Result of inverting computed eigenfrequencies of a solar model, using equation (13) to obtain an estimate c_m^2 (continuous line) of the square of the sound speed. Dashed line, square of the actual sound speed c^2 in the model. Units of speed, Mm s^{-1}.

Dispersion relation

A convenient form for analysing high-frequency p modes was introduced by Duvall[1]. He showed that if $\pi(n+\alpha)/\omega$ is plotted against ω/k_{hs}, where n is the order of the mode, ω is its frequency and k_{hs} is the horizontal component of its wave number vector in the photosphere, a value of the constant α could be found for which high-degree 5-min modes lie very close to a single curve. Thus,

$$\frac{\pi(n+\alpha)}{\omega} \simeq F(\omega/L) \tag{1}$$

where L is related to the degree l of the mode by

$$L^2 = l(l+1) = R_\odot^2 k_{hs}^2 \tag{2}$$

$R_\odot$ being the radius of the Sun. For the modes considered originally, $\alpha = 1.50$. This relation, with a somewhat different value of α, holds also for the eigenfrequencies of a solar model[5]. It is exact for a plane-parallel adiabatically stratified polytropic envelope[6,7] and deviations from it estimate an upper bound to the departure of the temperature gradient in the solar convection zone from its adiabatic value[2]. We now demonstrate that the relation is an asymptotic property of high-order acoustic modes in a spherical star, even when the stratification is not polytropic.

With respect to spherical polar co-ordinates (r, θ, ϕ) the vertical component v of velocity, say, of a linearized normal mode, has the functional form

$$v(r, \theta, \phi, t) = \mathrm{Re}[V(r)\, Y_l^m(\theta, \phi) e^{-i\omega t}], \tag{3}$$

where Y_l^m is a spherical harmonic of degree l and order m. Similar expressions hold for the perturbations in pressure and

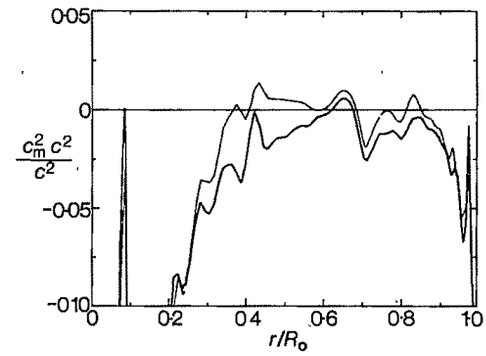


Fig. 3 Thick line, relative deviation $(c_m^2 - c^2)/c^2$ of the inferred squared sound speed c_m^2 shown in Fig. 2 from the actual value of c^2 in the theoretical model. Thin line, relative deviation from c^2 of the squared sound speed c_0^2 in Fig. 4 inferred from the solar data.

density. The amplitude function V satisfies a differential equation which admits, with appropriate boundary conditions, a discrete set of eigenfunctions that can be labelled with an integer n. The problem can be treated by standard asymptotic techniques when n is large[8–10]. The following simplified argument contains the essential physics, and to a good approximation yields the correct dispersion relation.

For modes with frequencies well in excess of the maximum buoyancy frequency in the Sun's interior (~0.5 mHz in standard theoretical solar models) the effect of buoyancy may be neglected. Furthermore, high-order modes produce a negligible perturbation to the gravitational potential. They may be characterized by a local wavenumber k whose vertical component $k_v(r)$ is much greater than the curvature and the inverse scale heights of the unperturbed structure of the Sun. The waves are therefore locally plane, satisfying

$$v(r, \theta, \phi, t) \propto e^{i(k\cdot r - \omega t)} \tag{4}$$

where r is the position vector and k and ω are related by the pure acoustic dispersion relation

$$\omega^2 = k^2 c^2 \equiv (k_v^2 + k_h^2) c^2 \tag{5}$$

with $k = |k|$. This simple relation is not valid close to the upper reflecting boundary of the cavity, where scale heights are comparable with k_v^{-1}. Nor indeed does it even predict the existence of that reflecting boundary. Where the relation is valid, however, it determines $k_v(r)$ according to

$$k_v^2 = k^2 - k_h^2 = \frac{\omega^2}{c^2} - \frac{L^2}{r^2} \tag{6}$$

Near the solar surface $\omega^2/c^2 \gg L^2/r^2$, unless L is very large, and $k_v^2 \gg k_h^2$. The waves propagate almost vertically, and the structure of the amplitude function V is essentially independent of the horizontal component k_h of the wave number vector. As r decreases, so does ω^2/c^2. Thus, according to equation (5), k_v^2 decreases with depth. The wave is refracted away from the vertical, and is eventually turned back at $r = r_t$, where

$$r_t^2/c^2(r_t) = L^2/\omega^2 \tag{7}$$

and propagation is locally horizontal.

From a comparison of the separated form of equation (3) with the local approximation in equation (4), it is evident that beneath the outer reflection point $V(r)$ is an oscillatory function where $r > r_t$. In particular, the difference in phase between the inner turning point $r = r_t$ and the outer reflection point $r = R_t$ is

$$\Delta\Psi = \int_{r_t}^{R_t} k_v \, dr \tag{8}$$

Had the mode been trapped between rigid surfaces the condition imposed by the waveguide would have been $\Delta\Psi = n\pi$, where n

is an integer. But the existence of evanescent regions above and below the cavity introduces an additional phase shift $\pi\alpha'$. Therefore, the dispersion relation is

$$\Delta\Psi = \int_{r_t}^{R_\odot} \left(\frac{\omega^2}{c^2} - \frac{L^2}{r^2}\right)^{1/2} \mathrm{d}r = \pi(n+\alpha') \tag{9}$$

Because the upper limit of integration is close to the surface of the Sun, we replace it by $R_\odot$ and absorb the small change in the integral into α', dropping the prime. The integral is now the product of ω and a function of ω/L alone, because, according to equation (7), r_t depends on ω and L only in the combination ω/L. Consequently the dispersion relation (9) becomes

$$F(w) \equiv \int_{r_t}^{R_\odot} \left(\frac{r^2}{c^2} - \frac{1}{w^2}\right)^{1/2} \frac{\mathrm{d}r}{r} = \frac{\pi(n+\alpha)}{\omega} \tag{10}$$

where $w = \omega/L$.

The phase shift introduced at the lower turning point is only weakly dependent on the structure of the solar model, because there the local dispersion relation (5) is a good approximation and the geometry of the deflection of the wave is adequately taken into account by the integrand in equation (10). Indeed, equation (9) may be derived directly from ray theory[11], in which $\Delta\Psi$ is expressed as an integral along a ray whose geometry is determined by equation (6).

For low-degree modes $r_t/R_\odot \ll 1$ and equation (10) may be approximated by

$$\omega \simeq \pi(n+\tfrac{1}{2}L+\alpha)\left(\int_0^{R_\odot} \frac{\mathrm{d}r}{c}\right)^{-1} \tag{11}$$

This can be compared with the dispersion relation for high-order acoustic modes of an isothermal gas confined in a rigid sphere, which is known exactly, and whose leading term is of the form in equation (11) with $L+2\alpha$ replaced by $l+1$. Thus, in this limit the form of equation (10) seems to be roughly correct, provided l is not too small.

To estimate the phase factor α it is necessary to account also for the phase shift on reflection at $r = R_t$. For this we consider the limit of high degree. In that case the modes are confined to the convection zone, which we approximate by an adiabatically stratified plane-parallel polytrope of index μ under constant gravity g. Then equation (10) becomes

$$\omega^2 = 2\mu^{-1}(n+\alpha)gk_{hs}, \tag{12}$$

which is identical to the exact dispersion relation under these conditions[6,7] if $\alpha = \mu/2$. Since conditions near $r = R_t$ are essentially independent of l, we deduce that this constant value of α is approximately valid for all l. Then equation (10) takes the form of equation (1). Moreover, if L were replaced by $l+\frac{1}{2}$, the approximation (11) would agree precisely with the leading term of previous asymptotic analyses[8,10] of low-degree p modes.

Observations

Measurements of $\omega(n, l)$ were made from two sets of Doppler observations using one or more of three different methods. The first data set[1] consists of a spectrum of prograde and retrograde sectoral and nearly sectoral modes with $105 \leq l \leq 892$. The resolution is ~ 10 in l and the uncertainty in the spatial scale is $\sim 0.5\%$. Frequencies were estimated by centroid analysis, and zonal-mode frequencies were deduced by averaging corresponding prograde and retrograde frequencies. The second data set[4] consists of a spectrum of zonal oscillations with $1 \leq l \leq 200$, with a resolution of ~ 3 in l and a scale uncertainty of $\sim 0.5\%$. For $l \leq 20$ individual modes could be isolated and identified, and their frequencies measured to ± 2 μHz. For $l > 20$ spline functions were fitted by eye to visual representations of sets of modes of like order n and used to determine the frequencies; we estimated that the frequencies determined in this way are correct

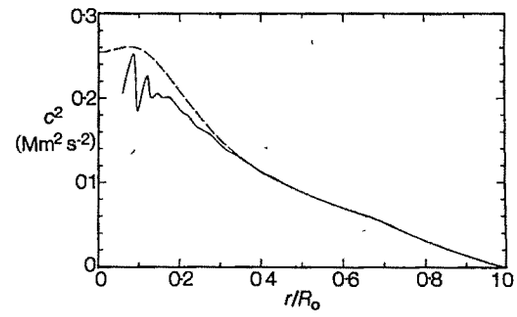


Fig. 4 Square of the interior sound speed c_0^2 in the Sun (continuous line) obtained by inverting the data in Fig. 1. The value of c^2 in solar model is included as a dashed line for comparison.

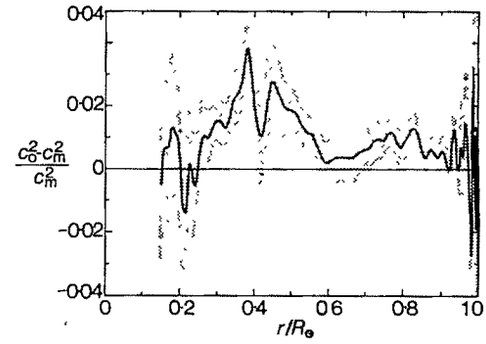


Fig. 5 Relative discrepancy $(c_0^2 - c_m^2)/c_m^2$ between the squared sound speeds in Figs 2 and 4 obtained by inverting corresponding oscillation frequencies of the Sun and the solar model. The shaded region lies within one standard deviation of that part of the error in the inferred values of c_0^2 resulting from random errors in the data It was computed from many theoretical realizations of the data obtained by adding random errors to the real solar data with the same variance as the error estimates that are indicated in the text.

to within ±10 μHz. When a mode was present in both data sets the average frequency was adopted in the subsequent analysis. A comparison of the original data sets revealed a systematic discrepancy that was larger than the estimated errors in either. The root of the discrepancy was traced to an error in the reduction of the high-degree data, which caused spatial scales to have been overestimated by 1%. Accordingly we increased the values of l in the original high-degree data set by 1%, although an increase of 1.4% would have minimized the discrepancy. In total, 2,820 modes were used, with lower turning points r_t in the range 0.06–0.994 $R_\odot$. Note that the quality of the estimates and the degree of smoothing changes at $l = 20$ and in the range 105–200.

The relation defined by equation (1) was fitted to the combined data, which are shown in Fig. 1. It was determined by choosing α such as to minimize the spread in the data, spread being measured by the sum of the squares of the altitudes of all triangles whose vertices are triplets of data points in Fig. 1 with consecutive values of ω/k_{hs}; for this purpose the base of a triangle is defined as the line joining the two points of a triplet with the extreme values of ω/k_{hs}. This yields $\alpha = 1.58$; other definitions of spread, such as the r.m.s. deviations of the data from an appropriately chosen smooth curve, gave similar results.

On the whole the scatter in the data decreases as ω/k_{hs} increases: those modes considered in ref. 1 having $\omega/k_{hs} \lesssim 0.1$ Mm s^{-1} have an r.m.s. deviation from a smooth curve of $\sim 0.4\%$, whereas the deviation is less than 0.1% when $\omega/k_{hs} > 1$ Mm s^{-1}. Theoretical eigenfrequencies of a solar model behave similarly. Therefore, it seems that much of the variation can be

attributed to the inaccuracy of our simple asymptotic analysis, which increases as l increases.

Inversion of dispersion relation

The first part of equation (10) can be inverted[11] to yield r as a function of c/r:

$$r = R_\odot \exp\left\{ -\frac{2}{\pi}\int_{c_s/R_\odot}^{c/r} [w^{-2}-(c/r)^{-2}]^{-1/2}\frac{\mathrm{d}F}{\mathrm{d}w}\,\mathrm{d}w \right\} \quad (13)$$

where c_s is the value of c at $r = R_\odot$. According to the second part of equation (10), the function F is equal to the observed quantity $\pi(n+\alpha)/\omega$. Consequently equation (13) can be used to determine $c(r)$ from the observations presented in Fig. 1 without any recourse to a solar model.

Given the simplified nature of the arguments leading to equation (10), the reliability of equation (13) may at first sight seem rather dubious. Therefore before using it to analyse the real solar data we tested it on a theoretical solar model. Adiabatic eigenfrequencies of the modes corresponding to those that had been observed on the Sun were computed, using the equilibrium solar Model 1 of ref. 12. These were fitted to the relation (1) in the manner described above, yielding $\alpha = 1.39$. A representation of the function F was then obtained by applying twice a 15-point running mean to the theoretical analogue of Fig. 1. The smoothing is necessary because deviations from the simple asymptotic law of equation (10) cause modes of different order to lie on somewhat different curves. The resulting estimate of $F(w)$ was extrapolated to $w = c_s/R_\odot$ with the power law Aw^a, with the coefficients $a = 1.035$ and $A = 1.70\times10^7$ (in c.g.s. units) chosen to match smoothly onto the lower end of the theoretical data. Its derivative was evaluated by centred second-order accuracy differencing of the smoothed representation of the data. Finally the expression on the right-hand side of equation (13) was evaluated using a difference scheme tailored to accommodate the square-root singularity in the integrand.

In Fig. 2 the square of the sound speed (c_m^2) inferred by this procedure is compared with the actual value of c^2 in the solar model. In a substantial region the result of the inversion is quite accurate. This is emphasized in Fig. 3, which shows the relative errors in the inferred values of c^2. For $r/R_\odot$ between 0.4 and 0.9, the error in c^2 is <2.5%.

In view of the breakdown of the assumptions of the asymptotic theory near the surface, the large error when $r > 0.9\ R_\odot$ is not surprising. The reason for the errors in the deep interior is less obvious, although it appears to arise in part from the fact that the inversion procedure is incremental, and can accumulate errors in the asymptotic analysis that occur nearer the surface.

Determination of solar sound speed

The inversion of the solar data in Fig. 1 was performed in exactly the same way as for the artificial data, except that the parameters definining the power law used to extrapolate F to the surface were $a = 0.846$, $A = 2.63\times10^6$. The results are presented in Figs 3–5.

The qualitative features of the inversions shown in Figs 2 and 4 are quite similar. A quantitative comparison is made in Fig. 5, in which the relative deviation of the square of the inferred solar sound speed (c_0^2) from that inferred by inverting the eigenfrequencies of the theoretical model (c_m^2) is plotted. In the region between 0.4 $R_\odot$ and 0.9 $R_\odot$, where the inversion is expected to work well, c_0^2 is within 3% of c_m^2. Furthermore, it is striking how the difference between c_0^2 and c_m^2 follows the errors in the inversion of the theoretical data, both of which are shown in Fig. 3. In particular, the behaviour is similar above 0.9 $R_\odot$ and below 0.4 $R_\odot$, where our inversion technique seems to fail. Systematic errors arising from inaccuracies in the simple asymptotic procedure are similar for the Sun and the solar model, suggesting that the error in Fig. 5 is substantially less than the discrepancies plotted in Fig. 3.

To test this hypothesis, we compared inversions of 5-min eigenfrequencies of two theoretical models of the Sun: Model A of Christensen-Dalsgaard *et al.*[13] and Model 1 (see ref. 12). The sound speeds in the two models differ substantially beneath the convection zone and thus provide a basis for assessing the significance of the deviations plotted in Fig. 5. Inversions were performed on 363 common modes whose frequencies lie in the range spanned by the solar observations. The relative difference between the squares of the sound speeds thus inferred is plotted in Fig. 6, together with the actual difference. Because relatively few modes were used, the errors in the inversions are rather greater than those illustrated in Fig. 3. However, they largely cancel in the difference plotted in Fig. 6, suggesting that except close to the solar surface and close to the centre, inversion errors contribute less to Fig. 5 than errors in the data. We conclude also from this comparison that the inversion technique is not sensitive to the detailed structure of the surface layers of the Sun. The sound speeds in Model A and Model 1 differ by up to 8% near the upper turning point R_t, and influence the frequencies of all 5-min modes whatever the value of l. However, as Fig. 6 demonstrates, those influences are successfully cancelled in that aspect of the function $F(w)$ that determines the sound speed at greater depths. This is achieved largely by absorbing the influence of the surface layers into the constant α, which takes different values for the two solar models.

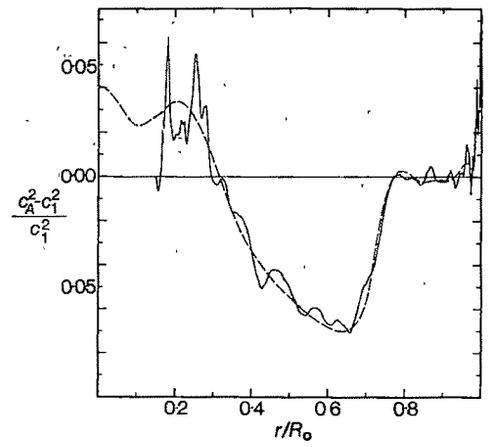


Fig. 6 Solid line is the relative difference $(c_A^2-c_1^2)/c_1^2$ between the inferred squared sound speeds c_1^2 and c_A^2 of Model 1 and Model A of the Sun obtained by inverting the frequencies of 363 5-min modes. Dashed line, actual relative difference.

We have also determined the effect of the spatial scale error in the original high-degree data by comparing inversions with and without the 1.01 scale factor imposed on l. At radii greater than 0.8 $R_\odot$ the inferred sound speeds differed by ~0.5%. At greater depths the difference decreased, and was <0.1% for $r < 0.6\ R_\odot$.

Discussion

It seems that in the region where we have succeeded in measuring $c(r)$ the differences between the solar values and those of the theoretical model are quite small. However, there is an indication that for r between 0.3 $R_\odot$ and 0.5 $R_\odot$ the sound speed in the Sun is generally somewhat greater than it is in the model. Deviations of the inferred sound speeds from a smooth curve are clearly similar in the two cases, and are partly dependent on mode selection. An earlier direct comparison between observed and calculated oscillation frequencies[14] suggested that there is an error in Model 1 somewhere between the turning points of the 5-min modes with $l = 20$ and with $l = 40$, which we find are located at $r/R_\odot \simeq 0.45$ and 0.70 respectively.

A change of curvature can clearly be seen in Figs 2 and 4 at $r \cong 0.7\ R_\odot$. This indicates the position of the base of the convection zone, which in the theoretical solar model is at $r = 0.71\ R_\odot$. In this respect the theory appears to mimic reality, and we deduce that the convection zone in the Sun is ~200 Mm deep. This value agrees with previous model-fitting calibrations by Berthomieu *et al.*[15] and by Shibahashi *et al.*[16], and is somewhat

greater than the value for the solar model preferred by Ulrich and Rhodes[17,18].

The relatively high sound speed deduced near $r = 0.4\ R_\odot$ deserves comment. A possible explanation is that the opacity used to construct the theoretical solar model is too low in the outer part of the radiative interior. An increase of ~20% in opacity at temperatures between $\sim 10^5$ and $\sim 4 \times 10^6$K, for example, would reduce the discrepancy to an insignificant level.

It has not yet been possible to determine the sound speed in the energy-generating core. This is particularly disappointing, for we had hoped to ascertain whether there had been substantial mixing in the chemically inhomogeneous layers. There are certainly grounds for believing, however, that the information is contained in the data[19].

It is of interest that the values of α obtained from the solar model and from the observations, namely 1.39 and 1.58, are significantly different, which indicates that errors in the theory of the surface layers have been made, either in the construction of the equilibrium model or in the oscillation calculation. This has also been found from comparing the observed and calculated frequencies directly[14]. A strength of the present approach is that it allows one to separate, in α, the effects of the surface layers where the physics of the oscillations is incompletely understood. The frequencies computed by Berthomieu *et al.*[15] and Shibahashi *et al.*[16] have $\alpha = 1.53$ and 1.51 respectively; the corresponding values of α computed from just those observed modes that are common with the calculations are 1.55 and 1.54. Thus, these models seem to represent the surface of the Sun more accurately. The frequencies published by Ulrich and Rhodes[18] have $\alpha = 1.40$, the corresponding value from the observations being 1.58.

Solar oscillation frequencies do not satisfy the relation in equation (1) exactly. The deviations, although small, are significant compared with the accuracy of the observations. Thus, we have not fully used the information contained in the data. To do so one must use either a higher-order asymptotic approach or a direct numerical calculation of the frequencies. In either case inversion would almost certainly be an iterative process. In general the result of such an analysis, like many a solution of a nonlinear problem, might depend on an initial guess of a model and may not necessarily be unique. On the other hand the JWKB asymptotic analysis used here does provide, in equation (13), a unique determination of the sound speed from the observed function F. The asymptotic analysis is valid if the scale of variation of the background state is much greater than all oscillation wavelengths considered, and that condition is indeed satisfied throughout most of the interior of any traditional theoretical model of the Sun. Therefore it is plausible that by using this asymptotic determination as an initial trial in a more accurate inversion, and imposing constraints of smoothness on the solution resulting from the iteration, a good model representing the large-scale structure of the Sun that satisfies the observed frequencies might be determined.

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Direct mutagenesis of Ha-*ras*-1 oncogenes by *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis in rats

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*Induction of mammary carcinomas in rats by a single exposure to a carcinogen during sexual development often involves malignant activation of the Ha-*ras*-1 locus. Each of the Ha-*ras*-1 oncogenes present in tumours induced by* N*-nitroso-*N*-methylurea, but not in those induced by 7,12-dimethylbenz(*a*)anthracene, became activated by the same G→A transition, the type of mutation induced by* N*-nitroso-*N*-methylurea. These results are consistent with the notion that Ha-*ras*-1 oncogenes are directly activated by the carcinogen during initiation of neoplasia.*

ELUCIDATION of the contribution of *ras* oncogene activation to the development of human neoplasia has been hampered by the unknown aetiology of most human cancers and the impossibility of experimental manipulations. Therefore, we have investigated animal tumour model systems that closely resemble human neoplasia. We have shown that transforming Ha-*ras*-1 genes can be reproducibly detected in mammary carcinomas induced in Buf/N rats by a single dose of *N*-nitroso-*N*-methylurea (NMU)[1]. Similar results have been obtained in other laboratories using other carcinogen-induced animal tumour systems[2-4] as well as with chemically transformed cell lines[5-7]. These carcinogen-activated *ras* oncogenes have the same type of activating mutations as those present in human tumours[1,8], thus validating these animal tumour systems as adequate models for studying the role of *ras* oncogene activation in human neoplasia.

Understanding the role that *ras* genes play in the multi-step process of carcinogenesis requires precise definition of the stage

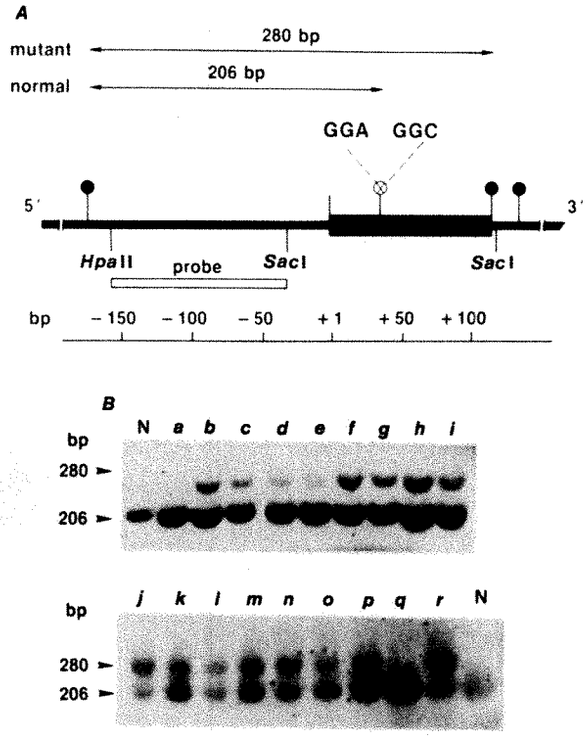


Fig. 1 *A*, Strategy for detection of *Mnl*I RFLPs created by mutations in codon 12 or 13 of the rat Ha-*ras*-1 locus. Wild-type *Mnl*I restriction sites (●) near the first exon (solid box, nucleotides +1 to +111) are indicated. The position of the polymorphic *Mnl*I site (GAGG, ⊗, nucleotides +35 to +38) relative to the sequences of the normal codon 12 (GGA) and codon 13 (GGC) is shown. The probe used is a 120-bp *Hpa*II to *Sac*I fragment (open box) comprising 5'-noncoding sequences derived from pNMU-1, a pBR322 derivative containing a transforming allele of the Ha-*ras*-1 locus[1] and allowing specific detection of this locus. Probes encompassing exon-coding sequences (for example, the 154-bp *Sac*I DNA fragment) detect an additional band, presumably derived from the Ha-*ras*-2 pseudogene, that interferes with detection of the *Mnl*I RFLPs. Arrows span the normal (206-bp) and polymorphic (280-bp) *Mnl*I restriction fragments detected by the 120-bp *Hpa*II/*Sac*I probe. ***B***, Use of *Mnl*I RFLPs to detect Ha-*ras* oncogenes in DNA isolated from NMU-induced rat mammary carcinomas: 40 μg of DNA isolated from normal breasts (N) and NMU-induced mammary carcinomas (*a*–*r*) were digested for 16 h with 40 U *Mnl*I using conditions recommended by the supplier (New England Biolabs). Digests were electrophoresed (18 h at 40 V) in horizontal 2.8% agarose gels (w/v) and blotted to either Zeta-Probe blotting membranes (Bio-Rad Laboratories) or nitrocellulose as described by Southern[23]. Membranes were hybridized under stringent conditions (50% formamide, 5×SSC, 42 °C) for 72 h to 10^7 c.p.m. ml^{-1} of ^{32}P-labelled probe obtained by nick-translation of the 120-bp *Hpa*II/*Sac*I DNA fragment described in *A*. Blots were exposed for 24 h to Kodak XAR-5 film at −70 °C in the presence of intensifier screens. Arrows indicate the positions of the normal (206-bp) and the polymorphic (208-bp) *Mnl*I restriction fragments. Co-electrophoresed ΦX174 DNA digested with *Hae*III served as size standards. Upper and lower panels represent two independent representative experiments.

at which they become activated. Recently, Balmain *et al.* have demonstrated the presence of transforming *ras* genes at the papilloma stage of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced skin carcinomas, suggesting that *ras* genes are activated early in tumour development[9]. Induction of rat mammary carcinomas by NMU does not proceed through identifiable preneoplastic stages[10]. We have taken advantage of two properties of this tumour system to investigate whether Ha-*ras*-1 oncogenes are involved in initiation of carcinogenesis. First, NMU is known to specifically (≥99%) induce G→A transitions (see refs 11, 12 for reviews). Here, we demonstrate that each of the Ha-*ras*-1 oncogenes present in NMU-induced mammary carcinomas became activated by G→A transitions in the second nucleotide of codon 12. No such mutations were observed in Ha-*ras*-1 oncogenes present in mammary carcinomas induced by DMBA, a carcinogen that does not induce specific G→A transitions[11]. These findings strongly implicate NMU as the agent directly responsible for the activation of Ha-*ras*-1 oncogenes. Second, the highly labile nature of NMU under physiological conditions has defined initiation of carcinogenesis as occurring within hours after administration of the single tumour-inducing dose of this carcinogen. Therefore, if NMU is responsible for the G→A mutations found in each Ha-*ras* oncogene, the process of oncogene activation must be concomitant with, and presumably contributing to, initiation of carcinogenesis.

Table 1 Malignant activation of the Ha-*ras*-1 locus in NMU-induced rat mammary carcinomas*

Strain	Mammary carcinomas Tested	Mammary carcinomas Ha-*ras*-1 oncogene	Normal breasts Tested†	Normal breasts Ha-*ras*-1 oncogene
Buf/N	38	31 (81%)	18	0
Sprague-Dawley	12	11 (92%)	8	0
Fischer 344	8	6 (75%)	ND	ND
Total	58	48 (83%)	26	0

* As determined by the *Mnl*I RFLP assay. Correspondence between *Mnl*I RFLPs and transforming activity was as high as 92% of tumour DNAs tested in more than one gene transfer assay. None of the normal breast DNAs was capable of inducing malignant transformation on transfection into NIH 3T3 cells.

† Includes four Buf/N and two Sprague-Dawley normal breasts obtained from tumour-bearing animals. ND, not done.

Mutagenesis in the Ha-*ras*-1 locus

We have previously reported the presence of transforming Ha-*ras*-1 genes in nine mammary tumours of inbred Buf/N rats induced by NMU[1]. One of these oncogenes was molecularly cloned and its activating mutation identified as a G→A transition in the second nucleotide of codon 12 (ref. 1). This mutation eliminates a GAGG sequence that is specifically recognized by the restriction endonuclease *Mnl*I, thus creating a diagnostic restriction fragment length polymorphism (RFLP). The first exon of the normal Ha-*ras*-1 allele is located in two *Mnl*I DNA fragments of 206 and 74 base pairs (bp) (Fig. 1*A*). Elimination of the *Mnl*I cleavage site generates a single 280-bp *Mnl*I DNA fragment. Wild-type (206 bp) and polymorphic (280 bp) DNA fragments were identified by Southern blot analysis using the 120-bp *Hpa*II/*Sac*I DNA fragment depicted in Fig. 1*A* as a probe.

Thirty-one of 38 DNAs isolated from NMU-induced tumours in Buf/N rats exhibited the diagnostic *Mnl*I RFLP (Fig. 1*B*, Table 1). In addition to the 280-bp polymorphic fragment, each tumour DNA exhibited the normal 206-bp *Mnl*I DNA fragment indicative of heterozygosity at this locus (Fig. 1*B*). Similar results were obtained with random-bred Sprague-Dawley (11 out of 12) and Fischer 344 (6 out of 8) rats, indicating the general validity of these observations. In total, 83% (48 out of 58) of all NMU-induced tumours tested scored as positive in the *Mnl*I/RFLP assay. Of these 48 tumour DNAs, 36 (75%) induced malignant transformation of NIH 3T3 mouse cells in gene transfer experiments, verifying that transfection is a reliable assay for the detection of *ras* oncogenes.

None of 20 normal breast DNAs obtained from 14 Buf/N and 6 Sprague-Dawley rats exhibited the *Mnl*I RFLP (Table 1). Moreover, the 10 tumour DNAs that did not exhibit the *Mnl*I polymorphism failed to transform NIH 3T3 cells even when repeatedly tested in gene transfer experiments. These findings indicate that malignant activation of Ha-*ras*-1 oncogenes in NMU-induced mammary carcinomas is the result of mutations specifically localized in a region no larger than four nucleotides.

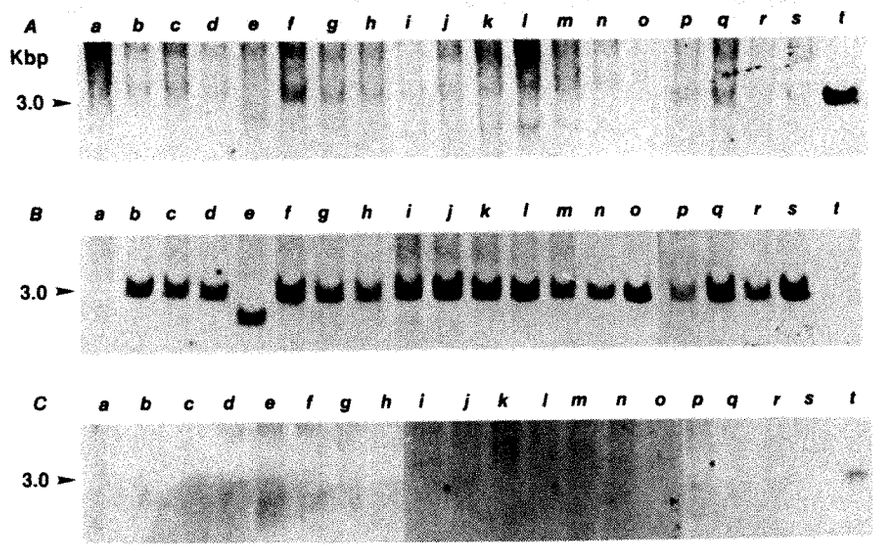


Fig. 2 Use of synthetic oligonucleotide probes to determine the point mutations responsible for malignant activation of the Ha-*ras*-1 locus in NMU-induced rat mammary carcinomas. *A*, Hybridization with probe Ha19-G^{35} (5′-TGGGCGCTGGAGGCGTGGG-3′), which recognizes nucleotides +26 to +44 of the normal Ha-*ras*-1 locus. *B*, Hybridization with probe Ha19-A^{35} (5′-TGGGCGCTGAAGGCGTGGG-3′), which detects Ha-*ras*-1 oncogenes activated by G→A transitions at nucleotide +35. *C*, Hybridization with probe Ha19-T^{35} (5′-TGGGCGCTGTAGGCGTGGG-3′), which detects Ha-*ras*-1 oncogenes activated by G→T transversions at nucleotide +35. *a*, DNAs isolated from NIH 3T3 cells; *b–s*, representative NIH 3T3 transformants derived from NMU-induced mammary carcinomas; *t*, NIH 3T3 cells co-transfected with the normal Ha-*ras*-1 gene and pSV2-neo[14] and selected for growth in the presence of G418. Arrowheads indicate the expected 3.0-kilobase (kb) *Hin*dIII DNA fragment of Ha-*ras*-1 containing all coding sequences except the first 12 nucleotides[1]. Abnormal migration is occasionally seen because the 3′ *Hin*dIII cleavage site maps outside the Ha-*ras*-1 locus and is occasionally lost during transfection. In control experiments, none of these DNAs hybridized to pBR322 or λ phage vector sequences. Co-electrophoresed DNA fragments of *Hin*dIII-cleaved λ*c*1857 DNA served as size standards. Results were compiled from two independent representative experiments.

Methods. 30 μg of each DNA was digested with 240 U of *Hin*dIII using conditions recommended by the supplier (New England Biolabs). Digests were electrophoresed (12 h at 50 V) in horizontal agarose gels (0.7% w/v) which were subsequently prepared for hybridization as described previously by Kidd *et al.*[24]. Dried gels were prehybridized for 1 h in hybridization buffer[24] containing 500 μg ml^{-1} of sonicated herring sperm DNA. Gels were subsequently hybridized for 18 h at 64 °C with individual oligonucleotide probes at a concentration of 5 ng ml^{-1} (2.5×10^9 c.p.m. μg^{-1}) in hybridization buffer containing 100 μg ml^{-1} sonicated herring sperm DNA. Hybridized gels were then washed with constant agitation as follows: four changes (300 ml) of 6×SSC for 15 min each at room temperature, 400 ml of 6×SSC for 30 min at room temperature; 500 ml of 6×SSC at 66 °C was then poured onto the gel and allowed to cool to room temperature (30 min). Finally, the gel was rinsed with 200 ml of 6×SSC at room temperature, dried and exposed to Kodak XAR-5 film for 18 h at −70 °C in the presence of intensifier screens. Labelling of oligonucleotide probes was performed under conditions allowing quantitative phosphorylation of 5′ termini with polynucleotide kinase. Reactions (300 μl) contained 0.01 nmol of individual nonadecamers, 1 nmol [γ-^{32}P]ATP (7,000 Ci mol^{-1}; NEN), 60 mM Tris-HCl (*p*H 7.8), 15 mM 2-mercaptoethanol, 10 mM $MgCl_2$ and 60 U T_4 polynucleotide kinase (New England Biolabs). Incubations were for 30 min at 37 °C and reactions were stopped by making mixtures 20 mM with respect to EDTA (*p*H 7.5). Labelled oligonucleotides were separated from unincorporated [γ-^{32}P]ATP by ion-exchange chromatography on Elutip-d columns (Schleicher & Schuell) according to the manufacturer's protocol. ^{32}P-oligonucleotides were eluted with 0.5 ml of 1 M NaCl, 20 mM Tris-HCl (*p*H 7.5), 1 mM EDTA and added directly to hybridization mixtures.

G^{35}→A mutations

We next examined whether the diagnostic *Mnl*I RFLPs detected in each of the Ha-*ras*-1 oncogenes present in NMU-induced mammary carcinomas were always the consequence of G→A mutations, which would implicate the mutagenic activity of NMU in the generation of these oncogenes. We used oligonucleotide probes to detect specific point mutations in genomic DNA[13]. We synthesized nonadecamers capable of identifying substitutions in position 35, the only nucleotide of the *Mnl*I cleavage site that can alter the coding properties of the critical codon 12 of the Ha-*ras*-1 gene. Oligomers included Ha19-G^{35} (5′-TGGGCGCTGG*AGGCGTGGG-3′, where the underlined nucleotides define the diagnostic *Mnl*I cleavage site present in wild-type DNA and G* is the deoxyguanosine residue at position 35), Ha19-A^{35} and Ha19-T^{35}. Ha19-A^{35} and Ha19T^{35} possessed the same sequence as Ha19-G^{35} except that the residue corresponding to position 35 (G* in Ha^{19}-G^{35}) was substituted by either a deoxyadenosine in Ha19-A^{35} or a thymidine in Ha19-T^{35}. The corresponding Ha19-C^{35} oligonucleotide was not synthesized because G^{35}→C transversions would have created a polymorphic *Pst*I cleavage site that was not detected in any of the tumours tested (data not shown).

Oligonucleotides probes were hybridized to *Hin*dIII-cleaved DNAs isolated from representative NIH 3T3 transformants obtained from each of 36 NMU-induced mammary carcinomas that scored positive in gene transfer experiments. NIH 3T3 transformants were selected because they usually contain amplified oncogene sequences and lack the normal Ha-*ras*-1 rat allele. Results of representative experiments are given in Fig. 1. At the discriminating temperature, each of the 36 NIH 3T3 transformants tested hybridized to Ha19-A^{35} but not to Ha19-G^{35} or Ha19-T^{35}, indicating that their transforming Ha-*ras*-1 oncogenes carried identical G→A transitions in position 35. DNA isolated from NIH 3T3 cells containing a normal Ha-*ras*-1 proto-oncogene (which was co-transfected with pSV2-neo[14])

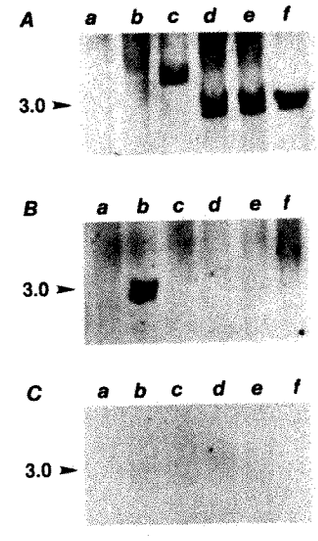


Fig. 3 Absence of point mutations in codon 12 of Ha-*ras*-1 oncogenes present in mammary carcinomas induced by DMBA. *A*, Ha19-G^{35} detects Ha-*ras*-1 genes with a normal codon 12 (G at position +35); *B*, Ha19-A^{35} detects G→A transitions in position +35; *C*, Ha19-T^{35} detects G→T transversions in position +35. Arrowheads indicate the expected position of the 3.0-kb *Hin*dIII fragment as deduced from co-electrophoresed DNA fragments of *Hin*dIII-cleaved λ*c*1857 DNA. *a*, DNAs isolated from NIH 3T3 cells; *b*, NIH 3T3 transformant derived from an NMU-induced mammary carcinoma; *c–e*, representative NIH 3T3 transformants derived from DMBA-induced mammary carcinomas; *f*, NIH 3T3 cells co-transfected with the normal Ha-*ras*-1 gene and pSV2-neo[14] and selected for growth in the presence of G418. These DNAs were digested with *Hin*dIII and electrophoresed in 0.7% (w/v) agarose gels. Dried gels were hybridized to each of the three nonadecamer probes using the same conditions as described in Fig. 2 legend.

Table 2 Malignant activation of the Ha-*ras*-1 locus in NMU- and DMBA-induced rat mammary carcinomas

Carcinogen	Oncogene detection*	Ha-*ras*-1 oncogene	$G^{35} \rightarrow A^{35}$ mutation
NMU	48/58 (82%)	48/48 (100%)	36/36 (100%)
DMBA	3/14 (21%)	3/3 (100%)	0/3 (0%)

These results demonstrate that the same point mutation is responsible for malignant activation in NMU- but not in DMBA-induced rat mammary carcinomas

* Results of NMU-induced tumours include *Mnl*I RFLP and gene transfer assays.

hybridized to Ha19-G^{35} but not to Ha19-A^{35} or Ha19-T^{35}oligonucleotide probes (Fig. 2). These results demonstrate that each of the Ha-*ras*-1 oncogenes present in NMU-induced mammary carcinomas became activated by the same mutation, a G→A transition in the second nucleotide of the critical 12th codon.

Ha-*ras*-1 genes in DMBA-induced tumours

The striking specificity of our findings suggests that the activating $G^{35} \rightarrow A$ mutations are the direct consequence of the mutagenic activity of NMU. However, we also considered the possibility that the presence of p21 proteins carrying glutamic acid residues in position 12 confers a selective growth advantage on the neoplastic mammary cells. To resolve this question we examined the mutations responsible for the activation of Ha-*ras*-1 oncogenes in mammary carcinomas induced by DMBA. DMBA, unlike NMU, forms large adducts with deoxyguanosine and deoxyadenosine residues that lead to excision repair, thus generating point mutations of undefined specificity[11]. Representative NIH 3T3 transformants derived from each of the three DMBA-induced mammary carcinomas known to contain a transforming Ha-*ras*-1 oncogene efficiently hybridized to the Ha19-G^{35} probe but not to the Ha19-A^{35} or Ha19-T^{35} probes (Fig. 3). These results indicate that DMBA-induced Ha-*ras*-1 oncogenes do not contain point mutations in either the 12th or adjacent codons. In agreement with these observations, none of these oncogenes exhibit the polymorphic *Mnl*I RFLP (data not shown).

Activation of Ha-*ras*-1 oncogenes in DMBA-induced tumours probably involves mutations in codon 61 (refs 15, 16). Although we have not defined the nature of such mutations, note that codon 61 of the rat Ha-*ras*-1 proto-oncogene is CAA[1,17]. G→A transitions cannot occur in this codon because they will result in the generation of a terminator codon, TAA. Whatever the specific nature of DMBA-induced mutations, our results argue strongly against the possibility that the specific $G^{35} \rightarrow A$ mutations of NMU-induced Ha-*ras*-1 oncogenes are the result of a positive growth selection process or a unique DNA repair system present in mammary cells. Instead, the results indicate that malignant activation of the Ha-*ras*-1 locus in NMU-induced mammary carcinomas must be the direct result of the mutagenic effect of NMU.

Biological implications

Induction of mammary carcinomas in rats by an injection of a single dose of NMU is one of the animal tumour systems most amenable to molecular analysis[1]. Because of the specific mutagenic properties of NMU (G→A transitions) it has been possible to examine whether oncogenes can be the target of chemical carcinogens. Our results demonstrate that each of 36 NMU-induced Ha-*ras*-1 oncogenes carried the same activating $G^{35} \rightarrow A$ mutation (Table 2). These findings strongly support the concept that these mutations are caused by NMU-directed methylation of the O^5 position of the second deoxyguanosine (G^{35}) in the critical codon 12. If so, our observations represent the first identification of an oncogene as a target for a chemical carcinogen. Alternative explanations for the observed mutational specificity, such as specific G→A repair mechanisms in mammalian cells or frequent G→A substitutions during tumour development, are unlikely in view of our control studies with DMBA-induced Ha-*ras*-1 oncogenes (Table 2). Moreover, Guerrero *et al.* have recently shown that Ki-*ras* oncogenes reproducibly activated in mouse lymphomas induced by γ-radiation exhibit diverse activating mutations[8] as expected from the type of DNA lesions induced by this carcinogen[11].

Our findings have further biological implications. Efficient induction of mammary carcinomas can be readily achieved by a single dose of NMU[1,18]. Because of the highly labile nature of NMU, its mutagenic effect must occur within hours following its administration. Therefore, our results imply that malignant activation of the Ha-*ras*-1 locus by NMU-induced G→A mutations must be concomitant with the initiation of the carcinogenic process.

Several laboratories have shown that *ras* oncogenes cannot transform normal (primary) rodent embryo cells unless they acquire the capability of self-proliferation either spontaneously (that is, NIH 3T3 cells), by carcinogen treatment[19] or by transfection of other oncogenes such as c-*myc*[20], adenovirus E1a[21] or even Ha-*ras*[22] when linked to strong transcriptional enhancers. It might be postulated that initiation of carcinogenesis by NMU requires the concomitant activation of two or more cooperating oncogenes, only one of which, Ha-*ras*-1, can be detected in gene transfer assays. But it is equally plausible that hormone-dependent proliferation, a normal physiological process required for tumour induction and (in some cases) progression, might provide conditions that are adequate for the phenotypic expression of the NMU-activated Ha-*ras*-1 oncogenes.

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Note added in proof: We have now identified $G^{35} \rightarrow A$ transitions in each of the H-*ras*-1 oncogenes present in the 48 NMU-induced mammary tumours used in this study. In addition, we have localized the activating mutation of the three DMBA-induced H-*ras*-1 oncogenes in the two deoxyadenosine residues of codon 61 (CAA).

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Galactic mergers, starburst galaxies, quasar activity and massive binary black holes

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Many quasar-like objects show evidence for massive binary black holes[1]. The recent discovery[2] of a massive ($5\times10^6\ M_\odot$) object in the centre of the local group dwarf elliptical M 32 greatly raises the probability of forming such binaries through galactic mergers. Here I argue that the enhancement of all kinds of activity (quasar-like activity and star formation) in galaxies with companions is not so much a consequence of tidal interaction between the massive galaxies as the result of collisions with their dwarf satellites.

Recent studies have shown that interactions between galaxies play a role in stimulating nuclear activity[3]. Imaging studies of high luminosity quasars[4,5] reveal that more than 30% have normal companion galaxies very close to them (here I use the term 'normal' galaxy to refer to any galaxy with a mass of $10^{11}M_0$ or more and refer to companion galaxies with masses much less than this as 'satellite' galaxies). Surveys of normal galaxies show that close pairs of galaxies are more likely to show activity characterised by optical emission lines[6,7] and radio emission[8] than are isolated galaxies. These results support the idea that most or all normal sized galaxies have massive black holes in their centres[3]. For simplicity I will assume that all normal galaxies have massive ($\geq10^6\ M_\odot$) black holes in their centres (it makes little difference here if the percentage is actually as low as 20%, as could be the case). The enhanced nuclear activity in pairs of galaxies would seem also to support an idea proposed by Toomre and Toomre[9] that close encounters may drive gas into the nuclear regions of a galaxy and either feed the central black hole directly or lead to a burst of star formation within the inner nuclear disc[7] which could indirectly increase the fuel supply rate. In addition to the enhanced nuclear activity close pairs of galaxies have enhanced star formation rates (as evidenced by far infrared emission[10]).

There are two problems with the Toomre and Toomre scenario. First, although a close pair of galaxies (with a projected distance of ~3 Holmberg diameters or less) is more likely to be active (as a Seyfert or radio galaxy), the majority of such galaxies (>75%) do not show nuclear activity. The mechanism is therefore less than 100% efficient or there is some other factor involved. The second problem is that although close encounters can wreak havoc on the outer parts of galaxies, the motions of the stars and gas in the tightly gravitationally bound cores are unaffected.

The solution I propose to these problems is that it is not actually the detected neighburing galaxy that is triggering quasar activity (or star formation) but instead a direct collision with one of the several dwarf satellite galaxies in the system. There is then no need to invoke some subtle action at a distance (tidal?) effect. Although the statistics of these dwarf satellite galaxies are poorly known, since they are difficult to detect beyond our local group, there are probably several (2–10) associated with each normal galaxy like our own[11]. I will here use the term 'dwarf' galaxy to refer to ellipticals and irregulars with absolute magnitudes in the approximate range $-14>M_V>-18$ (and hence ignore systems such as the Draco dwarf spheroidal). In our own local group there are two magellanic irregulars associated with our galaxy and four dwarf ellipticals associated with M31 in Andromeda.

In the scenario I propose here the statistical enhancement of quasar-like activity in close pairs of galaxies arises because of the increased probability of a merger with an unseen dwarf satellite galaxy when two normal galaxies are close. A 10^8–10^{10} $M_\odot$ dwarf galaxy near a Seyfert is hard to detect with present techniques and the only sign of a collision or merger might be the enhanced quasar-like activity and/or a burst of star formation (perhaps creating a 'starburst' galaxy). I suggest that the type of activity produced is a function of the Hubble type of the colliding satellite galaxy and the impact parameter. The amount of visible damage is also obviously going to depend on the mass of the dwarf. If a magellanic irregular galaxy hits a spiral galaxy anywhere the most probable result is a burst of star formation. If it hits the nucleus the resulting gas input and star formation could result in quasar-like activity as well. On the other hand the impact of a small elliptical galaxy, devoid of dust and gas, on the outer regions of a spiral galaxy will probably be much less spectacular. Perhaps only a distortion of the disc and spiral arms of the galaxy will occur (a dwarf elliptical satellite has typically 1% of the mass of a normal spiral and that the stars themselves do not collide).

The collision of a dwarf elliptical with a nucleus containing a dormant (or almost dormant) black hole will be more interesting. Unlike magellanic irregulars, the majority of dwarf ellipticals have strong central mass concentrations[12]. Tonry has produced powerful evidence that the 'spike' in the centre of M 32 is caused by a $5\times10^6\ M_\odot$ black hole. It would seem likely that the spikes in the luminosity profiles of other dwarf ellipticals are due to similar black holes. In a collision, if the impact parameter is small enough, the nucleus of the dwarf elliptical will be captured by the nucleus of the larger galaxy. The two black holes will go into orbit[1,13]. The distribution of stars in phase-space around each black hole will be severely perturbed, resulting in an increased fuelling of one or both black holes. I have proposed elsewhere[1] that the displaced broad-line peaks seen in many objects are the result of such orbital motion and argued that the velocities seen are consistent with the evolution of supermassive binary black holes. The discovery of a black hole in a dwarf elliptical now makes the creation of such binaries much more probable since there are an order of magnitude more dwarf galaxies than normal galaxies and the probability of mergers is correspondingly greater.

Detailed computer simulations of mergers of galaxies of all Hubble types and sizes taking into account both stars and gas need to be undertaken, as collisions of normal galaxies with dwarf satellite galaxies offer a likely explanation of both starburst galaxies and quasar-like activity. The Hubble Space Telescope could well reveal the results of the collisions when high spatial resolution studies of active galaxies are made.

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Evidence for a 150–200-km thick Archaean lithosphere from diamond inclusion thermobarometry

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Occurrences of diamondiferous kimberlites in southern Africa are concentrated within the boundaries of the Kaapvaal craton where crustal rocks are as old as 3,500 Myr (ref. 1). Garnet inclusions from diamonds that erupted in the Finsch kimberlite and kimberlites of the Kimberley group, situated in the southern part of the craton, have Rb/Sr and Sm/Nd model ages of 3,200–3,300 Myr (ref. 2). These kimberlites were erupted ~100 Myr ago[3,4]; thus the diamonds are xenocrysts. The localization of diamond-bearing kimberlites in the craton and the eruption of Archaean diamonds in late Cretaceous time are evidence that these diamonds crystallized and were immobilized in a root extending to depths of 150–200 km. Our applications of thermobarometry to silicate inclusions in the diamonds suggest that ambient temperatures at those depths 3,000 Myr ago were 900–1,200 °C, similar to the range predicted from present-day heat flow[5].

Monomineralic inclusions of olivine, garnet and pyroxenes are common in diamonds, and separate inclusions of two minerals in a single diamond have been found in many specimens[6]. Equilibration temperatures for these inclusion sets can be estimated by application of the same methods of geothermometry that have been widely used for garnet peridotite xenoliths[7–9], but it is important to recognize a marked difference in the circumstances of equilibration of the xenoliths and the inclusions.

Mineral grains in mantle rocks erupted as xenoliths were in contact with each other (and possibly with pore fluids) and had the potential for chemical exchange at high temperature until the time of eruption. Mineral grains that are included and isolated in diamonds, however, have chemical compositions that were fixed at the time of crystallization of the diamonds. The isolation of diamond inclusions is manifest in the marked differences in Nd and Sr isotope ratios obtained for garnet inclusions in diamonds and similar garnets from disaggregated mantle rocks found in kimberlite concentrates[2].

A crystallization history that is different from that of the inclusions has been experienced by three garnet lherzolite xenoliths from southern Africa that have diamond intergrown with primary silicate grains[10,11]. These and numerous non-diamondiferous, low-temperature garnet peridotites from many kimberlite pipes have estimated temperatures and depths of origin that approximate a continental shield geotherm[7,12]. The blocking temperatures for exchange equilibria used for peridotite thermometry have been estimated to be 700 °C (ref. 13) and 900 °C (ref. 14) on the basis of observed compositional profiles and experimental diffusion data. All three diamond-bearing lherzolites have equilibration temperatures in the range 1,050–1,150 °C (refs 7, 10). The least favourable estimate for the rate of equilibration at such temperatures[14] makes it probable that these peridotites reached chemical equilibrium within 10–100 Myr of the time of eruption. Thus, the times at which compositional relations were established for Archaean diamond inclusions and for the diamondiferous peridotite xenoliths erupted 100 Myr ago are believed to differ by ~3,000 Myr.

The assumption that mineral grains forming separate inclusions in a single diamond were in chemical equilibrium at the time of crystallization is supported by the fact that multiple inclusions of one mineral have the same composition[15]. The relative homogeneity of xenolith minerals[16] and the fact that coexisting olivine, garnet and pyroxene have systematic partition relations for most major and minor elements[7] are strong evidence of chemical equilibration for these rocks.

Diamond crystallization temperatures provide an upper limit for ambient temperatures at depth in the mantle at the times of diamond crystallization. The nature of the crystallization events in which natural diamonds have formed is not known; they may have been igneous or metasomatic or both. Nevertheless, these events can only have raised surrounding temperatures to values that were locally above ambient. The lower range of crystallization temperatures estimated for diamond inclusions is thus an approximation for ambient temperatures.

Comparative evaluation of element exchange reactions that can be used as thermometers for garnet-bearing, Mg-rich mineral assemblages has shown that application of the diopside–solvus method yields superior accuracy and precision[7]. The solvus cannot, however, be used for eclogitic assemblages or for diopside-free rocks and diamond inclusion sets. With such assemblages, comparable results can be obtained with Mg/Fe partition reactions, although in application to peridotites these Mg/Fe thermometers produce a scatter in estimated equilibration temperatures that is probably caused by the presence of small but unknown amounts of iron (III) (ref.7).

Two Mg/Fe partition relations that have wide applicability in the study of diamond inclusions are clinopyroxene–garnet[17] and olivine–garnet[18]. The effects on both of pressure and of the non-ideal substitution of Ca in garnet have been evaluated. The precision that can be obtained with the olivine–garnet thermometer has been estimated to be ±50 °C for favourable conditions of temperature (<1,300 °C) and composition[18], and the uncertainty associated with the garnet–diopside experimental data is a comparable value of ±5% of the temperature[17].

Peridotitic garnets included in diamonds are commonly Cr-rich with Cr_2O_3 contents ranging up to 15 wt%. The effects of this solid solution on the garnet–olivine (Mg/Fe) thermometer have not yet been evaluated. Garnets in low-temperature lherzolite xenoliths have an overlapping range for Cr_2O_3 of 2–9 wt%, and for most of these rocks, temperatures estimated with the garnet–olivine thermometer agree with those based on the diopside solvus within 100 °C (ref. 19). There is no discernible relationship between estimated equilibration temperature and Cr content of garnet for the peridotite and diamond-inclusion assemblages. Hence, an effect of Cr solid solution on the garnet–olivine thermometer seems unlikely to be important.

Analyses for 31 olivine–garnet inclusion pairs from Finsch diamonds show that 29 of the garnets are low-Ca pyropes[15], typical of the most common peridotitic garnets found included in diamonds. These are different specimens but are similar in composition to the garnet inclusions for which Archaean ages were obtained[2]. Crystallization temperatures estimated for these Finsch olivine–garnet inclusion sets have the range 900–1,300 °C at an assumed pressure of 50 kbar, within the diamond stability field[19]. Only two of the pairs, however, have crystallization temperatures above 1,150 °C. Most, with temperatures of 900–1,150 °C, are believed to best reflect crystallization in near ambient conditions.

The olivine–garnet (Fe/Mg) thermometer has a small pressure sensitivity, ~5 °C $kbar^{-1}$. Estimates for the crystallization temperatures of the 29 lower-temperature inclusion pairs thus plot in a pressure–temperature (P–T) band 250 °C wide with a slope of 5 °C $kbar^{-1}$ lying in the diamond stability field (Fig. 1). One diamond inclusion set from Finsch contains the assemblage enstatite–olivine–garnet[20], and estimation of equilibration pressure using the Al content of the enstatite, as well as temperature using the olivine–garnet thermometer, can be made for this assemblage. A point for the three-phase set plots in the diamond stability field and within the P–T band established for the olivine–garnet pairs. Estimates for the crystallization temperatures and depths of these inclusions plot close to a shield geotherm calculated for a heat flow of 40 mW m^{-2} (ref. 5).

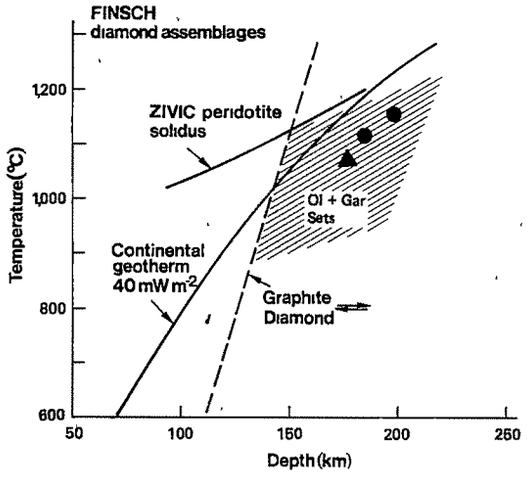


Fig. 1 Estimated equilibration conditions for diamondiferous assemblages from the Finsch kimberlite compared with the diamond-graphite equilibrium curve[35], a continental geotherm calculated for a heat flow of 40 mW m^{-2} (ref. 5), and the solidus for peridotite in the presence of H_2O and CO_2 (ZIVIC)[29]. The band defined by ruled lines is for temperature estimates made by the (Fe/Mg) olivine-garnet method[18], for 29 pairs of olivine and garnet inclusions. ▲, Olivine-garnet-orthopyroxene assemblage in a single diamond[20] for which temperature was estimated by the (Fe/Mg) olivine-garnet thermometer and pressure was obtained by the Al-enstatite method[7,36]. ●, Diamondiferous lherzolite xenoliths[10]; temperatures for these were estimated from the diopside solvus[37], and pressures, from the alumina content of enstatite[7,36].

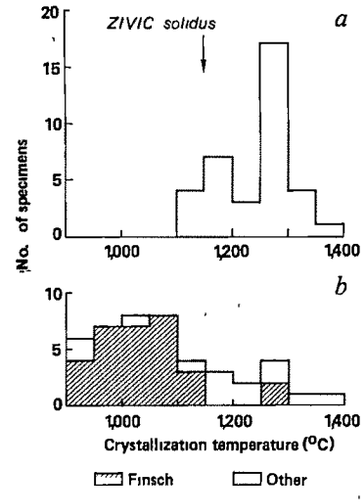


Fig. 2 Equilibration temperatures for sets of diamond inclusions of both eclogitic (*a*) and peridotitic (*b*) parageneses estimated for a pressure of 50 kbar (160 km). Eclogitic inclusions are from diamonds erupted in the Roberts Victor, Orapa and Premier kimberlites. Peridotitic inclusions are from diamonds originating at Finsch, Jagersfontein, Koffiefontein, Roberts Victor, Premier and an unknown locality. Temperatures for eclogitic inclusions were estimated with the garnet-clinopyroxene (Fe/Mg) thermometer[17]. Those for peridotitic inclusions were estimated primarily with the olivine-garnet (Fe/Mg) relation[18], but the diopside solvus[37] and garnet-clinopyroxene (Fe/Mg) thermometer[17] were also used.

Equilibration conditions estimated for two diamond-bearing garnet lherzolite xenoliths from the Finsch pipe also plot in the diamond stability field in proximity to the inclusion data (Fig. 1). The fact that the equilibration conditions for most of the inclusions and the lherzolites are similar and concordant with the shield geotherm suggests that these P-T conditions were near ambient and that they have changed little in ~3,000 Myr.

Whether there are age differences either for diamonds of eclogitic and peridotitic affinities or related to their location in the Kaapvaal craton is not yet established, but all data available indicate Precambrian ages for diamond crystallization. Diamonds with garnet inclusions from the Finsch and Kimberley pipes dated at 3,200–3,300 Myr (ref. 2) have a peridotite paragenesis. Diamonds with both eclogitic and peridotitic affinities are present in the Premier kimberlite, and sulphide inclusions in Premier diamonds have yielded a model Pb age of 1,200 Myr (ref. 21); the paragenesis of the dated specimens, however, is unknown. Isotope analyses for Pb in clinopyroxenes from eclogite xenoliths from the Roberts Victor kimberlite define an apparent age of 2,500 Myr (ref. 21), whereas Sm/Nd and Rb/Sr determinations for other Roberts Victor samples indicate a model age that may be as much as 4,200 Myr (ref. 22). Eclogite xenoliths studied in these investigations do not contain diamonds, but many other Roberts Victor eclogites are diamondiferous. Ancient, alluvial diamonds of unknown paragenesis and primary source occur in the 2,500-Myr-old Witwatersrand conglomerates in the central part of the Kaapvaal craton[23]. High $^3He/^4He$ values found in some South African diamonds of unknown specific source suggest diamond crystallization in the early Archaean[24]. A model argon age of 3,100 Myr has been obtained for a single diamond from Arkansas[25], but similar measurements have not yet been performed on South African diamonds.

Crystallization temperatures estimated for diamonds from seven Kaapvaal kimberlites show the large range of 900–1,400 °C (Fig. 2). There is a difference in the estimated temperatures for diamonds with eclogitic and peridotitic inclusions; temperatures for the eclogitic suite are confined to the range 1,100–1,400 °C, whereas the peridotitic suite has the full range 900–1,400 °C, but with a concentration in the range 900–1,100 °C. Geographical differences, however, appear in these data. Most of the inclusion sets with crystallization temperatures <1,100 °C are from the Finsch pipe (Fig. 2). Other suites of diamonds with inclusions of both peridotitic and eclogitic parageneses (in separate diamonds) from the Premier and Roberts Victor kimberlites have higher crystallization temperatures, predominantly >1,200 °C (refs 26, 27). Differences in mean carbon isotope composition between diamonds from the Finsch and Premier kimberlites have also been noted[28].

The beginning of melting for peridotite (or kimberlite) in the presence of H_2O and CO_2 at 50 kbar is ~1,150 °C (ref. 29). This fact led Boyd and Finnerty[19] to suggest that peridotitic diamonds with crystallization temperatures below 1,150 °C might be of sub-solidus origin. An obvious corollary to this suggestion is evident from the data in Fig. 2; that is, eclogitic diamonds and perhaps some peridotitic diamonds with crystallization temperatures >1,150 °C might be igneous. Diamonds with crystallization temperatures in the lower part of the range shown in Fig. 2 are believed to approach ambient conditions most nearly, regardless of the nature of the fluid from which they were precipitated.

Evidence from many xenolith suites supports the concept of a peridotite-rich, cratonic lithosphere with a thickness of 150–200 km. Recent seismic studies have provided data that are concordant with this model[30], and evidence for seismic velocity drops in the depth range 150–200 km (ref. 31) may correlate with geotherm inflections. Jordan[32] has suggested that thick continental tectospheres developed as early as 2,000–2,800 Myr.

Burke and Kidd[33] have argued that the rarity of minimum-melting granites in the Superior Province is evidence that Archaean gradients beneath shields were not much steeper than are those of the present time. Analogous evidence led Davies[34] to propose that Archaean cratons were underlain by relatively cool 'root zones' or lithosphere that extended to a depth of 200 km where the palaeotemperature was near 1,500 °C.

The results obtained from the chemical and isotopic studies of diamond inclusions are evidence that the Kaapvaal craton, and perhaps other cratons, were stabilized earlier (3,300 Myr) than hitherto believed. Moreover, these data appear most concordant with thermal models for the Earth during the Archaean in which increased heat generation from radioactive sources was lost primarily through the ocean basins (see ref. 34).

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Geochemistry of Holocene volcanic rocks associated with ridge subduction in Baja California, Mexico

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Although the geochemistry of magmatism associated with the subduction of oceanic lithosphere is well understood[1–6], the geochemical signature of lavas produced shortly after subduction of an oceanic spreading centre has not been characterized. The Baja California peninsula in Mexico provides an ideal scenario to study the latter process. Geophysical models indicate that, in response to oblique collision of the ancestral East Pacific Rise (EPR) with the western seaboard of North America at ~29 Myr, subduction of the Farallon plate was succeeded by the progressive southwards development of a transform fault system[7–9]. At 12.5 Myr a considerable length of the EPR was simultaneously subducted beneath Baja, thus terminating subduction processes along this segment[10]. By 3.5 Myr the locus of transcurrent faulting had switched from the west of Baja to within the developing Gulf of California, coupling Baja to the Pacific plate[11–13]. We present here data for Holocene volcanic rocks from Baja California Norte which show that post-ridge subduction volcanism has a distinctive geochemistry. These results have important consequences for the interpretation of ancient orogenic belts.

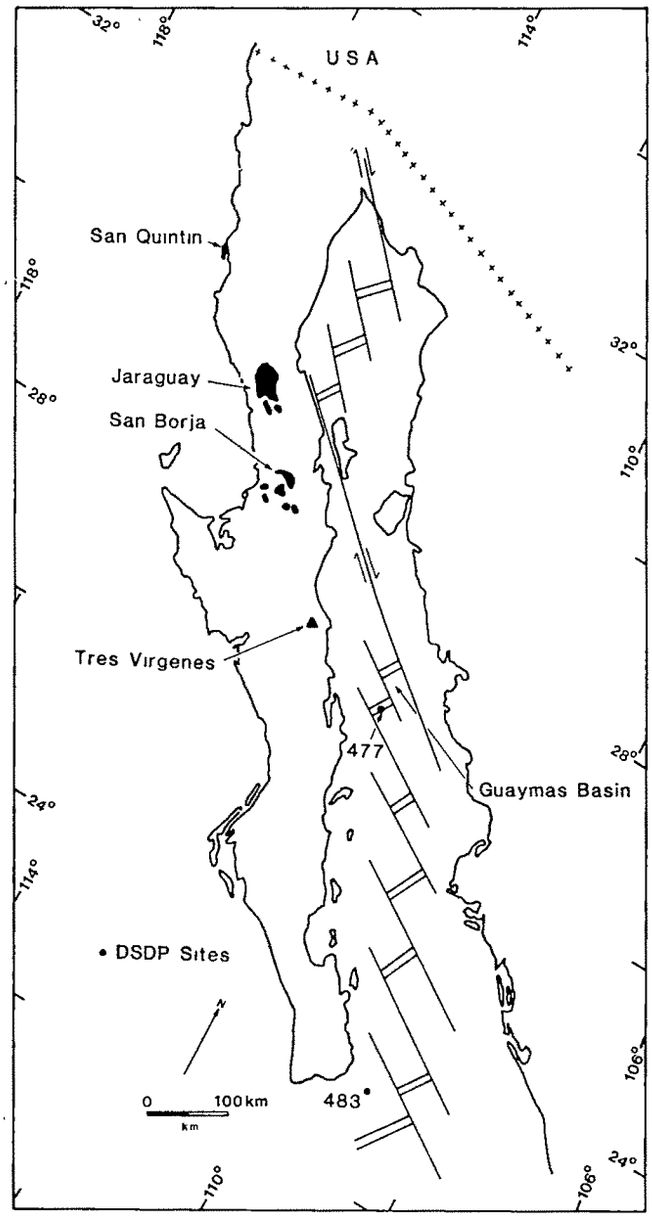


Fig. 1 Map depicting areas of Upper Cenozoic-Holocene volcanism in Baja California showing the three volcanic fields of Baja California Norte, and the Tres Virgenes volcano of Baja California Sur.

In sympathy with the changing tectonic regimes, there are variations in the geochemistry of associated volcanism. Calc-alkaline lavas related to subduction were widespread until ~15 Myr (refs 11, 16, 17) since when there has been an increase in tholeiitic and alkaline products[11,14–18], although the currently active Tres Virgenes stratovolcano[19] is also calc-alkaline[20]. As shown in Fig. 1, Holocene volcanism in northern Baja California is confined to three main areas, namely around San Quintin, Jaraguay and San Borja, although there are isolated flows elsewhere in the region[16,17,21,22]. Additionally, the lavas at San Quintin contain the only known occurrences on the peninsula of lherzolite nodules[23].

All the rocks studied form blocky flows erupted from small cones, and are fresh and sparsely porphyritic (<5%). They are morphologically very young: flows at San Quintin which overlie

Table 1 Representative analyses of Holocene lavas from Baja California Norte, the Gulf of California and Isla Cook, Chile

	San Quintin		Jaraguay		San Borja	Guaymas Basin*	Gulf of California†	Isla Cook‡
Sample	SQ1.1	SQ9.1	J14.1	J14.3	SB19 1	477A-2-2	483-24-1	4-151
SiO_2	46.26	47.17	54 64	56.30	55.92	49.00	48.40	57.65
TiO_2	2.93	2.19	1.09	1 18	2.15	1.76	2 41	1.23
Al_2O_3	15.74	15.32	15.86	16.28	15.59	17.40	14.53	17.22
Fe_2O_3‖	2.07	1.73	0 86	0.89	0.95	9 66§	13 67§	2.03¶
FeO‖	10 33	8.64	4.31	4.45	4.76	—	—	2.57¶
MnO	0.20	0 17	0 08	0.08	0.08	0.15	0.22	0.08
MgO	6.79	9.04	6.19	6.20	5.00	7.23	6.60	4.60
CaO	8 27	9 26	8 10	8.00	8.13	11.81	11.50	8 79
Na_2O	3.70	3.19	4.31	4 26	4 29	3.10	2.64	4.23
K_2O	2.02	1.47	1.83	1 84	2.26	0.25	0 12	0.43
P_2O_5	0.80	0.51	0.53	0.53	0.85	0.23	0.20	0.35
LOI	−0.05	−0.23	0 68	0.49	0.46	—	0.06	0.51
Total	99.06	98.46	98.48	100 50	100.44	100.59	100.35	99.69
Sr	758	565	2,123	2,068	2,609	241	109	2,045
Rb	37	28	7.7	8.9	9.3	1	<1	1
Ba	470	377	812	804	1,050	22	37	132
Zr	301	203	158	166	257	123	144	195
Nb	60	41	4.7	4.9	10.4	4	4	14
Y	36	26	8.3	7 9	11.4	29	49	11
Cr	87	277	209	205	84	191	140	114
Ni	86	162	157	150	85	66	61	60
La	43	31	30	30	45	5.5	4.7	37
Rb/Sr	0.049	0 050	0.0036	0.0043	0 0036	0.0041	0.0092	0.0005
Sr/Zr	2.51	2.78	13.4	12.5	10.1	1.96	0.76	10 5
Sr/Ba	1.61	1.50	2.62	2.57	2.49	11.00	2.95	15.4
Zr/Nb	5.0	5.0	33.6	33 9	24.7	30.8	36.0	13.9
K/Rb	454	434	1,973	1,716	2,017	2,075	996	>3,000
La/Rb	1.2	1.1	3.9	3.4	4 8	5.5	>4 7	37
La/Nb	0.72	0.75	6.3	6.0	4.3	2.3	1.8	2.6
$(La/Y)_N$	7.3	7.2	21.7	22.8	23 9	1.92	0.87	20.5

* Guaymas Basin basalt (64/477A/2/2, 64–68 cm)[14]
† Basalt from mouth of Gulf of California (65/483/24/1, 96–100 cm)[24].
‡ Magnesian andesite, Isla Cook, Chile[32].
§ Total ion as Fe_2O_3.
‖ $Fe_2O_3/FeO = 0.2$.
¶ Measured Fe_2O_3 and FeO.

midden deposits dated at 5,000–6,000 yr BP (ref. 16) have a much greater vegetation cover than either the Jaraguay or San Borja lavas, suggesting an even younger age for the latter. Moreover, a lava underlying the Jaraguay flows discussed here has yielded a whole rock K–Ar age of 2.69 Myr (unpublished data). Petrographically, the lavas from San Quintin are distinct in having olivine as the dominant phenocryst phase with subordinate labradorite and rare clinopyroxene. Groundmasses contain olivine, magnetite, plagioclase microlites and augite. In contrast, most of the lavas from San Borja contain only sparse olivine crystals, and clinopyroxene is the major, and often only, phenocryst phase. Pilotaxitic groundmasses comprise plagioclase microlites, magnetite, clinopyroxene and an iron-rich mesostasis. The Jaraguay and a few San Borja flows are intermediate between the two in having dominant phenocrystal olivine, subordinate clinopyroxene and trace plagioclase. Plagioclase microlites, clinopyroxene and an iron-rich mesostasis form the groundmass.

Representative analyses of the lavas from the three volcanic fields are presented in Table 1, together with samples drilled from the Guaymas Basin in the central part of the gulf of California[14], and from the mouth of the gulf[24]. The lavas from San Quintin are distinct from those of Jaraguay and San Borja, being nepheline-normative alkali basalts whose elemental variations reflect fractional crystallization of olivine + Cr spinel[22]. Zr/Nb (4.5–5.4), K/Rb (410–482) and La/Nb (0.66–0.75) ratios are lower than N-type mid-ocean ridge basalts (MORB) but comparable to other suites of alkali basalts[25]. Chondrite-normalized La/Y ratios ($(La/Y)_N$) of 6.8–7.8 indicate slight light rare-earth elements (LREE) enrichment, but there is no marked depletion of the heavy rare-earth elements (HREE). In contrast, rocks from Jaraguay are olivine or quartz normative basaltic andesites. The most striking geochemical feature is their high Sr contents (2,068–2,152 p.p.m.) which results in low Rb/Sr (0.0031–0.0043) and high Sr/Zr (12.5–13.5) ratios. Rb is also depleted with respect to K_2O, the K/Rb ratios varying from 1,716 to 2,314 (Fig. 2). Furthermore, all the large-ion lithophile elements (LILE) are enriched relative to the high field strength elements (HFSE) (for example, La/Nb = 5.2–6.7). Inter-HFSE ratios, however, have MORB-like affinities (for example, Zr/Nb = 29.3–37.0; Hf/Ta = 13.8–15.1). The lavas are strongly LREE enriched (Fig. 3) with $(La/Yb)_N = 45.7–47.9$ and $(La/Y)_N = 17.5–22.8$; moreover, Yb and Y show pronounced depletion with $Yb_N = 3.5–3.6$ and $Y_N = 3.95–4.75$. The high MgO contents of 6.2% at 55% SiO_2 classify the rocks as magnesian basaltic andesites; high Ni (150–157 p.p.m.) and Cr (199–221 p.p.m.) concentrations preclude much low-pressure fractionation.

The Holocene lavas from the San Borja volcanic field are also magnesian basaltic andesites with MgO ~5.0% at 55% SiO_2; these rocks, however, have undergone low-pressure fractionation of olivine + clinopyroxene + Cr spinel as indicated by their generally lower Ni and Cr contents (68–140 p.p.m. and 144–242 p.p.m. respectively). They show features of the Jaraguay lavas; high Sr (2,163–2,609 p.p.m.) and Ba (946–1161 p.p.m.) concentrations, high Sr/Zr (9.5–10.7), K/Rb (1,484–2,177) and La/Nb (4.2–5.9), and low Rb/Sr (0.0035–0.0046) ratios. $(La/Y)_N$ ratios (18.4–23.9) are very fractionated, with $Y_N = 4.3–6.7$. Zr/Nb ratios (23.3–39.5) overlap those of Jaraguay but are often a little more enriched (Fig. 2).

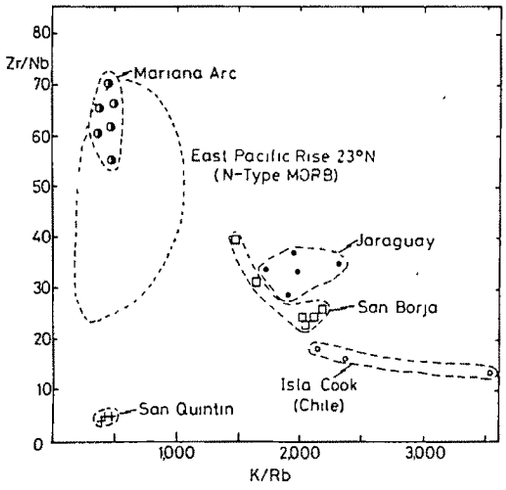


Fig. 2 Zr/Nb versus K/Rb for Holocene lavas from San Quintin, Jaraguay and San Borja volcanic fields of Baja California Norte and Isla Cook, southern Chile. MORB from EPR 23° N (ref. 24) and arc lavas from the Marianas[4] are shown for comparison.

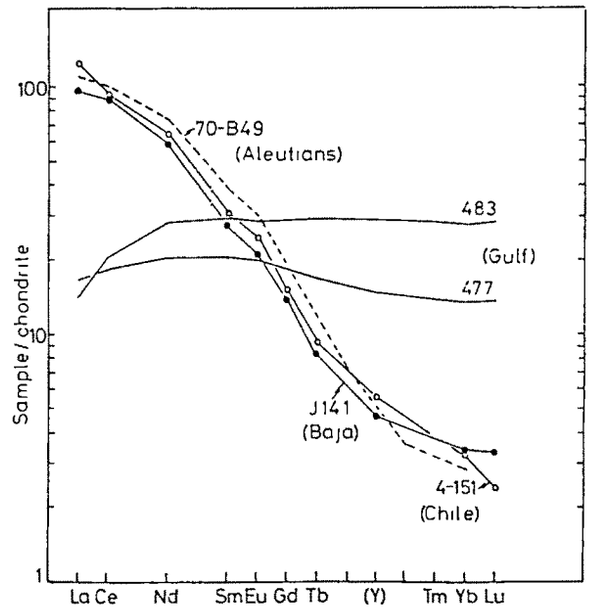


Fig. 3 Chondrite-normalized REE plot for the lavas from Jaraguay (J14.1) (unpublished data); the Gulf of California (Site 483: Gulf Mouth[24], Site 477; Guaymas Basin[14]; seeTable 1); Isla Cook, Chile (4–151)[32], and western Aleutian Ridge (70–3 49)[31]. All analyses by instrumental neutron activation analysis

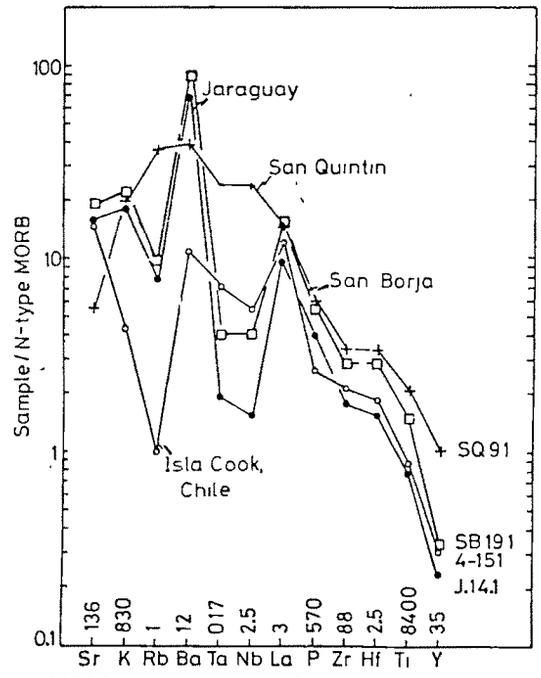


Fig. 4 MORB-normalized trace element plot for the Holocene lavas of Baja California Norte and Isla Cook, Chile[32] (Table 1). MORB-normalizing values from ref. 43.

The depletion of Y and HREE in the San Borja and Jaraguay lavas attests to the importance of garnet as a residual phase during melting, assuming that the source was not already HREE depleted. MORB-normalized plots for the three lava types (Fig. 4) reveal that whereas San Quintin is similar to other within-plate alkali basalts[5,25], Jaraguay and San Borja have pronounced Nb depletions relative to LILE, a characteristic of subduction-related magmatism[4–6]. Interestingly, recent basalts recovered from the Guaymas Basin[14] and from Isla Tortuga[18] (a tholeiitic seamount), both in the central part of the Gulf of California, also portray enrichments of LILE compared with those from the gulf mouth where there is active spreading[20]. It thus appears that this enrichment in alkalis and LREE is inherited from the subduction regime which was operative beneath north-west Mexico 12.5 Myr ago, and is superimposed on a MORB-type mantle.

High-magnesian andesites have been reported from other localities[26–37]. Those from the Bonin Islands, Mariana Forearc and Trench and Cape Vogel[26–30] have been ascribed to the boninite series. They differ from the Jaraguay and San Borja lavas in having much higher MgO (>9% at 55% SiO_2) and much lower TiO_2 and Zr contents, higher K/Rb and lower Rb/Sr ratios and very depleted REE concentrations (<20 times chondrite and usually <10). Petrographically boninites are also distinct in having phenocrysts of orthopyroxene or clinoenstatite, while lacking olivine. The magnesian andesites from Baja California are thus not members of the boninite series. However, magnesian andesites similar to Jaraguay and San Borja have been described from several volcanic arcs[31–35]. Those from the Setouchi volcanic belt in Japan are considered to be primary melts from hydrous mantle[34–37]. In the Aleutians, Kay concluded that the highly fractionated REE profiles (and, incidentally, the high Sr contents) of magnesian andesites (Fig. 3) were produced by eclogite melting. However, this cannot be the case in Baja as the enrichment of Sr and the depletion of Rb are too extreme. Magnesian andesites recently reported from Isla Cook in southernmost Chile[32,33] bear a close resemblance to the Jaraguay and San Borja samples, particularly in their high Sr and very low Rb contents (Table 1, Fig. 3). They have depleted Sr and Nd isotope systematics ($^{87}Sr/^{86}Sr = 0.70268–0.70280$; $^{143}Nd/^{144}Nd = 0.513136$), 'mantle' $\delta^{18}O$ (5.1–5.7) and Pb isotopes similar to other volcanic centres in the southern Andes[33,38]; they are thought to have risen through the crust with little interaction. It is not possible, with the available data, to preclude crustal contamination during the formation of the Jaraguay and San Borja lavas. It is, however, unlikely. First, the low Rb content and concomitant very low Rb/Sr, Rb/Ba and Rb/La ratios imply that assimilation of sialic upper crust has been minimal. Second, the low $^{87}Sr/^{86}Sr$ ratio (0.7037) reported for the Jaraguay lavas[9] precludes significant contamination either of the source by subducted sediment, or of the ascending magma. The isotope data for Jaraguay, Aleutians and southern Chile also rule out incorporation of pelagic, calcareous sediments in the magma sources to explain the very high Sr contents of these lavas.

Most interesting, however, is the apparent tectonic association of these magnesian andesites with the subduction of an actively spreading oceanic ridge. Isla Cook, for example, is sited on a transcurrent fault zone at the junction of the South American, Antarctic and Scotia plates where subduction has virtually ceased following the northward migration of the Chile Rise–Peru–Chile Trench triple junction in response to the oblique subduction of the Chile Rise. The magnesian andesites from the

Aleutians have an estimated middle Miocene age[31], which overlaps the final stages of the effects of subduction of the Kula Ridge[39]. The Setouchi high-magnesian andesites were erupted 12–14 Myr ago[37], a date which succeeds cessation of subduction of the Shikoku Basin crust at the Nankai Trough, during which process there was consumption of part of the marginal basin spreading centre[40]. Finally, the Baja Holocene lavas were erupted 12.5 Myr after subduction of the EPR. Although Moore[41] considers that the Baja California peninsula has moved northwest by ~240 km during the past 4 Myr, this movement has been along the Pacific margin and thus over regions of the mantle into which the ancestral EPR had already been subducted.

The association of these magnesian andesites (here termed Bajaite series rocks) with ridge subduction may result from increased thermal input into the mantle beneath the arc[42] and/or from modification of the chemical input from the subducting slab compared with periods of 'normal' subduction of oceanic lithosphere. It is evident that volcanism associated with ridge subduction has a distinctive geochemistry, namely the generation of magnesian andesites with fractionated REE profiles, low Rb/Sr and high K/Rb, Sr/Zr, La/Rb and La/Nb ratios superimposed on a MORB-type HFSE signature. The mechanism for producing such compositions is unclear, but probably involves garnet as a residual phase during melting of a source variably enriched in Sr, and depleted in Rb, relative to most island arc magma sources. The transcurrent fault-related setting of Baja California and Isla Cook may allow magmas to rise rapidly through the crust with insignificant contamination and only minor fractionation.

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Cenozoic weathering covers in Buchan, Scotland and their significance

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Isolated occurrences of chemically weathered rock or saprolite are common in many formerly glaciated areas around the North Atlantic[1–6]. These pockets of saprolite are widely regarded as remnants of Cenozoic deep-weathering covers which existed before the first extensive Northern Hemisphere glaciations at ~2.4 Myr (ref. 7) and their apparent survival has long been used as evidence for the local ineffectiveness of glacial erosion[2,4,8,9]. However, the scarcity of correlative deposits means that the age and significance of these fragmented saprolites are uncertain. I report here the results of a detailed survey which shows that in Buchan, Scotland, a combination of Cenozoic tectonic stability and extremely limited Pleistocene glacial erosion has allowed the preservation of deep-weathering covers of probable Miocene to early Pleistocene age on a scale not previously reported from any other formerly glaciated area.

Buchan is an area of subdued relief lying at the angle of north-east Scotland (Fig. 1). The geology consists largely of Precambrian to Dalradian metamorphic rocks intruded by Caledonian acid and basic igneous masses[10]. The only sedimentary rocks onshore are Devonian Old Red Sandstones but thick accumulations of Mesozoic and Palaeogene sediments lie offshore.

In Buchan, saprolites are continuous over wide areas[11] and maximum depths exceed 60 m in boreholes[12,13] (Fig. 2). All major rock types are affected and alteration decreases with depth, indicating that weathering is a product of subaerial rather than hydrothermal alteration.

Within major lithologies, saprolite characteristics vary widely and reflect important differences in the degree of chemical alteration. Data on granulometry and clay mineralogy, backed by geochemistry, indicate that two main weathering types exist. Most widespread is the gruss weathering type in which a relatively low degree of alteration has caused disintegration into a granular sand. Gruss is characterized by an abundance of little-altered primary minerals, low fines contents, low soluble base losses and heterogeneous and immature clay mineral assemblages (Table 1). The second weathering type, clayey gruss, is virtually confined to central Buchan. Here advanced alteration has reduced parent rocks to a sticky, clayey silty sand. Detrital primary mineralogy is dominated by quartz, fines contents are relatively high, and clay mineral assemblages consist of mature kaolinite–illite suites. At several sites, clayey grusses are rubefied due to the presence of haematite and goethite. This distinction between grusses and clayey grusses is comparable with others made for granite weathering covers in Europe south of the limits of Pleistocene glaciation[5,14,15].

The relative ages of the two weathering types can be established using evidence from correlative deposits, saprolite mineralogy and geomorphology. All deep weathering in north-east Scotland must predate the last glaciation as materials reworked from subjacent and adjacent saprolites form a major component of Devensian tills and congeliturbates[11,16,17]. Evidence that many gruss weathering profiles are of middle Pleistocene age or older is provided by: (1) the presence of weathered biotite and feldspar and inherited clays throughout the middle to late Pleistocene sequence of glacial, glacifluvial and periglacial deposits at Kirkhill[18,19]; and (2) the presence of hydromorphic iron and manganese minerals in weathering profiles now occupying free-draining sites[20]. These minerals indicate that weathering had already extended close to and probably below the water table before a major change in groundwater drainage status during the Pleistocene in response to regional drainage incision.

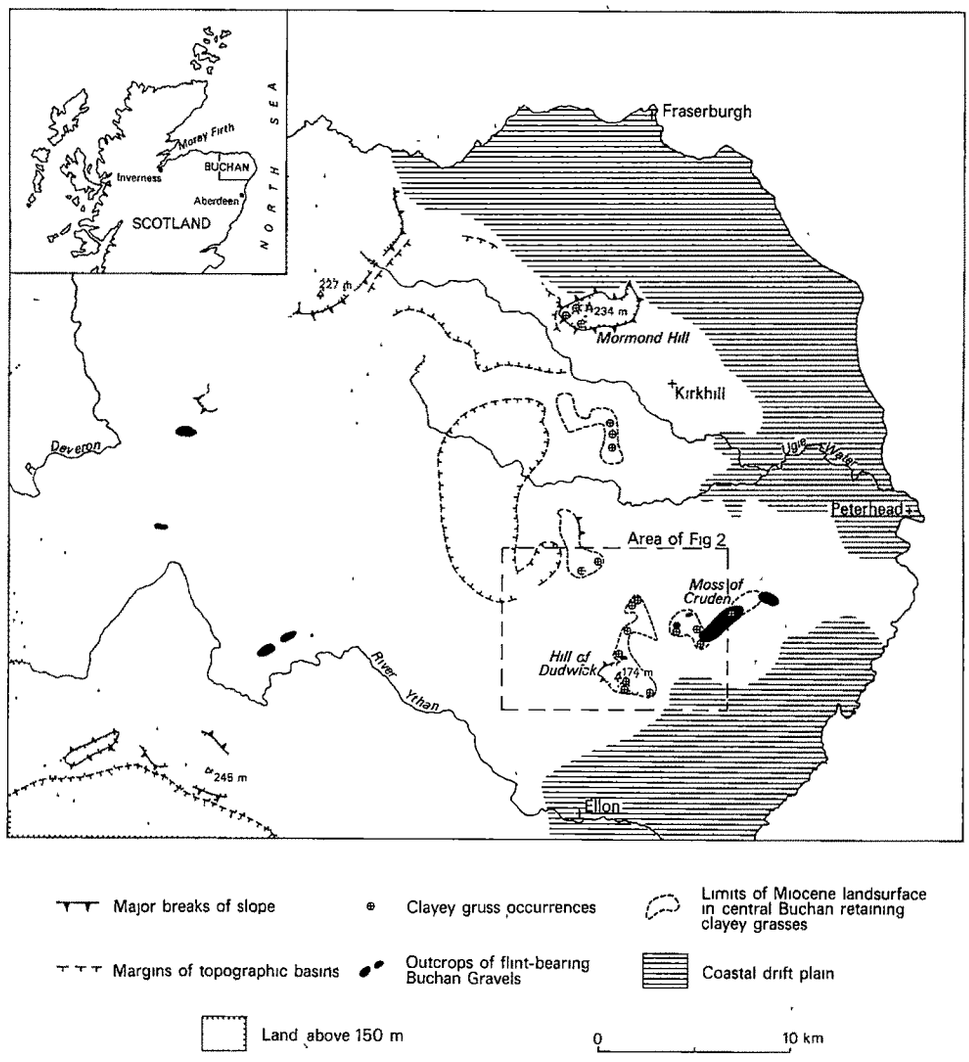


Fig. 1 Location and relief.

Table 1 Summary characteristics of weathering types

Rock type	Granite						Basic igneous			Quartz schist					Pelite	
Site and grid reference	Hill of Longhaven NK 084423	Cairnlea NJ 901537	Blackrigg NJ 912600	Mill Maud NJ 566067	Backhill NK 026407	Pittodrie NJ 693245	Silverford NJ 432253	Fedderate NJ 888505	Cruden borehole NK 028402	Moss-side NJ 949568	Howford NJ 954547	Mormond Quarry NJ 950568	Sunnyside NJ 983372	Howe of Dens NJ 975805	Kinmuck NJ 991341	Windy Hills borehole NJ 793393
Weathering type	G	G	G	G	G	CG	G	G	CG	G	G	CG	CG	CG	G	CG
% <2 μm	0.2	1.9	1.7	2.1	3.6	17.0	0.2	1.8	43.5	2.1	8.2	12.0	15.4	13.7	7.1	22.2
% <63 μm	2.5	14.4	12.7	17.5	26.1	45.0	6.7	11.3	75.5	9.1	41.3	32.0	43.0	49.8	20.1	94.7
Feldspar/quartz ratio	1.2	0.9	1.2	1.8	1.1	0.1	ND	ND	ND	0.5	0.7	0.1	0.1	0.2	ND	ND
% MgO	0.36	0.36	0.44	0.34	0.53	0.11	6.60	4.52	0.49	0.51	1.23	0.24	0.15	0.14	ND	0.18
% CaO	1.34	0.08	0.12	0.14	0.37	0.07	8.44	4.66	0.49	0.20	0.23	0.07	0.07	0.07	ND	0.10
% Na_2O	3.36	1.13	1.58	2.14	1.81	0.14	1.98	1.59	0.05	1.22	0.18	0.15	0.10	0.05	ND	0.55
% K_2O	6.03	5.3	4.65	5.63	3.56	3.00	0.56	0.55	0.45	2.71	3.84	1.24	0.63	2.80	ND	0.72
% Soluble base losses	5.9	53.4	58.6	48.6	ND	95.4	19.0	31.1	ND	ND	ND	ND	ND	ND	ND	78.9
Rubefaction	—	—	—	—	—	X	—	—	—	—	—	X	X	X	—	—
Etching of quartz	—	—	—	—	—	—	—	—	—	—	—	X	X	—	—	—
Clay mineralogy	I	H	I	K	K	K	I	Gi	K	Gi	I	K	K	K	M	K
	K			I	H	I	V	K	I	I	K	I	I	I	C	I
	H			C	V	Hm		I		K	H		Hm	Go	I	
	C				S						V					

C, Chlorite; Gi, gibbsite; Go, goethite; H, halloysite; I, illite; M, mixed-layers; S, smectite; V, vermiculite; G, gruss, CG, clayey gruss; ND, not determined.

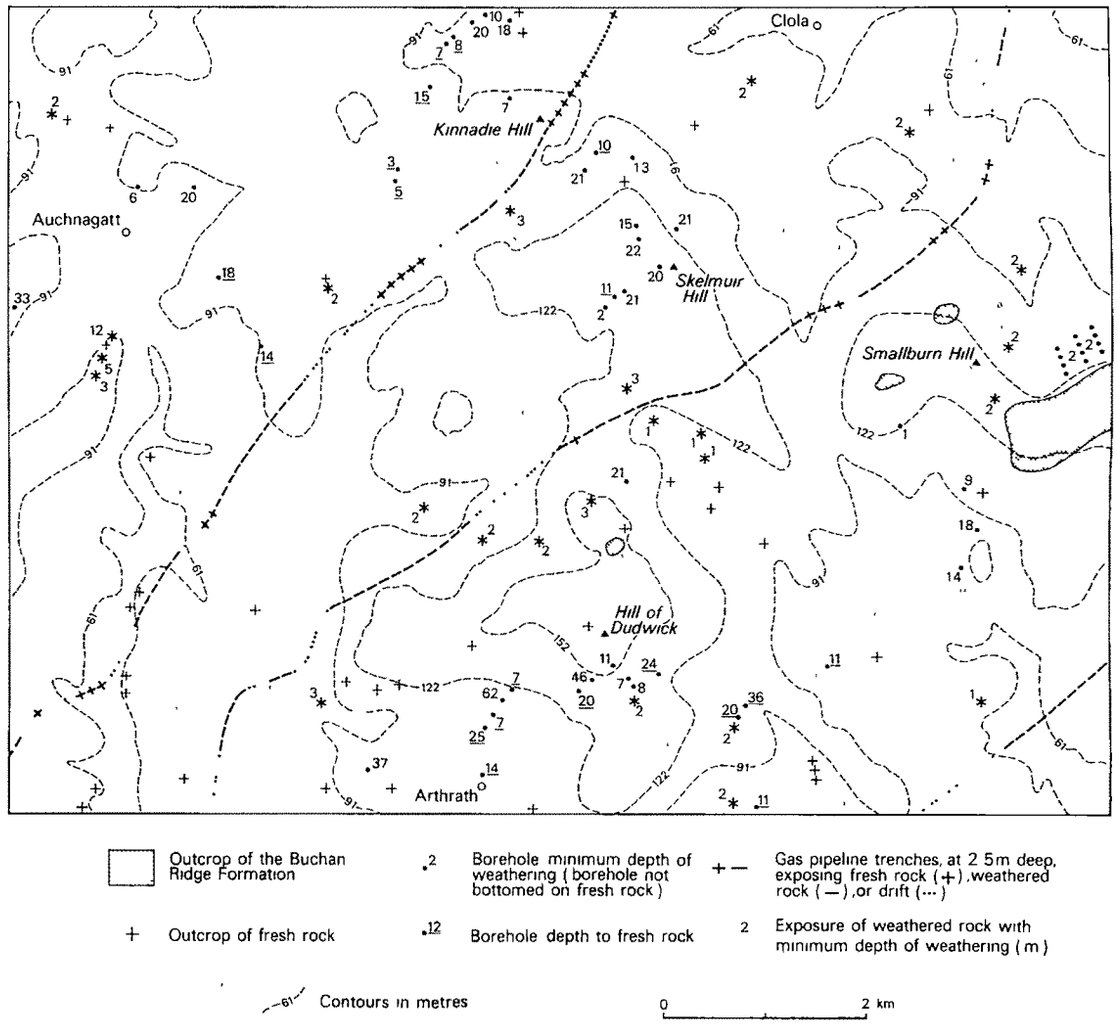


Fig. 2 Deep weathering patterns in central Buchan.

Suggestions that deep weathering is wholly of interglacial age[21] may also be rejected in view of the disparity between rates of rock alteration during the present interglacial[2], the relatively short durations (~10–30 kyr) of previous interglacials[22] and observed depths of weathering. Formation before the glacial Pleistocene is indicated.

The dominance of kandite and illite clay mineral assemblages in acid igneous and metamorphic grusses[20] and hydrous mica-amorphous iron oxide assemblages in basic igneous grusses[16] indicates weathering under humid temperate environments. However, the intensity of alteration shown by the clayey grusses requires warmer, probably subtropical climates[14,15]. Marine palaeotemperatures[23] and terrestrial palynology[24] indicate that subtropical climates last prevailed in the Scottish area during the middle Miocene (10 Myr). Major climatic cooling after this time[23,24] is reflected in the mineralogy of North Sea well samples, with appearance of chlorite and amphibole and increases in illite and feldspar contents[25,26], and in a transformation in styles of rock weathering throughout western Europe with the first development in the Tertiary of sandy saprolites retaining significant amounts of detrital feldspar and biotite[5,14]. I conclude that the clayey gruss weathering type predates the late Miocene and that the gruss weathering type developed continuously throughout the Pliocene and early Pleistocene.

The clayey grusses are virtually confined to a north–south belt of high ground in central Buchan (Fig. 1) which represents the remnants of a Miocene or older landsurface. Around Hill of Dudwick, the clayey grusses are juxtaposed with and underlie outcrops of the Buchan Ridge Formation. These intensely-weathered gravel deposits comprise clasts of rolled Cretaceous flint and Dalradian quartzite with a matrix of quartz sand and kaolinitic fines[27], and are widely regarded as Tertiary in age[28–30]. At Moss of Cruden, the gravels are >25 m thick and include clasts of little-travelled nodular flint. The preservation of such large volumes of flint suggests that Buchan has remained close to base-level since marine transgression in the late Cretaceous. This reflects the position of Buchan at the stable eastern extremity of the Scottish Highlands, an area repeatedly tilted westwards towards the rapidly subsiding North Sea basin in the Tertiary[31].

Tectonic stability has been a major factor in the extraordinary development and state of presevation of Cenozoic weathering covers. However, the survival of friable saprolites also demonstrates the singular ineffectiveness of Pleistocene glacial erosion in Buchan, where a succession of cold-based ice sheets have done little to modify Neogene landsurfaces[19].

Pockets of gruss-type saprolites occur widely in other glaciated areas bordering the North Atlantic[1–6]. Analogies with Buchan suggest that during the Neogene, lowland crystalline terrains were extensively weathered under temperate environments, with depths of weathering exceeding 30 m on interfluves. Where saprolites have survived glaciation and can be shown not to be the products of hypogene alteration[32] or exceptionally deep alteration down fault zones[12,20], it can be proposed that local Pleistocene glacial erosion has amounted to at most the removal of a few tens of metres of regolith and exposure of the basal surface of weathering. Such estimates can be reconciled with regional evidence of deep Pleistocene erosion deduced from the

volumes of offshore sediments[33,34] by recognizing the selectivity of glacial erosion. Enclaves of little-modified Neogene relief have survived in parts of eastern Canada[4,35], eastern Greenland[36], Fennoscandia[5,37–39] and the British Isles[2,9,40] where local factors, including remoteness from ice accumulation centres, low gradients and diffluent flow patterns, have combined to maintain basal ice below its pressure melting point and so prevent significant glacial erosion[36]. Finally, the thicknesses of saprolite reported here would suggest that material reworked from preglacial Cenozoic weathering profiles forms a major, but largely overlooked component of glacial tills[37,41].

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Isotope composition of sulphates generated by bacterial and abiological oxidation

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Oxygen isotope compositions of secondary sulphates provide a potentially powerful tool for elucidating mechanisms of sulphur oxidation. Such studies should reveal the consequences of various anthropogenic sulphur contributions to the environment, including gaseous emissions, aqueous solutions and elemental sulphur dust. At mining sites, metal sulphides may be oxidized *in situ*, giving rise to acid mine waters. The environment can receive these compounds from natural sources also, for example volcanoes, sulphurous springs and sour-gas seeps. There are many fundamental questions concerning the specific oxidation reactions and associated kinetics. In principle, isotope data from appropriate experiments can be used to address such questions. In the case of acid mine drainage, the approach of Taylor *et al.*[1] is noteworthy. Here we present a critical examination of their results and suggest a novel method of determining the kinetic isotope effects identified with the incorporation of O_2 and H_2O-oxygen by SO_4^{2-}.

Oxygen isotope data may provide information about redox reactions in the sulphur cycle when sulphur isotope data fail to do so. For example, SO_4^{2-} in subsurface water may be partially reduced to HS^- in deeper anaerobic enviroments, and the reduced product may be subsequently re-oxidized near the surface. The sulphur isotope composition may be conserved if processes such as metallic sulphide deposition or H_2S escape are absent; in this case there would be no sulphur-isotope evidence of the reduction–re-oxidation sequence. However, during re-oxidation, oxygen incorporated in the product SO_4^{2-} can be derived from both O_2 and H_2O molecules; this generally produces $\delta^{18}O$ values different from those of the original SO_4^{2-}. The above scenario has been documented for sulphates in several springs in western Canada[2–4].

Taylor *et al.*[1] presented a scheme whereby a plot (their Fig. 2) of $\delta^{18}O$ values of SO_4^{2-} versus those of associated H_2O can be used to evaluate the relative participation of two specific oxidation reactions. In our laboratory, $^{18}O/^{16}O$ analyses have been done on sulphate produced by oxidation of biogenic H_2S (near sulphurous springs)[2,3] and of metal sulphides (in acidic springs)[3]. It would seem that the approach of Taylor *et al.*[1] could be applied to these data. However, when this approach was attempted, it became apparent that their Fig. 2 required refinement.

Taylor *et al.*[1] cited three reactions identified with the oxidation of FeS_2:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (1)$$

$$Fe^{2+} + \tfrac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2}H_2O \quad (2)$$

$$FeS_2 + \tfrac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (3)$$

In reaction (1) all sulphate oxygen (O_s) is derived from water molecules, whereas in reaction (3) 87.5% of the sulphate oxygen is derived from molecular oxygen and 12.5% from water molecules. Sulphates produced by the two reactions have different $\delta^{18}O_s$ values because of the generally large difference between the $\delta^{18}O$ values of O_2 and H_2O ($\delta^{18}O_a$ and $\delta^{18}O_w$, respectively).

Even if reactions (1) and (3) apply, there are two reasons why the isotope composition of oxygen in the sulphate does not necessarily reflect the stoichiometric proportions. First, a reactant atom need not end up in the product; it may instead enter a solvent molecule or another ion, depending on the reaction mechanisms and isotope exchange reactions. Second, reaction (2), the rate-limiting step for reaction (1), generates H_2O with a $\delta^{18}O$ value similar to that of O_2, which may affect the isotope balance of reaction (1). In the absence of data bearing on these possibilities, the stoichiometry of reactions (1) and (3) will be assumed to apply to the isotope composition, as done by Taylor *et al.*[1].

In their Fig. 2, Taylor *et al.*[1] plotted $\delta^{18}O$ values for SO_4^{2-} in acid mine waters ($\delta^{18}O_s$) against those for associated H_2O

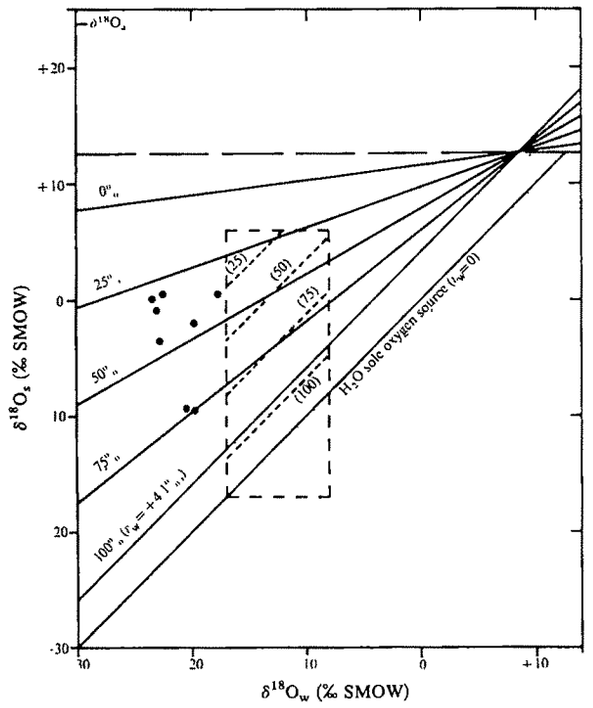


Fig. 1 Expected $\delta^{18}O_s$ values for secondary sulphates produced by oxidation of pyrite plotted against the $\delta^{18}O_w$ value of associated water, for relative contributions by reaction (1) ranging from 0 to 100%. Broken lines are from Taylor *et al.*[1]. Points represent data for acidic springs in western Canada.

($\delta^{18}O_w$). They drew a line representing zero difference between $\delta^{18}O_s$ and $\delta^{18}O_w$, labelled 'H_2O sole O_2 source', and a parallel line, offset by about +3.5‰ and labelled 100%, representing sulphates produced exclusively by reaction (1). The offset reflects the estimated kinetic isotope effect for the reaction. Three further lines, corresponding to 75, 50 and 25% contribution by reaction (1) were drawn parallel to the first two lines.

When we plotted $\delta^{18}O_s$ values for dissolved sulphates from acidic spring waters in western Canada, associated with lower $\delta^{18}O_w$ values (−23‰ standard mean ocean water, SMOW) than the data of Taylor *et al.*[1], an unrealistic contribution from reaction (3) was indicated. On further reflection, it was realized that the lines labelled 75, 50 and 25% should not be parallel. This can be readily appreciated by assuming a hypothetical reaction whereby all oxygen in the product sulphate is derived from O_2. In that case, the plot would be a horizontal line, as $\delta^{18}O_s$ would be independent of $\delta^{18}O_w$. Whereas the line for 100% contribution of sulphate oxygen by reaction (1) should be parallel to the line for 'H_2O sole oxygen source' and have a slope of unity (Fig. 1), decreasing contributions by reaction (1) would produce lines with progressively decreasing slopes.

The above considerations are incorporated in the isotope balance equation

$$\delta^{18}O_s = Y(\delta^{18}O_w + \varepsilon_w) + (1-Y)\,[0.875\,(\delta^{18}O_a + \varepsilon_a) + 0.125(\delta^{18}O_w + \varepsilon_w)] \quad (4)$$

in which Y is the fraction of SO_4^{2-} produced by reaction (1); ε_w and ε_a represent shifts in $\delta^{18}O_w$ and $\delta^{18}O_a$ values due to kinetic isotope effects during incorporation of O_w and O_a into sulphate. Using $\varepsilon_w = +4.1$‰ (ref. 1), $\varepsilon_a = -11.2$‰ (ref. 1) and $\delta^{18}O_a = +23.8$‰ (ref. 5), equation (4) can be rewritten as

$$\delta^{18}O_s = \delta^{18}O_w(0.875\,Y + 0.125) + (11.5375 - 7.4375\,Y) \quad (5)$$

Lines for 75, 50, 25 and 0% contribution by reaction (1), calculated using equation (5), are plotted in Fig. 1; they converge and intersect at the point where $\delta^{18}O_s = +12.6$‰ and $\delta^{18}O_w = +8.5$‰. This convergence means that at low latititudes, where $\delta^{18}O_w$ values are closer to SMOW, it is more difficult to differentiate contributions from individual reactions than at higher latitudes, where $\delta^{18}O_w$ values may be lower than −30‰.

The 75, 50 and 25% lines from Fig. 2 of Taylor *et al.*[1] have been added to our Fig. 1, together with $\delta^{18}O$ data from acidic springs in western Canada. It is readily seen that extrapolation of the parallel lines of Taylor *et al.*[1] would indicate very different proportions for the relative contributions by reactions (1) and (3) to these sulphates.

Figure 1 also suggests that oxidation of FeS_2 in contact with water having a $\delta^{18}O_w$ value of +8.5‰ should produce sulphate having a $\delta^{18}O_s$ value equal to +12.6‰, independent of the relative contributions by reactions (1) and (3). It should be possible to verify this point experimentally; this in turn would provide a check on the values for ε_a and ε_w, as well as on the validity of the assumption that the stoichiometry of reaction (3) applies, or whether the overall approach is too simplified.

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Fluorescent bands in massive corals result from terrestrial fulvic acid inputs to nearshore zone

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The discovery of yellow-green fluorescent bands in the skeletons of massive corals from inshore waters of the Great Barrier Reef, and the temporal and spatial correlations of these bands with adjacent river flow[1], prompted an investigation into the substances responsible for the phenomenon. We have determined that all regions of the coral skeleton contain fulvic acids[2,3] which impart a blue background fluorescence. The yellow-green fluorescent bands found only in inshore coral appear to result from enhanced concentrations of low-relative molecular mass (<10,000) fulvic acids from adjacent river input. These compounds are ubiquitous, and the yellow-green banding phenomenon can be expected to occur in massive corals located near to significant river systems. In the widely distributed genus *Porites*[4], the great age of the largest colonies[1] means that extremely long, highly resolvable records of coastal runoff exist throughout tropical nearshore seas.

Fluorescence spectra of coral core sections were obtained using a modified version of the apparatus described previously[1]. The new version employed a 150-W mercury arc excitation beam, passed through a precision 360-nm bandpass filter and chopped at 285 Hz. A quartz optical fibre focused this beam into a 3-mm spot on the coral surface. Another optical fibre passed the fluorescence emitted from the coral surface into the monochromator and detector system of a Varian AA6 atomic absorption spectrophotometer. This instrument was tuned to the chopper frequency to eliminate interference from ambient light.

The fluorescence spectra for the banded and background regions of the coral (Fig. 1) are very broad, with maxima at 440–460 nm. These are indicative of humic and fulvic acids, mixtures of complex polyphenolic compounds with a wide range of relative molecular mass ($M_r < 5\times10^2$–5×10^4). Fulvic acids are generally in the lower M_r range ($<5\times10^2$–3×10^4) and are defined as the fraction of the humic material soluble at *p*H 1 (ref. 2). The ubiquitous blue fluorescence found in marine and terrestrial waters[5] is thought to result from the presence of these

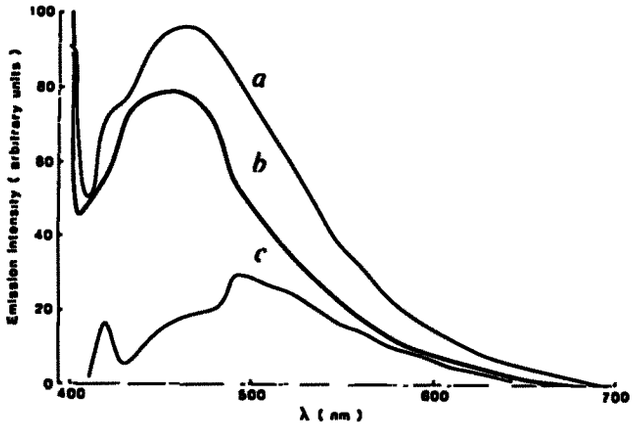


Fig. 1 Emission spectra of the, *a*, 'yellow-green band'; and *b*, background regions of an inshore *Porites* spp coral skeleton. The 'yellow-green band' corresponds to the large January 1974 flow recorded in the nearby Burdekin River[1]. *c*, Direct subtraction of *b* from *a*

humic compounds. In particular, the high fluorescence efficiency and refractory nature of terrestrial humics has enabled their use as conservative tracers of fluvial input to coastal waters[3,6]. The molecular structures and the nature of the fluorescing chromophore(s) of these compounds are poorly understood[2,5], although their general fluorescent characteristics and strong metal-complexing nature have been well documented[7,8]

The following simple experiments were performed to test the hypothesis that the fluorescence in the inshore-growing corals was a consequence of humic inclusions in the crystal structure of the skeleton. All solutions were adjusted to *p*H 6.5 before obtaining fluorescence spectra using an Aminco-Bowman spectrofluorometer.

(1) Dissolution of coral samples in dilute HCl gave solutions which showed broad fluorescence excitation and emission spectra typical of humic compounds[5]. Excitation (light absorption) maxima occurred in the 320–340-nm region with distinct shoulders at 250–270 nm. Emission maxima occurred at 410–440 nm The greater wavelength of emission maxima for the intact corals (Fig. 1) is compatible with the greater degree of crosslinking and structural rigidity conferred on the humic molecules when contained in a solid matrix[2].

(2) Solutions of similar fluorescence characteristics to the above were obtained by hot alkaline (0.1 M NaOH) extraction of the crushed coral samples, denoting the existence in the skeleton of organic acid functional groups.

(3) Addition of excess Fe^{3+} to solutions obtained by acid dissolution or alkaline extraction of the coral shifted the emission maximum (+25 nm), with 30% reduction in intensity. These observations are consistent with the formation of a strong humic material-Fe^{3+} complex, and the quenching of fluorescence of these compounds by Fe^{3+} (ref. 3).

(4) Extracts of coral samples, acidified to *p*H 1, were passed through Amberlite XAD-2 resin which has a strong affinity for adsorption of humic compounds in the acid form[9] It was found that 90% of the fluorescent components were adsorbed onto the resin and could be eluted subsequently with NH_4OH solution *p*H 11.

(5) All of the fluorescent material in the coral was apparently soluble at *p*H 1, as evidenced by unchanged total fluorescence after 0.2-μm filtration. Following the usual definition[2] we shall refer to this material as fulvic acid

Comparison of the emission spectra (Fig. 1) for the background and 'yellow-green band' areas of the coral shows that their maximum differences occur in the 480–530-nm region. These differences, although small, combined with the human eye response around 540–560 nm, result in the yellow-green appearance of the bands and their remarkable visible contrast

Table 1 Apparent M_r distribution (% in each size class) of the dissolved organic carbon in acid extracts of the yellow-green fluorescent band and background regions of a coral skeleton[1]

Apparent M_r	Yellow-green Distribution* (%)		Yellow-green Fluorescence Ex	Em	Background Distribution* (%)		Background Fluorescence Ex	Em
<1,000	32.4	32.1	320	415	25.5	21.3	330	415
10^3–10^4	30.6	27.9	325	430	13.2	18.1	320	415
>10^4	37.0	40.0	325	415	61.3	60.6	332	420

Extract solutions were adjusted to *p*H 6.5 and passed through a 0.2 μm filter before ultrafiltration using Amincon UM-2 (M_r 1,000) and UM-10 (10,000) membranes[10] The fluorescence characteristics of the resulting fractions are expressed as Ex (wavelength of maximum excitation) and Em (wavelength of maximum emission (nm))

* Results of duplicate analyses

with the blue background fluorescence. We consider that the fulvic acid composition in the two distinct regions of the skeleton is sufficiently varied to impart these small but visually prominent differences in their emission characteristics. We investigated the M_r distribution of the compounds present in acid extracts of the two different regions of the skeleton. We employed Amincon ultrafilters with nominal M_r cutoffs of 10^3 and 10^5 daltons, using a similar procedure to that described by Cole *et al.*[10] The dissolved organic carbon (DOC) content of initial, filtrate, and concentrate solutions was determined using a Graentzel DOC analyser[11]. Table 1 shows that the yellow-green fluorescing region of the coral contained significantly greater ($P<0.05$) proportions of fulvic acids in the $<10^3$ and 10^3–10^5 daltons size classes than did the blue-fluorescing background region of the coral.

The synchrony of deposition of the yellow-green fluorescent bands with adjacent fluvial inputs, and the lack of such bands in mid- and outer-shelf corals[1] allows us to postulate that the enhanced fulvic acid levels in the yellow-green bands are of terrestrial origin. These terrestrial fulvics probably differ considerably in structure and chemical characteristics from the fulvic compounds generally present in sea water[12].

We were able to induce yellow-green fluorescence in the fast-growing branching coral *Acropora formosa* by incubating the colonies in local sea water to which fulvic acid extracted from a local alluvial soil had been added at either 10 or 15 mg l^{-1}. This demonstrated conclusively that the inclusion of these ambient compounds can impart the unusual fluorescence characteristics to coral skeletons.

The global distribution of massive species of *Porites* combined with the ubiquity of fulvic compounds and their ingress to nearshore environments via fluvial pathways, suggests that the fluorescent banding in inshore corals will be a general phenomenon. In accordance with previous arguments[1], it implies that centuries of rainfall/runoff analogues are available in corals from a global range of locations.

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Distinctive mammal-like reptile from Mexico and its bearing on the phylogeny of the Tritylodontidae

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The skull of an unusual new mammal-like reptile of the family Tritylodontidae, recently discovered in the Jurassic of northeastern Mexico, is described here as *Bocatherium mexicanum*. Tritylodontids are the last survivors of the great radiation of mammal-like reptiles that also gave rise to mammals at or near the Triassic–Jurassic boundary (~200 Myr ago). These superficially rodent-like herbivores coexisted with early carnivorous mammals for some 50 Myr before becoming extinct in the late Jurassic. Although eight genera are recognized, their interrelationships have remained unclear because it has been impossible to determine the direction of morphological change within this morphologically stereotyped group. Unusual specializations of the Mexican skull now permit character polarities within the family to be determined and a cladogram to be constructed. *Bocatherium* shows a close relationship to *Bienotheroides*[1] of China and *Stereognathus*[2,3] of Great Britain. It is the first mammal-like reptile and probably the oldest terrestrial vertebrate from Mexico.

Controversy remains as to the closest relatives of tritylodontids; they are either the terminal taxon of the gomphodont cynodonts, a group showing herbivorous adaptations similar to those of tritylodontids[4], or very close relatives of the earliest true mammals, with which they share many similarities[5]. The eight genera described so far are: *Tritylodon*[2] and *Tritylodontoideus*[6] from southern Africa; *Bienotherium*[7], *Lufengia*[8] and *Bienotheroides*[1] from China; *Stereognathus*[2,3] from Great Britain; *Oligokyphus* from western Europe[9] and Arizona[10]; and *Kayentatherium*[11] from Arizona. *Stereognathus* is mid-Jurassic (Bathonian)[3] and *Bienotheroides* is mid- or late Jurassic[1,12] whereas the remaining taxa are considered to be of early Jurassic age[13]. Fragmentary postcranial remains from the Los Colorados Formation of Argentina, probably late Triassic (Norian), seem to represent the oldest-known tritylodontid[14]. We have been prompted to reassess the intrafamilial relationships of tritylodontids by the discovery of a nearly complete skull with lower jaws from northeastern Mexico (collected by J.M.C. in November 1982). The specimen pertains to a new genus and species which is described below.

Division Synapsida (Theropsida)[15]

Family Tritylodontidae

Table 1 Measurements of *B. mexicanum* type (mm)

Basal skull length	51.1	
Right cheek teeth	Length	Width
M1/	4 2	5.2
M2/	4.1	5.1
M3/	4.0	5.0
M4/	4.1	5.0
M/1	5.2	3.8
M/2	5.2	3.9
M/3	5.2	4.0

Genus *Bocatherium*, gen. nov.

Diagnosis. *Bocatherium* is unique among tritylodontids in having a large boss on the lateral surface of the dentary anterior to the masseteric fossa. It differs from other tritylodontids with the exception of *Bienotheroides* and *Stereognathus* in that: (1) its maxilla is reduced to a simple, nearly cylindrical bone lacking a medially directed horizontal lamina contributing to the secondary palate, a dorsally directed facial process in contact with the nasal, and a laterally directed lamina underlying the jugal on the anterior root of the zygomatic arch; (2) its upper postcanine teeth have only two cusps in each of three longitudinal rows (other tritylodontids have at least three cusps in the central row). *Bocatherium* is distinguished from *Bienotheroides* in having a slender, rather than extremely deep, zygomatic arch. It is distinguished from *Stereognathus* (known only from isolated teeth and a left maxilla with three teeth) in having upper postcanine teeth with an oval rather than quadrangular outline in crown view.

Bocatherium mexicanum, spec. nov.

Diagnosis. As for genus.

Type. IGM 3492, Instituto de Geologia, Universidad Nacional Autonoma de Mexico, Mexico City; a skull with lower jaws, lacking the right zygomatic arch, the roof and much of the sides of the braincase.

Horizon and locality. Approximately the middle of the 400-m-thick exposed section of the La Boca Formation in Huizachal Canyon near the towns of Huizachal, Anacahuita and La Joya, 15 km south-west of Ciudad Victoria, Tamaulipas State, Mexico. The La Boca Formation consists of terrigenous sediments which lie between Permian and late Jurassic (Oxfordian) marine sediments[16–18]. The occurrence of a tritylodontid suggests a Jurassic age for that part of the formation from which the fossil was collected. However, late Triassic plant fossils are reported from discontinuous beds assigned to the La Boca Formation at a locality 12 km to the north[16]. The conflicting ages probably indicate that the La Boca Formation contains strata of both

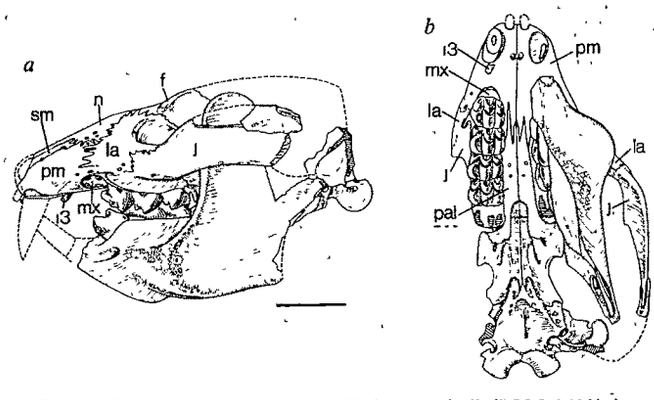


Fig. 1 *Bocatherium mexicanum.* Holotype skull (IGM 3492) in lateral (*a*) and ventral (*b*) views. Right mandible removed in *b*. Key: f, frontal; i, incisor, j, jugal; la, lachrymal; mx, maxilla; n, nasal; pal, palatine; pm, premaxilla; sm, septomaxilla. Scale bar, 1 cm.

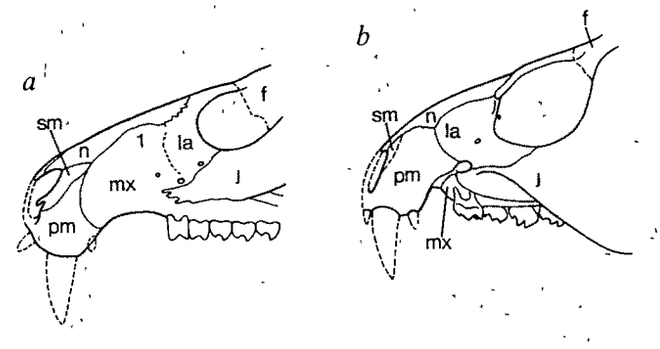


Fig. 2 Lateral views of tritylodontid facial regions representing the primitive pattern of facial bones exemplified by *Bienotherium* (*a*) and the derived pattern exemplified by *Bienotheroides*[1] (*b*). Not to scale. Key: 1, the facial process of the maxilla; other abbreviations are as in Fig. 1.

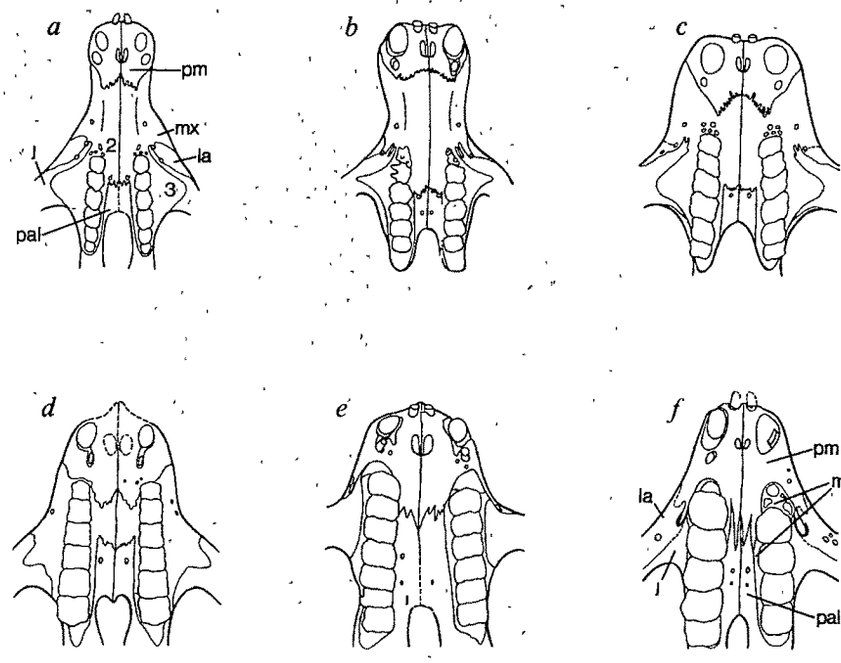


Fig. 3 Ventral views of tritylodontid skulls *a*, *Oligokyphus*[9]; *b*, *Tritylodon*; *c*, *Bienotherium*; *d*, *Kayentatherium* (H.-D. Sues, unpublished); *e*, *Bienotheroides*[1]; *f*, *Bocatherium*. Key: 2, palatine process of maxilla; 3, zygomatic process of maxilla; other abbreviations as in Fig. 1. Cusps on teeth omitted. Not to scale.

Triassic and Jurassic age, as has already been suggested by a palaeomagnetic study of rocks in this area, which identified three diachronous units within the La Boca Formation[19]. The Huizachal Canyon beds have the youngest pole position of the three, but a pole position has not been determined for rocks of the plant locality.

Bocatherium mexicanum (Fig. 1) is one of the smallest tritylodontids (Table 1), only slightly larger than *Lufengia minor*[8], but the only known skull may represent a sub-adult individual. Immaturity is suggested by the unusual depth and robustness of the dentary and by the relatively small number and large size of the cheek teeth, features characterizing juvenile but not adult *Bienotherium yunnanense* (J.A.H., unpublished). However, unlike immature individuals of other tritylodontids (see, for example, Fig. 3*d*), the teeth are of equal size rather than the posterior teeth becoming increasingly larger (Table 1). In overall morphology, *Bocatherium* resembles other tritylodontids, differing most notably in the bulbous swelling on the dentary anterior to the masseteric fossa. Basicranial and braincase structure, as preserved, are typically tritylodontid[9,20].

In the pattern of bones on the side of the face and on the palate, *Bocatherium* is strikingly different from all other tritylodontids (Figs 2*a*, 3), indeed, from all other cynodonts (cladistically including mammals), except *Bienotheroides*[1] and *Stereognathus* (J.A.H., unpublished). In these three genera, the maxilla is reduced to a nearly cylindrical element holding the roots of the teeth; thus, it lacks the usual thin laminae extending upward onto the face, medially onto the secondary palate and laterally below the jugal. *Bocatherium* and *Bienotheroides* (Figs 2*b*, 3), and by inference *Stereognathus*, show the following specialized features. The premaxilla is greatly enlarged so that it is in contact with the nasal and the anteriorly expanded lachrymal on the side of the face and the anteriorly expanded palatine on the secondary palate. The jugal is expanded anteroposteriorly on the lateral surface of the maxilla to form a long flat vertical contact. This contact facet is seen on the type specimen of *Stereognathus ooliticus*, usually described as a right maxilla[2,3] but actually a left (J.A.H., unpublished). Opening onto the face at the junction of the jugal, premaxilla and lachrymal is a large infraorbital foramen, the medial wall of which is formed by a prominent groove on the maxilla (visible in *S. ooliticus*).

The crown pattern of the upper postcanine teeth of these three genera differs from that of all other tritylodontids in having two cusps in each of three longitudinal rows, with very small accessory cuspules at the anterior end of each row. The crowns are oval in occlusal outline in *Bocatherium* and *Bienotheroides*, quadrangular in *Stereognathus*.

Knowledge of the extremely derived condition of the facial and palatal bones in these three genera permits the remaining genera known from adequate skull material to be arranged in a morphocline (Fig. 3). (*Tritylodontoideus*[6] is too poorly preserved to be included.) The most derived early Jurassic taxa are *Kayentatherium* (H.-D. Sues, unpublished) and *Lufengia* (J.A.H., unpublished), in which the premaxilla and palatine closely approach each other on the palate so that the maxillary component is greatly reduced. The premaxilla is in contact with the nasal in *Lufengia* juveniles (known adult skulls are damaged in this region) but not in *Kayentatherium* and other early Jurassic genera (Fig. 2*a*). The palatine of *Bienotherium* shows the primitive unexpanded condition, but the premaxilla extends well back

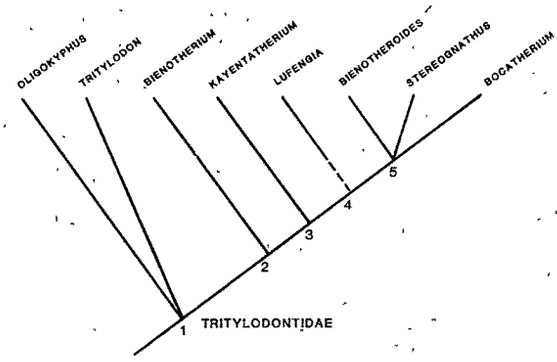


Fig. 4 Cladogram showing proposed relationships of tritylodontid genera. Synapomorphies for each node are as follows: 1, Second upper and first lower incisors enlarged; first upper incisor very small; canines absent; cheek teeth with divided roots; postcanines with three (upper) or two (lower) longitudinal rows of crescentic cusps; postorbital bar and prefrontal and postorbital bones absent. 2, Premaxilla extends further posteriorly on palate; snout relatively short with no post-incisive constriction. 3, Premaxilla extends posteriorly between anterior postcanines, and palatine extends anteriorly beyond level of palatine foramina so that palatine process of maxilla is reduced. 4, Premaxilla in contact with nasal, and facial process of maxilla reduced in area (in *Lufengia* juveniles; adult condition uncertain). 5, Facial, palatine and zygomatic processes of maxilla absent; upper cheek teeth with two principal cusps in each longitudinal row.

on the palate (J.A.H., unpublished). All of the above genera are similar in possessing the presumably derived condition of a short snout lacking a constriction behind the incisor region. *Tritylodon* (J.A.H., unpublished) and *Oligokyphus*[9] have a short premaxilla and a long narrow snout with a mid-length constriction; these features are found both in early mammals[21] and in gomphodont cynodonts (J.A.H., unpublished) and thus are considered primitive for tritylodontids. The interrelationships of tritylodontid genera (Fig. 4) suggest that a small number of cusps in the upper postcanine teeth is a derived condition.

The close relationship of *Bocatherium* to *Bienotheroides* and *Stereognathus* suggests that the Mexican form may also be post-early Jurassic in age; it is certainly younger than late Triassic. A Jurassic age for the upper part of the La Boca Formation is consistent with the stratigraphical[18] and palaeomagnetic[19] evidence. We believe *Bocatherium* to be the oldest terrestrial vertebrate known from Mexico. If the upper part of the La Boca Formation proves to be middle Jurassic in age, *Bocatherium* will be the first terrestrial vertebrate of this age in North America[22,23].

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Neo-darwinian evolution implies punctuated equilibria

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The two central elements of neo-darwinian evolution[1,2] are small random variations and natural selection. In Wright's view, these lead to random drift of mean population characters in a fixed, multiply peaked 'adaptive landscape', with long periods spent near fitness peaks[3,4]. Using recent theoretical results[5], we show here that transitions between peaks are rapid and unidirectional even though (indeed, because) random variations are small and transitions initially require movement against selection. Thus, punctuated equilibrium[6,7], the palaeontological pattern[8,9] of rapid transitions between morphological equlibria, is a natural manifestation of the standard wrightian evolutionary theory and requires no special developmental, genetic or ecological mechanisms[10-13].

The simplest neo-darwinian model for the evolution of the population mean $\bar{x}$ of a genetically determined character expresses the change $d\bar{x}$ in time interval dt as the sum of a natural selection term and a random variation term:

$$d\bar{x}(t) = F'(\bar{x}(t)) + \alpha\, dW(t) \qquad (1)$$

$F(\bar{x})$, a one-dimensional genotypic[3] or phenotypic[14] adaptive landscape, describes the mean population fitness. If, for example, the slope F' is positive (a rising landscape) at $\bar{x}(t)$, natural selection pushes $\bar{x}$ towards larger values. The parameter α gives the magnitude of random variations relative to that of natural selection. The random process $W(t)$, a standard brownian movement with zero mean drift, represents, for example, genetic drift and short-term environmental fluctuations showing no obvious trend. We assume, for simplicity, that α is independent of $\bar{x}$.

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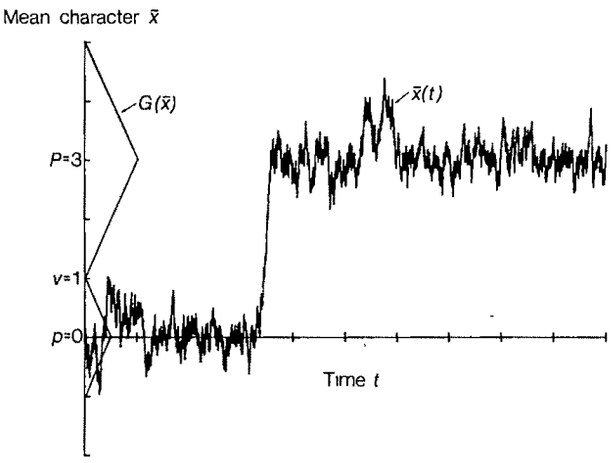


Fig. 1 Punctuated equilibria in a numerical solution of the discrete time equation, $\bar{x}(t+1) - \bar{x}(t) = G'(\bar{x}(t)) + sw_t$. The landscape G has a lower peak at $\bar{x}=0$, a valley at $\bar{x}=1$ and a higher peak at $\bar{x}=3$; its slope, G', takes only the values $+0.01$ or -0.01. w_t Represents the sign of random variables, s their magnitude. For each t, w_t is independently $+1$ or -1 with equal probability; $s=0.07$. The jagged line plots $\bar{x}(t)$ for 5,000 time units. As theory predicts, during the transition between peaks, $\bar{x}$ moves from 0 to 1 as if the direction of natural selection were reversed, that is, at the same speed that $\bar{x}$ moves by natural selection from 1 to 3.

Suppose that the landscape is doubly peaked: as $\bar{x}$ increases, F rises to a peak at $\bar{x}=p$, declines to a valley at $\bar{x}=v$, rises to a second higher peak at $\bar{x}=P$, then declines. Let the initial value $\bar{x}(0)$ be smaller than v. To mimic the fossil record, suppose $\bar{x}(t)$ is observed, not continuously, but at some finite (perhaps large) number N of epochs $0 < t_1 < \ldots < t_N$, perhaps chosen by another random process modelling stratigraphic sampling. There is a natural timescale τ_α associated with transitions from the smaller to the larger peak which increases dramatically as α decreases. We suppose that the intervals between observations are, for small values of α, proportional to τ_α.

Under these assumptions[5], for small α, the observed $\bar{x}(t_i)$ values behave quite differently from $\bar{x}(t)$ itself, which is intrinsically continuous. As α approaches 0, the observed values become fixed exactly at p for some random (exponentially distributed) time, then jump (apparently) instantaneously to P

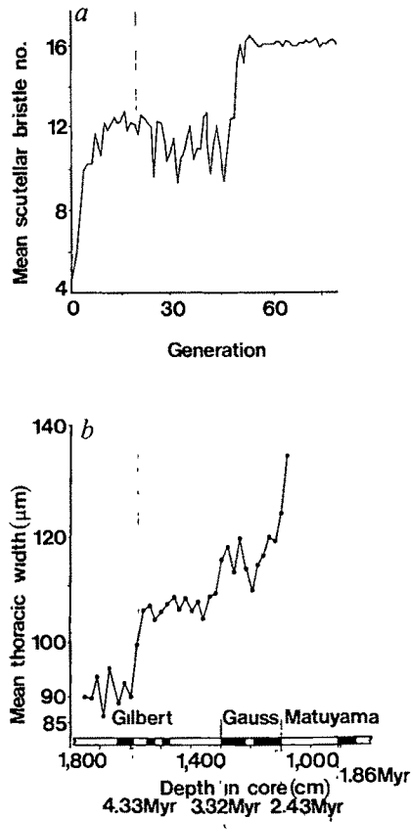


Fig. 2 *a*, Response to selection for scutellar bristle number in female *Drosophila* (presumably *melanogaster*; species not given[16]) according to MacBean *et al.*[16], as simplified by Parsons[18]. *b*, Increase in thoracic width of the Antarctic radiolarian *Pseudocubus vema* during ~2.5 Myr according to Kellogg[17], as simplified by Parsons[18]

and remain there (apparently) indefinitely. Analogous results hold if F has more than two peaks[5] or if $\bar{x}$ is multidimensional[15].

To understand this result, note that a movement of $\bar{x}$ from p to v goes against the influence of natural selection and, for small α, is very improbable. Improbable events eventually occur (with timescale τ_α) and then by the most likely of the improbable alternatives. Here it is most likely[5] that small random variations combine to push $\bar{x}$ from p to v as if the direction of natural selection were reversed, hence rapidly and unidirectionally. Once at v, the move to P is by natural selection. The rapidity of the second part of the transition is well recognized[4], whereas that of the first part seems largely unknown.

Figure 1 is a computer simulation illustrating the theoretical results about equation (1). For comparison, Fig. 2 shows examples[16,17] (from ref. 18) of observed punctuated equilibria with two different timescales.

In a changing landscape, punctuation can be triggered by selection, rather than drift, either by a rapid (for example, environmental) change[19] or after a gradual change eliminates the currently occupied peak[13]. The introduction of a rare selectively superior mutant into a population from which it was previously absent can lead to punctuation[20,21] corresponding to transition from an unstable equilibrium in a fixed landscape. Our analysis is the first to show that transitions between stable equilibria, triggered by drift in an unchanging adaptive landscape, should have fossil records indistinguishable from punctuations triggered by selection.

Gradual evolution is only possible in an unchanging landscape either if α is not small (violating neo-darwinian assumptions) or if sampling intervals are as short as the timescale of natural selection (unusual in fossil records). Even assuming many peaks, the suggestion[20,22] that stepwise changes will appear to be gradual on a coarse timescale seems to be inconsistent with Wright's model: an increased timescale of observation implies[5] that more local peaks are skipped over apparently instantaneously and stasis is observed only at higher fitness levels.

Gradual evolution is possible in a changing landscape if peaks shift gradually and unidirectionally. Our analysis suggests a resulting palaeontological pattern of 'punctuated shifting equilibria' as the gradual evolution of a population following a moving peak is punctuated by rapid transitions between peaks. It is unclear whether such changing landscapes should occur commonly.

Models similar to that described in equation (1) are applicable directly to populations that are panmictic or have much migration and hence are geographically homogeneous. Because the timescale τ_α is a rapidly increasing function of population size, equation (1) predicts stasis and punctuation for a small to moderate population but only stasis for a large population.

The model in equation (1) may be applied to an isolated subpopulation with a smaller τ_α than the larger parent population. Allopatric speciation will occur if the subpopulation, after transition to a new peak, cannot interbreed with the parent population. Without requiring special 'genetic revolutions',[23] our analysis predicts that punctuated equilibria will be associated with the branching speciation of isolated subpopulations.

Generalizations of equation (1) incorporating spatial inhomogeneity in a local mean character correspond to Wright's notion of local populations in his shifting balance model[3,4], in which he suggested that speciation could occur without physical barriers to migration and gene flow[23]. Mathematically non-rigorous[24,25] and rigorous[26] results suggest that punctuation occurs on the timescale τ needed to form by random drift (against natural selection) a 'critical droplet' (that is[23], a geographical region where transition to a higher peak of fitness occurs) which is of sufficient size to resist swamping by gene flow; such a critical droplet spreads rapidly by selection. The timescale τ is a slowly decreasing function of population size[27] (for fixed spatial density) because a critical droplet can form in each local subrange[4,23]. Unlike τ_α, this τ need not exceed geological timescales even for very large populations.

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Interpolation in stereoscopic matching

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Anyone who has stared at a repeating wallpaper pattern, or a periodic pattern of tiles, has probably experienced the phenomenon of a false stereoscopic depth percept. This arises because of a mismatching in the two eyes of repeating elements in the pattern. The phenomenon is less likely to occur if an edge of the textured region is in view; the edge seems to fix the registration of elements. We describe here a stereogram which exemplifies this principle; it has a central, periodic region bounded on either side by edges with pre-assigned disparities. We find that the perceived depth of the central region is controlled by the edges. In certain conditions (when the period is spatially large), the edges simply impose one of the expected discrete matchings. In other conditions, however, we observe a striking phenomenon: interpolation in depth occurs between the edges, violating any possible feature-by-feature matching.

Our stereogram can be broadly described as a regular grid of points with horizontal disparities introduced at the two vertical edges (Fig. 1*A*). A single row of the grid is shown in Fig. 1*B*, where the convention is that the vertical alignment of the dots seen by the left and right eyes corresponds to the zero disparity; that is, if each dot were matched to that vertically above or below, they would be seen in the fixation plane. The row consists of a set of equally spaced dots, typically 6 arc min apart, but with the left-most dot moved inwards a certain fraction, S_L, of the inter-dot spacing in the left eye, and the right-most dot moved inwards by a fraction S_R of the inter-dot spacing in the right eye.

The stereoscopic percept depends on how points in the two eyes are matched. When the stereogram is inspected carefully, using a large inter-dot spacing (for example, Fig. 2 viewed close up), one of two possible percepts is seen, depending on the size of S_L and S_R. When S_L and S_R are small, the percept corresponds to the matching shown in Fig. 1*C*(1), and consists of a plane with two flaps tilted forwards at the edges [Fig. 1*D*(1)]. When S_L and S_R are large, the underlying matching is that of Fig. 1*C*(2), in which each point is paired obliquely; the percept here is of a grid lying in front of the fixation plane, with two unpaired points at the edges [Fig. 1*D*(2)]. Thus, changing S_L and S_R causes a change of matching throughout the central regular lattice. Such propagation of matching from a limited area throughout a periodic region has been shown to occur in stereograms studied by Julesz and Chang[1], of which ours can be regarded as a highly specialized case.

To explore the depth percepts more systematically, we added a test point beneath the grid (Fig. 1*A*) and asked subjects, in a forced-choice paradigm, to judge whether the test point lay in front of, or behind, the grid. The test point was placed at a fixed vertical position below the grid, and could be given any desired horizontal location, so that depths of particular regions of the grid could be explored. The test point was randomly given one of seven equally spaced disparities, chosen so that at one extreme the grid lay considerably in front of the test point, and at the other, behind it. By fitting a cumulative gaussian curve to the percentage of trials on which the grid was reported as lying in front of the test point, we could locate the apparent mean position of the region of the grid above the test point, and also determine the standard deviation in its perceived position[2]. Observers were first shown a fixation pattern which disappeared during the subsequent 160-ms presentation of the grid and test point.

When the (horizontal) inter-dot spacing in the central region was >6–8 arc min, we found that the grid lay either in the fixation plane [as expected according to Fig. 1*C*(1)], or moved forward to the depth expected of the oblique matching in Fig. 1*C*(2). The former was seen when the sum of the displacements, $S_L + S_R$, was small (<1 inter-dot spacing), and the latter when the sum was large (>1.5 inter-dot spacing). For intermediate values of the sum $S_L + S_R$, either depth percept might be seen on a given trial, with a probability depending on the sum $S_L + S_R$.

A radically different type of behaviour was seen when the inter-dot spacing was <6 arc min; it was then possible to obtain depth percepts which could not be accounted for by any discrete matching of points. For example, Fig. 3*A* shows a depth profile of a grid with $S = 0.2$, $S_R = 0.8$. This profile is slanted in depth: it runs continuously between the depths which would be expected from matching the end points (open circles), and none of the intermediate values is compatible with discrete pairings of points within the grid. It is as though the end points have been paired and the intermediate depths obtained by interpolation between these extremes. For comparison, Fig. 3*A* also shows profiles for $S_L = S_R = 0$ and $S_L = S_R = 0.67$; both of these are

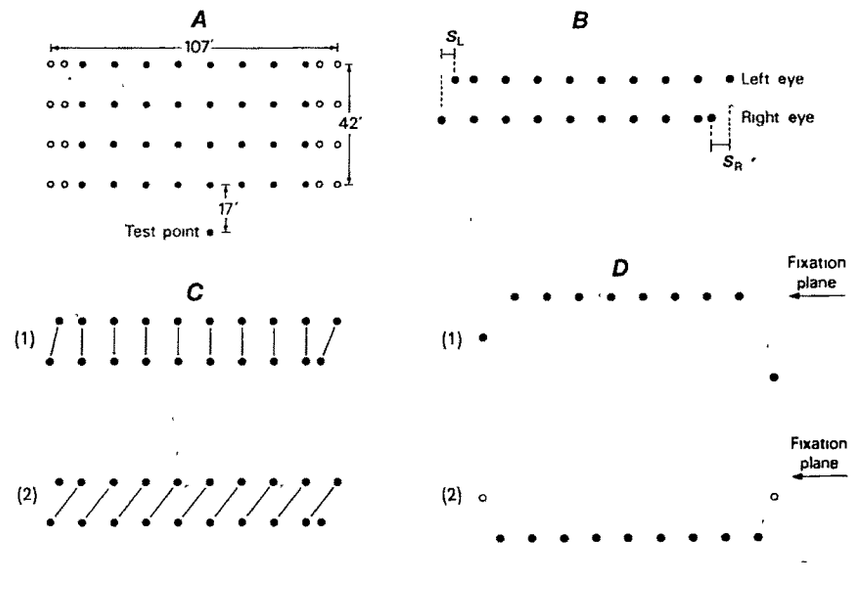


Fig. 1 *A*, The grid of our stereogram, showing the test dot. *B*, A single row from the grid, with the left-most dot in the left eye displaced inwards by a fraction S_L, and the right-most dot of the right eye displaced by a fraction S_R of the inter-dot spacing *C*, (1), (2), Two possible matchings of points of the left and right eyes in a single row. In (1) the dots are matched in sequence; in (2) the displaced dots at the ends of each eye's row are unmatched, and a point in the left eye is matched to a point in the right eye which is one step of the grid to its left. *D*, The depth percepts expected, based on conventional stereoscopic theory, from the matchings (1) and (2). The fixation plane (vertical matching in *C*) is indicated by an arrow In fact, a fixation target was always presented before the grid was shown, but was never shown simultaneously with it. In (1), two dots lie in front of the fixation plane. Here S_L is shown smaller than S_R, so the right-most dot should be farther in front than the left-most. In (2) the grid lies in front of the fixation plane (corresponding to a crossed disparity). The two monocular points (open circles) appear to lie behind the grid, although we do not know precisely what perceived depth should be assigned to them.

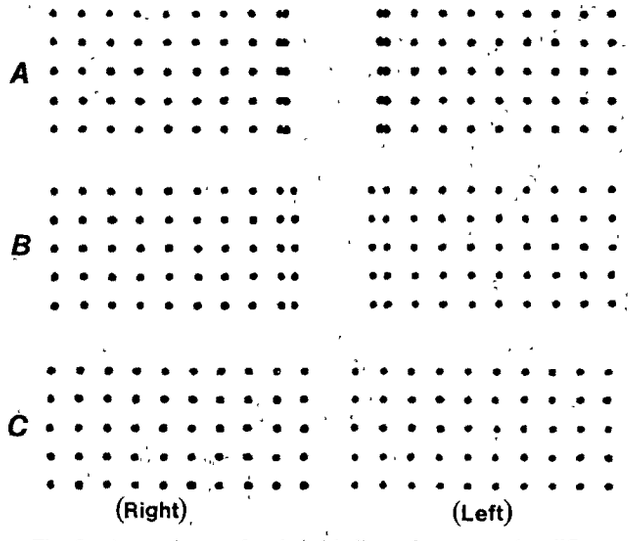


Fig. 2 A stereogram of grids (with dimensions somewhat different from those in Fig. 1*A*) to show the basic phenomena. The displacements S_L and S_R are equal: they have a large value in panel *A* and are zero in *C*. In *B*, a displacement has been chosen which should give an intermediate depth percept under interpolation conditions. Those who free-fuse by converging will see the grid correctly; those who diverge will see the depths reversed. When the stereogram is viewed quite close up (say 1 m), the discrete matchings of Fig. 1*C* and *D* should be seen. When it is viewed from a distance of ≥3 m, the inter-dot spacing comes within the interpolation range (<6 arc min), and *B* should appear at a depth between that of *A* and *C*.

flat, the former being close to the fixation plane, and the latter forward of it, though not as far forward as would be expected for the oblique matching.

These intermediate-depth percepts do not correspond to an ambiguous alternation between the two matchings, as demonstrated by the curves showing the proportion of trials in which the test dot lay in front of the grid, as a function of test dot disparity (Fig. 3*B*). If there were an alternation of percepts, these curves would be broad, encompassing the range from the fixation plane to the forward matching. However, they are very narrow, having a standard deviation of 10–15 arc s. The intermediate depths (such as curves *b–d* in Fig. 3*B*) are, in fact, as well localized as the extremes (curves *a*, *e*) which satisfy a 'conventional' matching. Depth percepts which lie between possible discrete matches have been described by Foley[3], and may be related to those we find, but his conditions were very different from ours, and the intermediate states far less localized.

Interpolation appears to be quite stable under prolonged inspection (for example, Fig. 2 viewed at ≥3 m), and, in particular, the percept does not depend on vergence. The viewing time of 160 ms used in our experiments was designed to eliminate changes of vergence during each presentation. In one experimental condition, the displacements *S* were changed randomly from trial to trial to prevent the observer from maintaining a biased convergence position in front of the fixation plane, and essentially similar results were obtained.

One might attempt to account for interpolation by saying that the stereo matching system has a coarser resolution than 6 arc min, and 'sees' only the ends of the grid when the inter-dot spacing is small. We can show that this is not the case by randomly perturbing points in the periodic region by the same amount in each eye; when this is done, the grid can be matched as a flat surface in the fixation plane (Fig. 4*A*), but the oblique matching should introduce disparity variations (Fig. 4*B*). There are general arguments for expecting that the matching which gives the more continuous depth profile will be preferred[4] and, indeed, even quite small random perturbations keep the grid in the fixation plane matching Fig. 4*A*, no matter how large the displacements *S*. For example, for an inter-dot spacing of 3.7 arc min, a 20-arc s (SM) or 40-arc s (GM) perturbation serves to

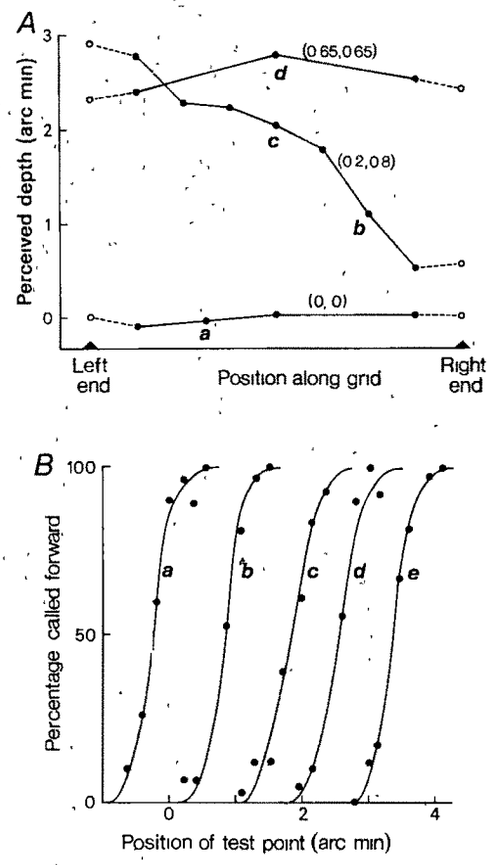


Fig. 3 *A*, Apparent depths for various values of S_L and S_R (shown in brackets) at selected positions along a grid of inter-dot spacing 3.7 arc min (test subject G J.M.). Each solid point represents the mean of a psychometric curve (see *B*) based on at least 200 trials. The open circles show the expected depths of the paired end points of the grid, calculated from their disparities. *B*, Psychometric functions for various points in *A* (curves *a–d*) and for the grid with $S_L = S_R = 1$ (*e*). The ordinate shows, for a given disparity of the test dot, the percentage of responses 'test dot in front of grid'. If a cumulative gaussian is fitted to these points using the probit method[2], its mean defines the apparent depth of the grid, and its standard deviation indicates the imprecision of this depth judgement

keep the grid in the fixation plane, even with the largest possible values of S_L and S_R. Stereoscopic matching is therefore sensitive to very small perturbations of the points in the grid.

What is the relationship between interpolation and the conventional point-by-point matching seen for larger dot spacings? To determine how they interact, we constructed a grid which expands slowly so that the spacing in the centre is in the range where discrete matching occurs, while the spacing at the edges lies well in the interpolation range (Fig. 4*C*). A displacement at the edges brings forward the whole grid, generating a vivid arch in depth. This implies that interpolation is not merely an interpretative stage which supplies appropriate depths after matching is complete, but can influence matches, as in the central region here.

We have seen that the edges of the grid have a privileged role in that they provide end points for interpolation. What counts as an edge? If we displace two dots in the centre of our grid, so making a small gap, the resulting percept exhibits two segments of interpolation (Fig. 4*D*), suggesting that the inner gap provides two 'edges' in this sense (dotted lines).

In summary, the stereo matching system appears to skip over regions with a periodic spacing of <6 arc min, but is alert to small irregularities within such a region. These irregularities might be interpreted either as gaps or as disparity discontinuities. Of the two possibilities, the second is the more attractive; it implies that it is not periodicity *per se* which is required for

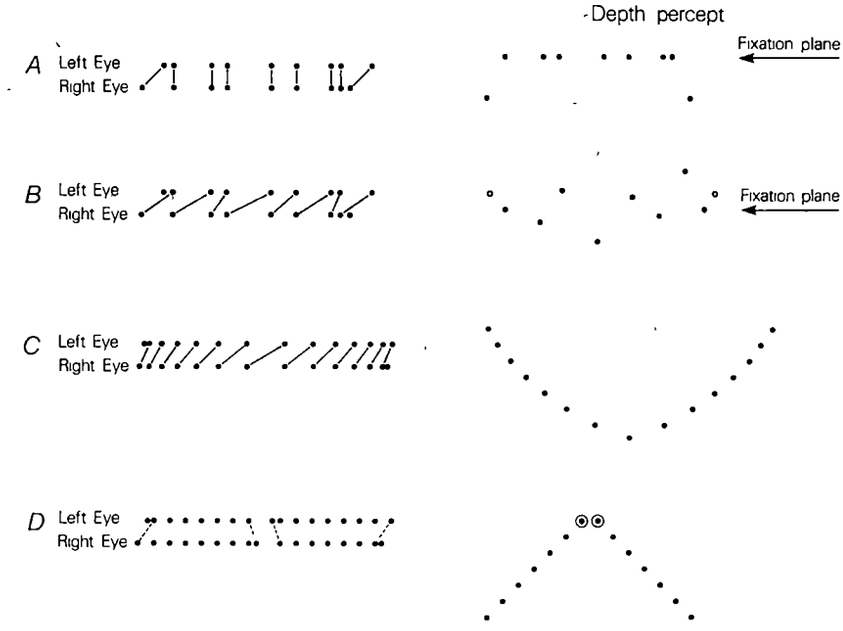


Fig. 4 *A*, In a modified version of the stereogram, a grid is constructed from several identical rows such as that shown here This row is derived from that shown in Fig 1*B* by leaving the outer two dots in each eye unchanged, but randomly perturbing the inner dots by the same amount in each eye. The lines show the matching which corresponds to Fig. 1*C*(1), and the depth percept is shown to the right. This is found for all dot spacings in the interpolation range (<6 arc min). *B*, An oblique matching, corresponding to Fig. 1*C*(2), in which each dot in the left eye is matched with a dot one further along in sequence in the right eye; this generates a ragged depth profile (shown on right) *C*, A modified version of the row shown in Fig. 1*B*. The spacing between dots at the edges is 3 arc min; this spacing increases gradually towards the centre, reaching a maximum of 11 arc min, and then decreases again, in a symmetrical fashion. The expected depth percept for the oblique matching shown is a forward-bending arch. *D*, A gap is created by displacing two dots near to the centre of the grid. In the left eye, a dot is moved half an inter-dot spacing to the right; in the right eye, the dot which would be paired with this in the fixation plane is moved to the left by the same amount. With the dot spacing in the interpolation range, two segments of interpolation are seen (shown on right). The fine broken lines indicate the matching which defines the 'edge' In fact, the perceived depth of these dots is somewhat variable between trials, and this is indicated by ringing of the dots in the diagram of the depth percept

interpolation, but simply the absence of disparity discontinuities. There is an obvious economy in making explicit matches only where a potential disparity discontinuity is registered.

These findings impose some constraints on models of stereoscopic matching. For example, the fact that disparities at the edges determine the matching within each grid in the absence of vergence movements argues against the coarse-to-fine vergence strategy postulated by Marr and Poggio[5]. Information might, in principle, be propagated inwards from the edges by a cooperative process of point-by-point matching[4,6], but it seems unlikely that such a mechanism operates under interpolation conditions.

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Changes in the dendritic branching of adult mammalian neurones revealed by repeated imaging *in situ*

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A major obstacle to understanding the mechanism of long-term change in the vertebrate nervous system has been the inability to observe the same nerve cell at different times during the life of an animal. The possibility that changes in neural connectivity underlie the remarkable flexibility of the nervous systems of mammals has therefore not been tested by direct observation. Here, we report studies in which we have visualized the same neurone in the superior cervical ganglion of young adult mice at intervals of up to 33 days. This collection of nerve cells is particularly accessible and therefore well suited to our approach. We find that the dendritic branches of the neurones examined change appreciably over intervals of 2 weeks or more; some branches retract, others elongate and others seem to form *de novo*. The apparent remodelling of these postsynaptic elements implies that the synaptic connections of these cells normally undergo significant rearrangement beyond what is usually considered to be the developmental period.

Sexually mature male mice (8-12 weeks old, 25-35 g, CF1 strain) were anaesthetized with chloral hydrate and mounted on a platform that could be moved in three axes for positioning and focusing. Either the right or the left superior cervical ganglion was exposed through a midline incision, care being taken to preserve the blood supply. The wound was superfused with sterile lactated Ringer's solution (Travenol) and the pool of fluid grounded with a chloride-treated silver wire. The ganglion surface was illuminated at an oblique angle by a plastic light guide (diameter 0.7 mm). Proper adjustment of the angle of illumination caused the neurones on the surface of the ganglion to appear as a mosaic of polygonal cell outlines (Fig. 1*a*). Individual neurones could be identified as a result of variations in size, shape, neighbour relationships and position with respect to blood vessels. A particular nerve cell was selected for study and 35-mm colour transparencies were taken of the relevant region of the ganglionic surface at a magnification of ×35-320. Following photography, the neurone that had been selected was impaled under direct vision at a magnification of ×125 with a microelectrode filled with a 6% solution of 5(6)-carboxyfluorescein in 0.44 M KOH (*p*H 7.0; resistance 80-150 MΩ). Resting potentials usually ranged from −40 to −60 mV; all cells had overshooting action potentials, occurring either spontaneously or elicited by current injection through the microelectrode. The illuminator was then turned off and the dye injected iontophoretically by passing hyperpolarizing current for 2 min (3-4 nA d.c.). Subsequent observations of the cell were made on a video monitor with a low-light level video camera (GE model 4TE56 SIT) and a ×40 water-immersion lens with a

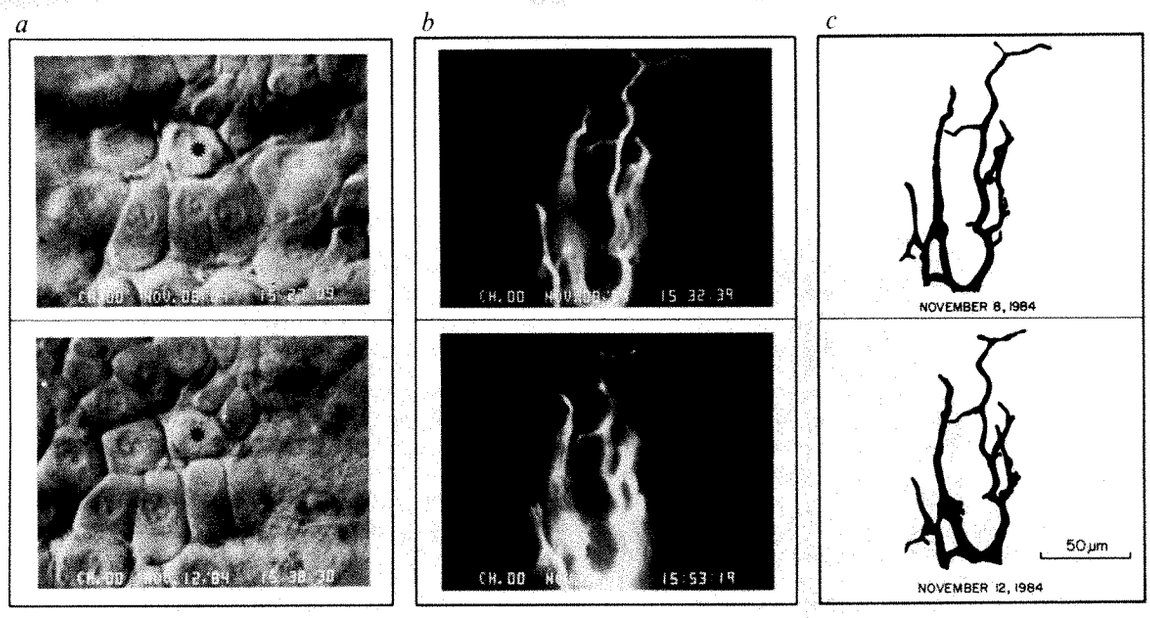


Fig. 1 Identification and imaging of the same neurone over a 4-day interval. *a*, Surface images of a mouse superior cervical ganglion. The same ganglion cells could be recognized at a second operation by virtue of their size, position and relationship to other landmarks such as blood vessels. The neurone marked with an asterisk was impaled with a microelectrode and injected with 5(6)-carboxyfluorescein on both days (see text). *b*, Fluorescent images of a portion of the dendritic arborization of the injected cell in comparable focal planes (this and subsequent photographs were taken from a video monitor). Parts of several large dendrites and their higher-order branches can be seen; the cell soma is just below the bottom of the frame. *c*, Five to ten images at slightly different planes-of-focus were combined to form reconstructions such as those shown here. Comparison of the initial and final images of this and other neurones observed at intervals of a few days showed relatively little change (see Table 1).

numerical aperture of 0.75 and a working distance of 1.6 mm. The dye-filled cell was imaged using standard epifluorescence optics (390–490 nm excitation, 515 nm suppression); low-level illumination was provided by a 6-V, 15-W tungsten bulb controlled by a rheostat and focused through a heat filter. After verifying that the intended cell had been successfully injected, a portion of its dendritic arborization, usually one primary dendrite and its branches, was chosen for study. Fluorescent images were recorded on Polaroid film (Fig. 1*b*). We photographed only a part of the dendritic arborization of each cell to minimize the time of exposure of the cell to the exciting wavelengths. The total period of exposure of the neurone to the exciting light was <1 min. After satisfactory pictures had been obtained the wound was closed and the animal was allowed to recover. The entire procedure usually took ~1 h.

Following an interval of 3–33 days, the animals were re-anaesthetized and the ganglion exposed as before. Projection of the transparencies made at the initial operation allowed us to identify the neurone that had been impaled previously. The cell was re-impaled and injected with dye in exactly the same manner as at the first operation (the dye is lost from the cell within a few hours). Comparisons of dendritic geometry were made either from single focal planes (Fig. 1*b*) or by reconstructing the relevant portion of the dendritic arborization from 5–10 photographs taken at slightly different depths of focus (Fig. 1*c*).

Single neurones from a total of 57 mice were examined by this method. In 44 of these animals we were successful at the second operation in identifying the portion of the ganglionic surface that had been studied previously. In the other 13 animals we were unable to locate the appropriate region; most of these failures occurred near the beginning of our series of experiments when we were less experienced at choosing locations and cell groupings that would be easy to identify subsequently. In 28 of the 44 animals in which the correct region was located, the cell of interest was identified and successfully re-impaled. Average resting potential and action potential amplitudes were not appreciably different from values obtained at the initial operation. In the 16 remaining cases the neighbouring cells could be identified but the cell of interest apparently had died and been removed by phagocytic action. Thus the survival rate of neurones impaled and imaged in this way was ~64%. The death of about a third of the neurones is not explained by illumination of the fluorescent dye, because in additional control experiments 4 out of 10 cells that were filled with dye but never illuminated also died. The fluorescent dye itself has been shown to be non-toxic in at least one other neural system[1], although prolonged or intense exposure to the exciting wavelength can kill dye-filled cells[2]. Therefore, it seems likely that impalement by a microelectrode is sufficient to cause the death of some cells.

The dendritic arborizations of neurones observed in this way showed shape changes that were, in general, proportional to the

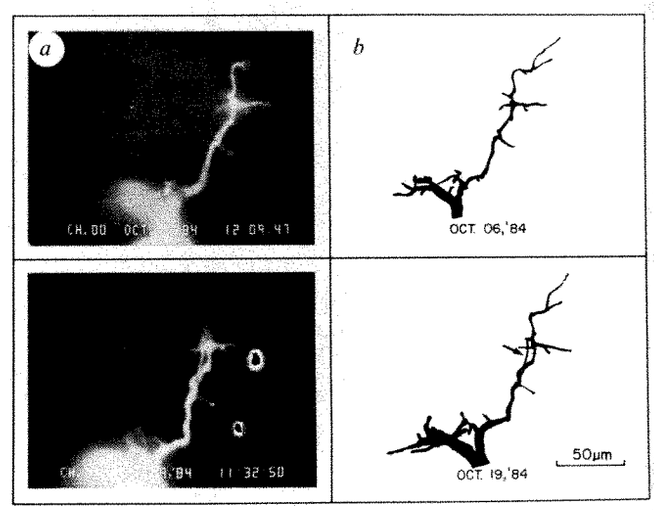


Fig. 2 Images of a neurone observed at an interval of 13 days. *a*, Fluorescent images of an identified neurone at the beginning and end of the period of observation; the plane of focus is approximately the same in both images. *b*, Reconstructions of this portion of the dendritic arborization of the neurone from six different focal planes at the beginning and end of the interval. Examination of higher-order branches showed changes that were more obvious than those observed at shorter intervals; for example, the branch marked by the arrow seems to have formed *de novo* about halfway along the major dendrite.

Table 1 Dendritic changes of mouse superior cervical ganglion cells over intervals of 3–33 days

Interval (days)	No. of cells studied	No. of branches unchanged	No. of branches extended	No. of branches retracted	Branches changed (%)
3–8	4	17	0	0	0
13–14	3	11	4	3	39
28–33	6	9	8	10	67

The classification of a branch as having extended, retracted or remained unchanged is based on an apparent change of >10 μm; measurements were made from camera lucida drawings (see Figs 1–3) using a digitizing pad attached to a computer. Only terminal dendritic branches reconstructed completely from multiple focal planes were included (13 of the 28 cells imaged at two different times).

interval between observations. Although the images obtained at the two different times were never exactly congruent (in part because of small differences in rotation and variations in the tension on the cell), the general configuration of the processes that had been viewed initially was easily recognized and always had the same overall arrangement. Thus, dye filling was complete, or at least consistent in what it revealed. Detailed examination showed differences in the dendritic geometry of many cells, involving the retraction of some branches and the extension of others. In some instances branches evident during the initial observation of the cell had disappeared altogether, whereas other dendritic branches appeared *de novo* in this interval. In most cases, the changes observed at longer intervals were more extensive than those observed at shorter intervals.

Thus, eight neurones that were visualized at intervals of 3–8 days showed relatively minor changes (Fig. 1). The 13 neurones that were imaged after 13–16 days often showed more obvious changes (Fig. 2). In seven animals the same cell was imaged after an interval of 28–33 days. Although the general configuration of the dendrites at the second viewing could still be recognized as similar to the initial pattern, in each case the changes were more extensive than those observed at the shorter intervals (Fig. 3; Table 1). Complete reconstructions of the relevant portion of the dendritic arborization were made in 13 of the 28 cells studied. To quantitate this progressive change, individual branches were measured and scored according to whether they had changed by more than 10 μm (see Table 1).

Our results show that the dendrites of the cells observed gradually change over periods of a few weeks. We cannot eliminate the possibility that such changes represent a response to the experimental procedure rather than normal remodelling of dendritic arborizations. However, two aspects of our results provide evidence against artificially induced changes in cell shape. First, the surviving cells remained healthy; resting potential and action potential amplitudes of the cells at the second observation were indistinguishable, on average, from values obtained during the initial impalement, and the dendritic arborizations, although changed, were normal in appearance. Second, and perhaps more importantly, the dendritic arborizations changed progressively over the period of observation (up to 33 days) (Table 1). Although the procedure used here might have caused some initial damage, it seems unlikely that such an effect would continue to contribute to the loss and gain of dendritic processes weeks later.

Our interpretation of these observations is that extension and retraction of dendritic processes is a normal occurrence in sympathetic ganglia of young adult mammals. As most synapses on superior cervical ganglion cells in the mouse are made on dendritic processes (ref. 3 and C. J. Forehand, unpublished observations), the dendritic changes we describe are presumably accompanied by commensurate changes in the synaptic connections of these neurones. It will be of interest to examine neurones in other parts of the nervous system where this same general approach may be feasible.

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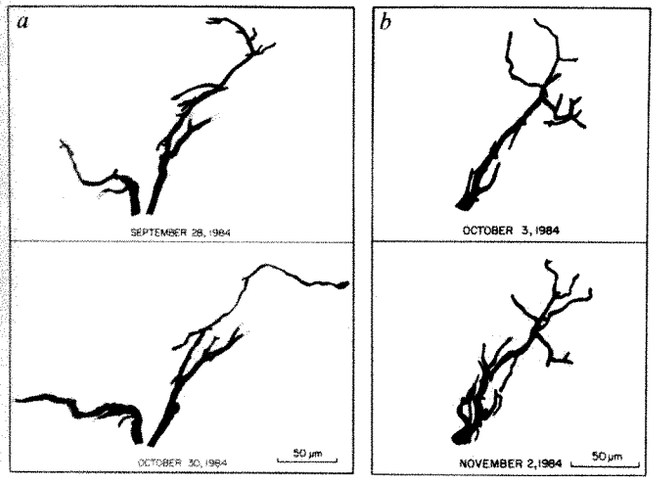


Fig. 3 Reconstruction of a portion of the dendritic arborizations of two ganglion cells observed after an interval of 1 month. *a*, A dendrite with several branches has withdrawn, whereas another branch has apparently extended more than 50 μm. Dashed lines indicate continuance of some branches to deeper levels that were not photographed. *b*, Similar retraction and extension of particular branches. Changes observed after 28–33 days were more prevalent and extensive than those observed at shorter intervals (compare Figs 1, 2; see also Table 1).

Axon guidance in cultured epithelial fragments of *Drosophila* wing

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Growing axons can be guided by a number of different cues: adhesive substrates[1,2], diffusible factors[1–3], electrical fields[1,2,4] and even factors intrinsic to the neurone itself[1,2,5] have all been shown to affect axon orientation and outgrowth *in vitro*. However, in most intact systems it has proved difficult to test directly the role played by these putative guidance cues. Here, we describe a system, the developing wing of the fruitfly, in which we have tested simultaneously two putative guidance mechanisms, physical constraints to axon growth (channels) and the position of neuronal somata (guideposts), using surgical techniques. We show that pioneer sensory axons can navigate correctly and form their normal stereotyped pattern of axon bundles in wing fragments that apparently lack both physical and neural cues. This technique allows access to the surface along which neuronal pathfinding takes place, making possible a wide range of experimental manipulations on the developing system.

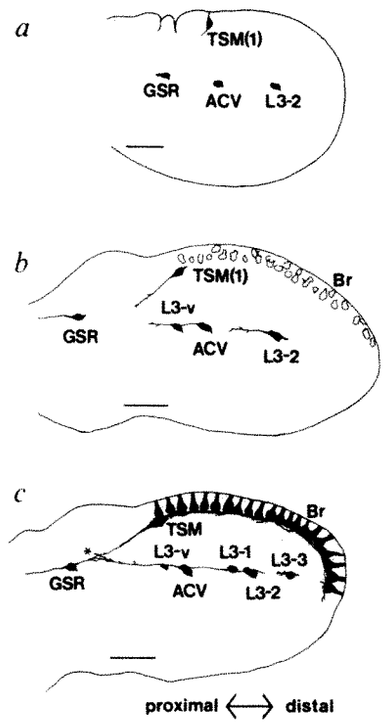


Fig. 1 Sensory neurones of a wing at 2 h after pupariation (*a*), or after having been removed at 2 h after pupariation and cultured for an additional 8 h (*b*) or 22 h (*c*). The earliest-arising neurones are those of the anterior cross vein campaniform sensillum (ACV), the second dorsal campaniform sensillum of the third vein (L3-2), the first twin campaniform sensillum (TSM(1)) and the giant campaniform sensillum of the radius (GSR); these neurones initiate axonogenesis just before (TSM(1) and GSR) or just after (ACV and L3-2) 2 h after pupariation. The ACV, L3-2 and TSM(1) neurones are each accompanied by an 'extra' neurone, E-1, E-2 and E-T respectively, which seem to develop similarly to the sensory neurones[8]; each of these cell pairs will be treated as a single unit designated by the name of the sensillum. Later-arising neurones initiate outgrowth at about the same times *in vivo* and *in vitro*, the ventral campaniform sensillum (L3-v) neurone at 5 h after pupariation, and the neurones of the first and third dorsal campaniform sensilla of the third vein (L3-1 and L3-3) and of the bristle sensilla (Br) at 12 h after pupariation[8,14]. Axon outgrowth is slightly slowed but essentially normal *in vitro*[14], although axons often fail to grow proximally beyond the junction of the first and third veins (asterisk; see text) after 22 h *in vitro*. Wings were cultured in Shields and Sang M3 medium supplemented with 10% fetal bovine serum[25] as described previously[14], except for the addition of 250 U ml^{-1} penicillin, 250 $\mu g\ ml^{-1}$ streptomycin, 0.63 $\mu g\ ml^{-1}$ Fungizone (Gibco) and 50 $\mu g\ ml^{-1}$ tetracycline-HCl (Sigma), and the exclusion of β-ecdysone from the medium. Distal is to the right. Scale bars, 50 μm.

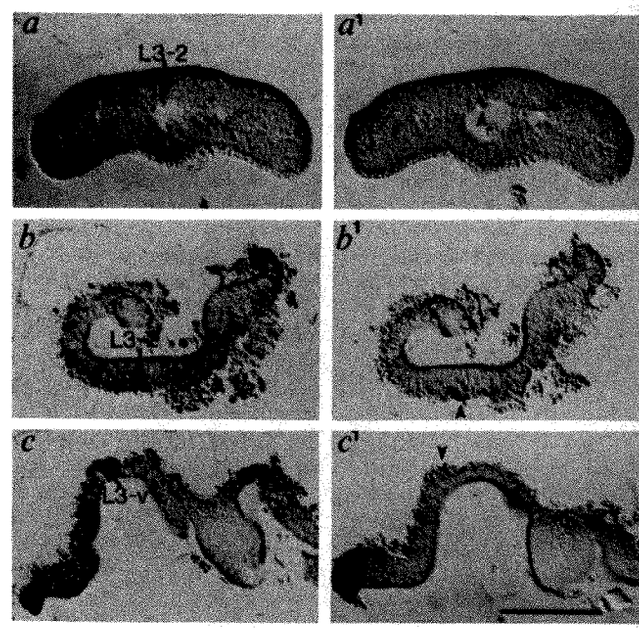


Fig. 2 Transverse sections through wings that had been removed at 2 h after pupariation and cultured an additional 8 h. Neurones were stained with anti-horseradish peroxidase (HRP) and an HRP-conjugated second antibody, and appear dark because of the HRP reaction product. *a*, a^1, Adjacent sections from a whole cultured wing, showing channels and the position of the soma of the L3-2 neurone (*a*) and axon (a^1, arrow) within the most central of them. *b*, b^1, Adjacent sections from a dorsal fragment generated at 2 h after pupariation, showing the position of the L3-2 soma (*b*) and axon (b^1, arrow); note the lack of channels or folds along the path of axonal outgrowth. *c*, c^1, Adjacent sections from a ventral fragment, showing the position of the soma of the L3-v neurone (*c*) and short axon (c^1, arrowhead); this fragment similarly lacks channels or folds along the path of axonal outgrowth. Three or more of each type of fragment (dorsal and ventral) have been completely sectioned; none shows any type of morphological irregularity which might act to guide growing axons in the distal wing blade. Fragmented wings for sectioning were reared and fixed after having been pinned flat onto Sylgard using glass needles; this reduces curling of the wings and does not affect axon guidance (8 h dorsal fragments, 6 of 6 cases). Wings were fixed and stained with anti-HRP as described previously[8], except that HRP-labelled goat anti-rabbit IgG (Cappel) was used as the second antibody, reacted with diaminobenzidine, cleared in 80% glycerol, embedded in JB-4 plastic, sectioned at 5 μm (in some cases (not shown) counterstained with toluidine blue) and viewed using Nomarski optics. Scale bar, 50 μm.

The wing of the fruitfly *Drosophila melanogaster* develops from an epithelial sac, the wing imaginal disk. During early stages of metamorphosis, the disk everts and flattens, bringing together originally separate epithelia to form the dorsal and ventral surfaces of the flat wing blade. Gaps are formed between the apposed inner epithelial surfaces, so that by 4 h after pupariation a system of channels loosely corresponding to the pattern of venation of the adult wing is created[6–8]. These channels are transiently lost when the wing inflates between 12 and 24 h after pupariation.[6,7] The pioneer sensory neurones of the wing (the neurones of specific large campaniform sensilla) are born (before 0 h after pupariation, from epithelial mother cells), differentiate (0–2 h after pupariation), initiate axonal outgrowth (1–3 h after pupariation) and complete the basic scaffolding of nerve bundles (12 h after pupariation) before this time[8,9] (Fig. 1). Thus, the first axons navigate through a system of channels between apposed epithelial cells, which might act to limit and guide axonal outgrowth. A similar role has been proposed for channels in the developing vertebrate central nervous system[10] and retina[11].

To test this hypothesis, the formation of channels between the wing epithelia was prevented by cutting the wing disk into dorsal and ventral fragments, after eversion but before the apposition of the two surfaces (2 h after pupariation); channels in such fragments could no longer be closed because the opposite epithelium was lacking. Axon outgrowth was assessed by rearing the fragments in culture for a further 8 or 22 h and then staining their neurones with antiserum generated against horseradish peroxidase[12]. No closed channels were observed in epithelial fragments cultured for 8 h; axons grew along the innermost surface of the epithelium and did not seem to be following any histologically detectable morphological irregularities, such as grooves or folds (Fig. 2). This technique therefore allowed us to analyse the pathfinding abilities of axons growing in a channel-free environment.

As demonstrated previously[13,14], whole wing disks reared *in vitro* show largely normal patterns of axonal outgrowth (Fig. 1). Neurones in dorsal and ventral wing fragments differed somewhat in their ability to reproduce this normal pattern[15].

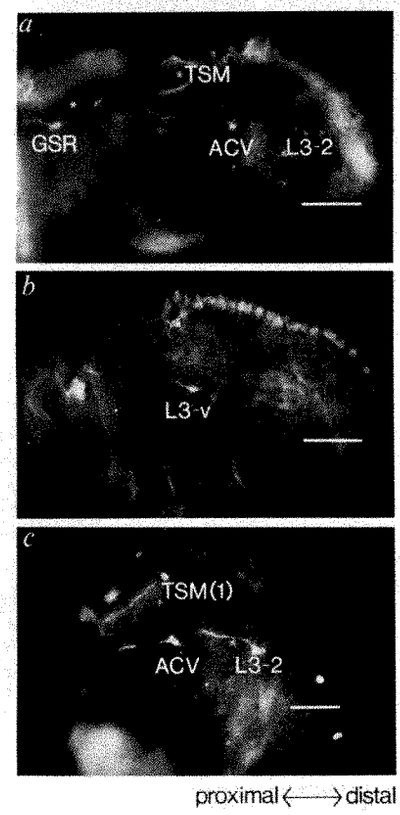


Fig. 3 Cultured wing fragments stained with anti-HRP. *a*, Dorsal wing fragment raised *in vitro* for 22 h. The nerve bundles of the first and third veins have formed normally and join at approximately their normal site just distal to the GSR neurone (compare with Fig. 1), although in this case they failed to grow to the GSR neurone (asterisk marks gap; see text). *b*, Ventral wing fragment, raised *in vitro* for 22 h. The L3-v neurone has formed a short bipolar projection along the proximo-distal axis of the wing; the proximal process is longer than the distal. *c*, Dorso-distal fragment, raised *in vitro* for 8 h. Although the fragment lacked both the GSR neurone and closed channels, the remaining ACV, L3-2 and TSM(1) neurones grew along the correct paths and directions (compare with Fig. 1). Fragments were fixed and stained as described previously[14]. Distal is to the right. Scale bars, 50 μm.

The earliest-developing neurones of the distal wing blade (ACV, TSM(1) and L3-2; see Fig. 1 legend) are all found on the dorsal surface. In dorsal fragments examined after 8 h in culture, the axons of these early neurones had grown in the correct direction along the normal paths (Table 1), either proximally (ACV and L3-2) or proximoposteriorly (TSM(1)). In fragments raised for 22 h, these neurones, together with the later-arising L3-1, L3-3 and Br neurones, formed largely normal nerve bundles (49 of 50 cases; Fig. 3*a*). The only commonly observed abnormality was that the ACV and TSM(1) axons failed to reach the GSR neurone (33 of 49 cases), even though they contacted each other at the usual, slightly more distal, point. This is also common in whole wings raised *in vitro*[14] (under current conditions, 19 of 33 cases) and probably results from damage to the wings or suboptimal culture conditions. In any case, initial outgrowth and most other elements of nerve formation were normal in dorsal fragments despite the lack of channels.

In contrast, axonal outgrowth of the only ventral neurone of the distal wing blade was both delayed and variable in the absence of the dorsal surface. In whole wings, this neurone, the L3-v, initiates axonogenesis only after the early dorsal axons have grown past it (~5 h after pupariation); its process then joins with and apparently follows the early axons. Although this fasciculation obscures the details of further L3-v outgrowth, dye fills in adults demonstrate that the L3-v forms a single, proximally directed axon[16]. In ventral fragments reared for 8 h,

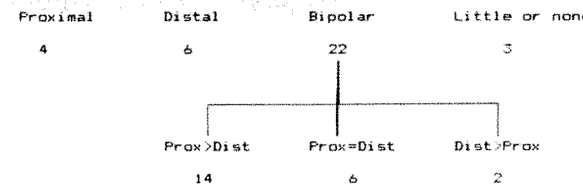


Fig. 4 Direction of outgrowth of the axon of the L3-v neurone in ventral fragments, generated at 2 h after pupariation and cultured for an additional 22 h. Significant outgrowth was taken as outgrowth longer than one neuronal cell diameter. Some branching of axons was observed, but in all cases outgrowth was oriented roughly along the proximo-distal axis of the wing. In five cases a region of dorsal epithelium lay along the anterior of the ventral fragment near the L3-v; in these wings (not included) the L3-v axon grew anteriorly into this region and sometimes seemed to contact the short nerve formed by the TSM neurones. Obviously infected cultures were not included in the data pool.

the L3-v formed no or only very short processes (<1 cell diameter, 20 of 20 cases). By 22 h it usually formed a short bipolar projection (Figs 3*b*, 4); in a few cases, exclusively proximal or distal outgrowth was seen. Because the normal growth pattern of this neurone is not known in detail, it is not certain that these results represent abnormal axonogenesis. However, the variable and generally stunted outgrowth seen in ventral fragments suggest that L3-v axonogenesis was to some extent disrupted by the removal of the dorsal epithelium. Nevertheless, important features of L3-v axonogenesis were retained in ventral fragments: outgrowth was still oriented approximately along the proximo-distal axis of the wing, and was usually, though not always, longer in the proximal than in the distal direction (Fig. 4). Thus, correctly directed axon outgrowth can occur even in a fragment in which the neuronal population is reduced to the greatest possible extent, namely a single cell differentiating in an expanse of epithelium.

Thus, it is apparent that both dorsal and ventral fragments must contain some types of guidance cues other than channels. For example, the filopodia of a neurone might seek out a more proximal 'guidepost' or 'landmark' neurone, which would act as a target for axon growth[17–19]. Guidepost neurones have been shown to have no necessary role in normal *Drosophila* wings[14,20] but might effectively guide neurones in dorsal fragments lacking channels. This possibility was tested by generating fragments, at or before the time of initial axon outgrowth (1.5–2 h after pupariation), from which both proximal tissue (containing a putative guidepost neurone) and the ventral epithelium had been removed. When such dorso-distal fragments were cultured for 8 h, the ACV and TSM(1) neurones still navigated along their normal paths and in their normal directions, even though they lacked both channels and the proximal putative guidepost neurone (GSR) towards which they normally grow (Fig. 3*c*, Table 1). Thus, the GSR neurone does not act as a necessary guidepost for these dorsal neurones, even in the simultaneous absence of obvious physical cues.

Table 1 Fraction of neurones showing normal axonal outgrowth in dorsal and dorso-distal wing fragments

Fragment	ACV	L3-2	TSM(1)
Dorsal	17/17	13/14	14/14
Dorso-distal	11/11	8/8	8/8

Fragments were cut at 2 h after pupariation and cultured for an additional 8 h. Not all neurones could be observed in each fragment. Dorso-distal fragments all lacked the GSR neurone. The single instance of abnormal L3-2 outgrowth consisted of a lengthening of its normally short distal process; this abnormality has also been observed in whole wings raised *in vitro*. Obviously infected cultures were not included in the data pool.

Although there is strong evidence that guidepost neurones can direct growing axons in specific instances in developing insects[18,21,22], even in these cases initial axon outgrowth seems to be directed, as here, by some other cue(s)[18,21]. A one-dimensional gradient of neural adhesivity may provide one such cue[23,24]. However, some features of the axonal pattern in fly wings, such as the angled outgrowth of the TSM(1) axon, are not easily explained by a single gradient, and might require the reading of information in two dimensions. An alternative mechanism that would require less from the neurone would be to possess adhesive cues localized selectively along the normal nerve pathways of the wing[16]. Finally, polarity information intrinsic to each neurone[5] might also guide outgrowth, particularly during its initial stages; extrinsic cues might then be needed only to induce large changes in the trajectory of the growing axon. As the surface along which the neurones of the fly wing navigate can now be exposed without disrupting outgrowth significantly, it may be possible to manipulate this surface directly, to study a variety of putative guidance mechanisms.

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Position-dependent properties of retinal axons and their growth cones

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The formation of the very orderly neuronal projection from the retina to the optic tectum is not yet understood, but several mechanisms are thought to be involved in a coordinated fashion. These mechanisms may include mechanical or chemical guidance in channels, guidance by spatial gradients of positional markers, gradients of temporal (maturation) markers or specific inter-axon interactions (see ref. 1 for review). The last-mentioned mechanism could explain the fibre order found in optic nerve and tract. It requires that some or all growing retinal axons can distinguish between retinal axons of various origins and grow preferentially along retinal axons originating from the same area as themselves. The *in vitro* experiments described here show that growth cones from the temporal half of the chick retina grow preferentially along temporal axons, whereas growth cones from nasal retina do not distinguish between nasal and temporal axons.

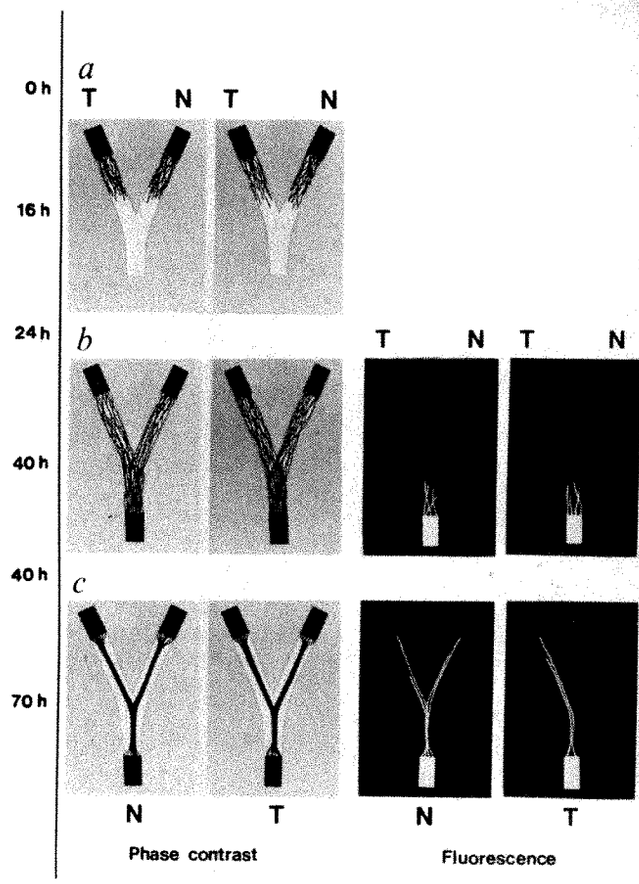


Fig. 1 Schematic description of the experiment. *a*, Phase-contrast diagram of axon growth, with first explantations at 0 h; *b*, explantation of fluorescent explants at 24 h; *c*, addition of α-laminin at 40 h. Axons visualized at 16, 40 and 70 h. T, temporal; N, nasal. **Methods.** Nasal and temporal explants (0.2 × 2 mm) of chick retina were prepared as described previously[6]. They were placed at time 0 onto laminin-coated round coverslips and incubated in culture medium at 37 °C. Laminin was applied to the coverslips by first treating them with 3-(triethoxysilyl)propylamine and then incubating them in laminin (40 $\mu g\ ml^{-1}$) for 3 h at 37 °C. The laminin serves as substrate for growing axons which are visible in phase contrast (*a*). To restrict axonal growth to the indicated Y-shaped field, border lines were drawn with a hypodermic needle on the laminin surface. Axons from nasal and temporal explants joined at the stem of the Y after ~20 h. At ~24 h, a fluorescent explant (either temporal or nasal) was placed at the foot of the Y which was reached by the axons a few hours later. At 40 h the Y-shaped field was crowded with axons as seen in phase contrast and a few fluorescent axons growing up the stem of the Y could be seen in fluorescence (*b*). At about that time, anti-laminin (provided by Dr R. Timpl) was added to the culture. After ~1 h, all fibres were detached from the laminin-coated coverslip and the axons spanned Y-shaped bundles joining the explants. Now the further growth of fluorescent axons (which can be inhibited by antibodies against the cell adhesion molecule N-CAM; ref. 7) could only occur along pre-existing axons. As seen in *c*, temporal fluorescent axons strongly prefer to grow along temporal axons, whereas nasal axons grow along both nasal and temporal axons.

Two 6-day-old chick embryo retinal explants were placed on a laminin-coated coverslip as described in Fig. 1 legend. One explant was taken from the temporal side of the area next to the optic fissure, whereas the other was taken from the nasal side. The axons were forced to grow within predetermined tracks scratched into the laminin so that the axons of both explants met and joined to form a 'Y'-like configuration. (The growth rate of nasal and temporal axons was about the same.) About 24 h later, a third rhodamine-stained fluorescent explant of either nasal or temporal origin was placed near the growth cones at the base of the Y. After a further 16 h, when most of the nasal and temporal axons had reached the labelled explant and some of the fluorescent axons were already leaving it, all axons were

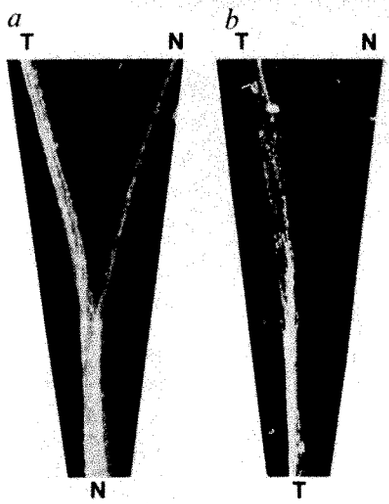


Fig. 2 *a*, Nasal fluorescent axons growing along both the nasal and the temporal axon bundles. The fraction of fluorescent fibres on either bundle is roughly proportional to the thickness of these bundles. *b*, Temporal fluorescent axons growing exclusively along the temporal axon bundle. The nasal axon bundle has been made barely visible by weak dark-field illumination. T, temporal; N, nasal.

detached from the laminin substrate by the addition of anti-laminin.

Because axons are very elastic, they form a structure similar to a tightrope once they lose their contact with the substrate. The only substrate remaining to support further growth of the fluorescent axons was the bundle of axons coming from the two unstained explants; axons had the choice of growing along nasal or temporal fibres within this bundle. Therefore, the preference of the labelled axons became apparent where the bundle bifurcated.

As shown in Figs 1 and 2, all temporal fibres grow towards the temporal explant, that is, they grow preferentially along temporal axons. The results are the same whether the temporal explants stem from more central or more peripheral positions in the retina. On the other hand, nasal fibres show no preference and grow towards either explant. The fraction of nasal fibres growing towards either of the explants is approximately proportional to the relative thickness of the fibre bundle coming from the explant. In Fig. 2*a*, for example, the number of axons growing towards the temporal explant is relatively high because the bundle of temporal fibres was relatively strong.

Of 61 experiments of this type, 53 could be evaluated; in the other 8 cases too few (or no) fibres passed the bifurcation point. The stained explants of the successful experiments were of nasal origin in 34 cases and of temporal origin in 19. Temporal axons grew exclusively towards the temporal explant, whereas nasal axons always grew towards both explants. The fraction of fluorescent axons growing along either unstained bundle was roughly proportional to the relative thickness of these bundles.

According to our results, axons of temporal origin exhibit position-specific properties of self recognition; temporal growth cones recognize temporal axons. From previous observations it is known that temporal axons show a strong tendency to fasciculate with each other (in contrast to nasal axons[2,3]), in good agreement with the observations reported here. Until now, however, it has not been clear whether this difference in behaviour results from different properties of the nasal and temporal growth cones, or their axons, or both. Our experiments indicate that both axons and growth cones of temporal retinal ganglion cells are different from nasal axons and growth cones.

We have also performed analogous experiments concerning the dorso-ventral axis. Explants were taken from dorsal and ventral positions relatively far apart (half the meridian) from both temporal and nasal retina. These experiments have not revealed any position-specific effects. Anatomical investigations have shown that chick retinal axons are already sorted out in the dorso-ventral dimension when they arrive at the tectum, whereas no sorting of nasal and temporal fibres has been detected[4]. Therefore, differences between dorsal and ventral axons in our *in vitro* assay might have been expected rather than differences between nasal and temporal axons, but the opposite is in fact the case. Our finding that temporal axons have a strong preference for growing along other temporal axons *in vitro*, however, is in excellent agreement with *in vivo* observations in the frog[5]. In contrast to the nasal fibres, the temporal axons of frog fasciculate with each other in the optic tract and form a dense bundle. Whether and how these position-specific properties of axons and growth cones are used during the formation of the chick retino-tectal projection is not known.

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Synapsin I is a spectrin-binding protein immunologically related to erythrocyte protein 4.1

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The membrane-associated cytoskeleton is considered to be the apparatus by which cells regulate the properties of their plasma membranes, although recent evidence has indicated additional roles for the proteins of this structure, including an involvement in intracellular transport and exocytosis (see refs 1–3 for review). Of the membrane skeletal proteins, to date only spectrin (fodrin) and ankyrin have been purified and characterized from non-erythroid sources. Protein 4.1 in the red cell is a spectrin-binding protein[4,5] that enhances the binding of spectrin to actin[4,6,7] and can apparently bind to at least one transmembrane protein[8–10] Immunoreactive forms of 4.1 have been detected in several cell types, including brain[11–14]. Here we report the purification of brain 4.1 on the basis of its cross-reactivity with erythrocyte 4.1 and spectrin-binding activity. We further show that brain 4.1 is identical to the synaptic vesicle protein, synapsin I, one of the brain's major substrates for cyclic AMP and Ca^{2+}–calmodulin-dependent kinases[15,16]. Spectrin and synapsin are present in brain homogenates in an ~1:1 molar ratio. Although synapsin I has been implicated in synaptic transmission, no activity has been previously ascribed to it.

Antibodies raised against pig erythrocyte 4.1 recognize only protein 4.1 in pig erythrocyte ghosts (Fig. 1). Cross-reactive polypeptides in brain (rat, pig or calf) have an apparent relative molecular mass (M_r) of 75,000 and 73,000 (judged by SDS-gel electrophoresis with a discontinuous buffer system[17]).

The cross-reactive polypeptides were extracted from myelin-depleted brain membranes under conditions similar to those used for the extraction of erythrocyte protein 4.1. Thus, they were virtually inextractable in solutions of low ionic strength but were liberated readily from the membranes by treatment with solutions of higher ionic strength (for example >0.2 M KCl). The extracted protein failed to bind DEAE-cellulose (in 10 mM Na-phosphate at *p*Hs as high as 8.2) or hydroxylapatite in the presence of 1 M KCl. However, it was

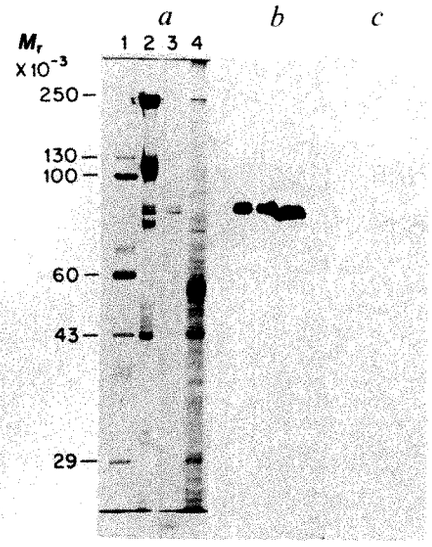


Fig. 1 Identification of a brain protein immunologically related to erythrocyte 4.1. Antibodies were raised in rabbits against SDS-denatured protein 4.1 that had been purified by standard procedures[4,5] and were affinity-purified on 4.1 immobilized on nitrocellulose strips after SDS-gel electrophoresis and blotting[38]. In *a*, *b* and *c*, proteins were electrophoresed on 9% SDS-polyacrylamide gels[17] and stained with Coomassie blue (*a*) or transferred to nitrocellulose paper[27] (*b*, *c*) for reaction with anti-red blood cell (RBC) 4.1 antibodies and ^{125}I-protein A (*b*) or non-immune IgG (*c*). Lanes 1, M_r standards: filamin (250,000), vinculin (130,000), α-actinin (100,000), catalase (60,000), actin (43,000), cabonic anhydrase (29,000); lanes 2, pig RBC ghosts; 3, pig RBC 4.1; 4, pig brain post-nuclear supernatant.

retained by carboxymethyl-cellulose and by hydroxylapatite at low ionic strength and *p*H 6.8. These properties are those of a very basic protein[18] and allowed the scheme for purification shown in Fig. 2. The purified brain 4.1 displayed anomalous migration between different SDS gel systems. Using a continuous gel buffer[19] we obtained subunits of M_r 88,000 and 85,000: in this system, brain 4.1 migrates more slowly than erythrocyte 4.1, as has been observed previously[12].

The spectrin-binding activity of brain 4.1 was shown using 'blot binding' techniques in which fractions were taken from various stages of purification of brain 4.1 and were electrophoretically separated, transferred to nitrocellulose and incubated with ^{125}I-labelled erythrocyte spectrin (Fig. 2). As a control for nonspecific binding, additional unlabelled spectrin was included in some of the incubations to displace specific binding. Brain 4.1 at later stages of the purification specifically binds ^{125}I-spectrin on the nitrocellulose (Fig. 2*a*,*b*), but this method was not sensitive enough to detect brain 4.1 in early stages of the purification. Other basic proteins (notably myelin basic protein) do not bind ^{125}I-spectrin.

Conversely, ^{125}I-brain 4.1 was incubated with blots on which erythrocyte and brain spectrins were immobilized (Fig. 3). As controls for nonspecific binding, we used heat-denatured ^{125}I-brain 4.1. As additional internal controls for binding of a basic protein (brain 4.1) to an acidic protein (spectrin), actin, tropomyosin and total erythrocyte ghost proteins were included in the blots. Again, we find specific binding of the ^{125}I-brain 4.1 to spectrin. One characteristic of erythrocyte 4.1 is that it binds to both α- and β-subunits of erythrocyte spectrin[7]. Blots in which ^{125}I-brain 4.1 was incubated with isolated α- and β-subunits of brain spectrin showed specific binding to both. Preliminary results indicate that brain 4.1 and erythrocyte 4.1 compete for the same site on brain spectrin, as brain 4.1 displaces binding of ^{125}I-brain spectrin to erythrocyte 4.1 on blots (not shown). The interaction between brain 4.1 and brain spectrin is also demonstrable by affinity chromatography (not shown).

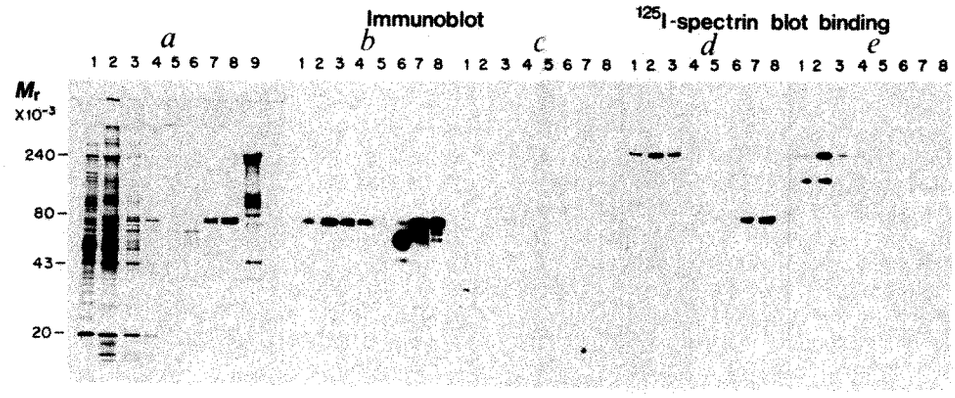


Fig. 2 Purification of brain 4.1. Fractions from the purification procedure were analysed on 5–15% polyacrylamide gels, which were stained with Coomassie blue (*a*) or transferred to nitrocellulose[27] (*b*–*e*). Nitrocellulose replicas were reacted with: *b*, anti-RBC 4.1; or *c*, non-immune IgG followed by ^{125}I-protein A. In *d* and *e* they were incubated with ^{125}I-RBC spectrin (20 nM, 2×10^6 c.p.m. ml^{-1} as described in ref. 27) in the absence (*d*) or presence (*e*) of a 20 fold excess of unlabelled spectrin. Lanes 1, myelin-depleted pig brain membranes; 2, 0.25 M KCl extract; 3, first hydroxylapatite (HA) column eluate; 4, DEAE supernatant; 5, carboxymethyl-cellulose (CMC) flow-through; 6, 30 mM NaCl eluate; 7, 60 mM NaCl eluate from CMC; 8, second HA column, $MgCl_2$ eluate; 9, M_r standards (human erythrocyte ghosts). In *b*, lane 8 contains half the amount of protein loaded in the corresponding lanes in *a* and *c*–*e*.

Methods. Myelin-depleted membranes[27] from pig brain (350–450 g) were washed twice with 40 mM KCl, 2 mM EGTA in buffer A (10 mM Na-phosphate, 1 mM dithiothreitol (DTT), 1 mM NaN_3, 1 μg ml^{-1} leupeptin, 1 μg ml^{-1} pepstatin A, 1 μg ml^{-1} chymostatin, 200 μg ml^{-1} phenylmethylsylphonyl fluoride (PMSF), 1 mM diisopropyl fluorophosphate (DFP) *p*H 7.4) and were brought to 1:1 with buffer A containing 0.25 M KCl and 2 mM EGTA. Incubation at 37 °C for 30 min released most of the cross-reactive peptides, which were recovered in the supernatant after centrifugation at 30,000*g* for 30 min. The protein extract was concentrated 10-fold by precipitation with ammonium sulphate at 65% saturation and resuspended in, and dialysed against, 1 M KCl, 1 mM EDTA, 0.1% Tween 20 in buffer A lacking DFP. After centrifugation at 180,000*g* for 90 min, the supernatant was made 5 mM in $MgCl_2$ and applied to a 20-ml column of hydroxylapatite (fast flow, Calbiochem) equilibrated with 1 M KCl, 10 mM Na-phosphate, 5 mM $MgCl_2$, *p*H 7.4. The void volume, containing the cross-reactive peptides, was dialysed extensively against 1 mM EDTA in buffer A lacking DFP, followed by batchwise absorption with 10 ml DEAE-cellulose (Whatman DE53, equilibrated with buffer A, lacking DFP): cross-reactive peptides were recovered in the supernatant after centrifugation at 1,000*g* for 2 min. The supernatant was applied to a 10-ml column of carboxymethyl-cellulose (Whatman CM32), equilibrated with buffer B (10 mM Na-phosphate, 1 mM EDTA, 0.1% Tween 20, 0.5 mM DTT, 1 mM NaN_3, *p*H 7.4), then the column was washed with buffer B and eluted stepwise with 30 mM NaCl in buffer B and 60 mM NaCl, 5 μg ml^{-1} leupeptin in buffer B. Peak fractions from the second step were dialysed against buffer C (1 mM Na-PIPES, 0.1 mM DTT, 0.05% Tween 20, 1 mM NaN_3, 1 μg ml^{-1} leupeptin *p*H 6.8) and applied to a 2-ml column of hydroxylapatite (high-resolution (Calbiochem), equilibrated with buffer C). Cross-reactive spectrin-binding peptides were eluted with 5 mM $MgCl_2$ in buffer C. Yields of 2–5 mg were typically obtained. The protein was typically 90–95% pure when prepared from pig brain, but a contaminant of M_r 48,000 was present as 10–15% of the total protein when this method was applied to calf brain. This yield represents ≥2% of the total brain 4.1 in a homogenate, major losses occurring because of incomplete extraction from membranes, proteolysis and adsorption to surfaces.

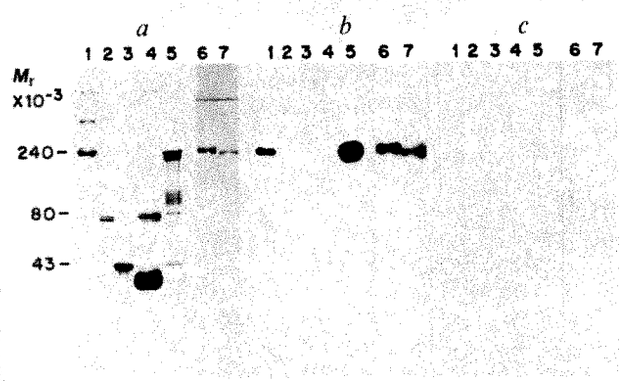


Fig. 3 ^{125}I-brain 4.1 specifically binds to erythrocyte and brain spectrin, α- and β-subunits. After SDS-gel electrophoresis on 5–15% gels, proteins were either stained with Coomassie blue (*a*) or electrophoretically transferred to nitrocellulose (*b*, *c*). Lanes 1, pig brain spectrin; 2, pig brain 4.1; 3, rabbit skeletal muscle actin; 4, rabbit skeletal muscle tropomyosin; 5, human erythrocyte ghosts; 6, brain spectrin α-subunit; 7, brain spectrin β-subunit. Actin, tropomyosin and ghost proteins were included as additional controls for nonspecific interaction between acidic proteins and a basic protein (brain 4.1). Brain spectrin and its subunits were prepared as described previously[27]. Nitrocellulose replicas were incubated with ^{125}I-brain 4.2 (10 nM, 2×10^6 c.p.m. ml^{-1}) in blot buffer (4% (w/v) bovine serum albumin (Pentex), 0.15 M NaCl, 10 mM Na-phosphate, 1 mM EDTA, 0.2% Triton X-100, 1 mM NaN_3, *p*H 7.1) for 18 h at 4 °C. In *c*, the ^{125}I-brain 4.1 had been denatured by heating to 60 °C for 20 min before incubation with the nitrocellulose paper. Unbound ^{125}I-brain 4.1 was removed by washing the replicas five times with blot buffer lacking albumin.

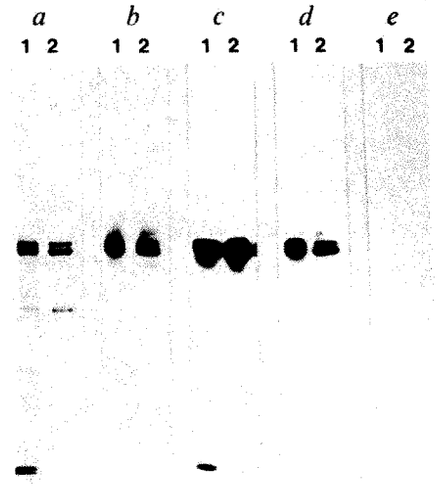


Fig. 4 Synapsin I cross-reacts with anti-RBC 4.1 and anti-brain 4.1 antibodies and binds ^{125}I-spectrin. Samples of bovine synapsin I (lanes 1) and bovine brain 4.1 (lanes 2) were electrophoresed on polyacrylamide gels which were stained with Coomassie blue (*a*) or transferred to nitrocellulose paper (*b*–*e*). The nitrocellulose paper was reacted[27] with: *b*, anti-RBC 4.1 and ^{125}I-protein A; *c*, anti-brain 4.1 and ^{125}I-protein A; *d*, ^{125}I-brain spectrin (20 nM, 2×10^6 c.p.m. ml^{-1}); and *e*, ^{125}I-brain spectrin in the presence of a 40-fold excess of unlabelled spectrin.

Brain and erythrocyte 4.1 are the products of different genes, with distinct two-dimensional peptide maps (not shown). Similarly, mammalian brain spectrin[20,21] and brain ankyrin[22] have fingerprints distinct from their erythrocyte counterparts. However, the two components of brain 4.1 give rise to nearly identical fingerprints (not shown). Limited chymotryptic digests of brain 4.1 resolved on SDS gels show ~10 peptides which cross-react with erythrocyte 4.1, suggesting a substantial degree of antigenic homology (not shown).

Like erythrocyte 4.1 (ref. 23), brain 4.1 is a substrate for cAMP-dependent kinase. When brain membranes were incubated with [γ-^{32}P]ATP and 10 μM cAMP, a pair of polypeptides became labelled which are specifically immunoprecipitable with anti-brain 4.1 antibody. In the absence of cAMP, these polypeptides do not become appreciably labelled (data not shown).

We conclude that the protein we have purified is closely related to erythrocyte 4.1, because: (1) it has cross-reactivity with anti-4.1 IgG; (2) it consists of two basic, sequence-related polypeptides; (3) it binds spectrin; and (4) it is a subtrate for cAMP-dependent protein kinases.

The properties of brain 4.1 are strikingly similar to those of synapsin I, a protein associated with synaptic vesicles[24]. Synapsin I was initially identified as a major substrate in brain for cAMP-dependent protein kinase[15] and is a doublet of M_r ~80,000 on SDS gels[25].

The physical properties of brain 4.1 and synapsin I are similar: synapsin I is basic (*p*I 10.3) with two polypeptide components that give rise to similar peptide maps[25]. Both brain 4.1 and synapsin I[26] are released from brain membranes in solutions of >0.15 M KCl. Immunoprecipitated ^{32}P-brain 4.1 co-migrates on SDS gels with a pair of ^{32}P-polypeptide that seem to be those defined by Johnson *et al.*[15] as synapsin I (not shown).

Standard synapsin I, prepared by extraction of brain membranes of *p*H 3, isoelectric precipitation of contaminants at *p*H 6, followed by hydroxylapatite and gel-filtration chromatography[25] (a gift of Dr H. C. Palfrey) has identical electrophoretic mobility on SDS gels to brain 4.1 (Fig. 4). Synapsin I and brain 4.1 were immunologically cross-reactive, as they both reacted on SDS gel blots with anti-red cell 4.1 and anti-brain 4.1 IgG, but not with non-immune IgG. Furthermore, they both bind ^{125}I-brain spectrin on gel blots, the binding being displaceable with unlabelled spectrin.

Finally, two-dimensional ^{125}I-iodo peptide maps of synapsin I and brain 4.1 are identical in all but two (minor) spots out of some 30 visible on the maps (Fig. 5), the variant spots differing only in their mobilities on the plates. The variations in the two spots need not imply variations in primary structure, but may reflect different post-translational modifications. Differing phosphorylation states, or modification to the proteins that occurred during their preparation, might give rise to such variation. The proteins clearly have very closely related amino-acid sequences and we believe that they are one and the same.

Synapsin I has previously only been studied as a substrate for protein kinases and its function was unknown. Here we report the first evidence that synapsin is a spectrin-binding protein which seems to be related to erythrocyte membrane skeletal protein 4.1.

An estimate of the stoichiometry of spectrin and synapsin I can be made: in whole brain homogenates, spectrin dimer (M_r = 500,000) comprises 2.4% of the total protein[27] and synapsin I (M_r = 75,000) 0.4% (ref. 28). These values correspond to 48 pmol spectrin and 53 pmol synapsin per mg total protein, that is, close to a ratio of 1:1. This stoichiometry is similar to that of the corresponding proteins in the erythrocyte[29].

The subcellular organization of spectrin in neurones has not yet been determined precisely. However, both spectrin and synapsin I are enriched in synapses[24,30] and are prominent components of isolated synaptosomes[26,31]. Spectrin is also a prominent component of the postsynaptic density[32]. Initial work suggested that synapsin I was a component of the postsynaptic density[33], but DeCamilli *et al.*[24] concluded it was not, although they could not eliminate the possibility that their antibody was excluded from the postsynaptic density. Bearing in mind that synapsin is present stoichiometrically with spectrin, this point is open to re-examination. Furthermore, recent work[12,34] suggests that although proteins immunoreactive with erythrocyte spectrin and 4.1 (presumably brain spectrin and synapsin) co-localize in neurones, they occur predominantly in the neuronal perikarya.

A spectrin–synapsin linkage may play a part in neurotransmission. Spectrin may afford a linkage (via synapsin) between

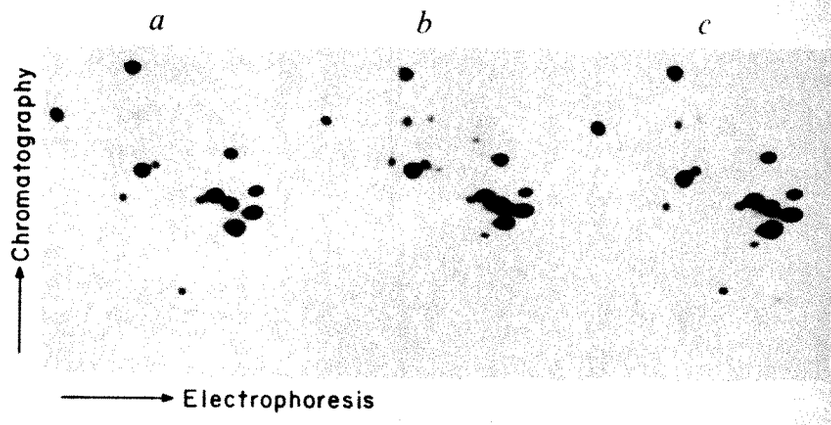


Fig. 5 Brain 4.1 and synapsin I have very similar peptide maps. *a*, Brain 4.1; *b*, synapsin; *c*, a mixture of brain 4.1 and synapsin. Peptide maps of bovine brain 4.1 and bovine synapsin I were prepared by the method described in ref. 27.

synaptic vesicles and the plasma membrane. However, spectrin has also been identified as an axonally transported protein, sometimes associated with membrane-bounded organelles[35]. Thus, an additional or alternative role for spectrin and synapsin might encompass linkage (or regulation of linkage) of synaptic vesicles to cytoskeletal elements. Such cytoskeletal elements need not be only actin. Although erythrocyte 4.1 seems to enhance the binding of brain spectrin to actin[36], our preliminary results have not so far revealed such a role for synapsin. However, synapsin may bind microtubules (unpublished data), which are also present in synaptosomes[31].

Erythrocyte 4.1 is able to bind at least one transmembrane protein. Binding of synapsin to a transmembrane protein of the synaptic vesicle may be of importance in regulation of neurotransmitter secretion, as synapsin is released from its membrane binding site on phosphorylation of synapsin by cAMP- or Ca^{2+}/calmodulin-dependent protein kinases[26], which phosphorylate synapsin in response to hormonal or electrical stimulation of nerves[37].

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Expression of human growth hormone-releasing factor in transgenic mice results in increased somatic growth

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The neurohumoral regulation of growth hormone secretion is mediated in part by two hypothalamic peptides that reach the anterior pituitary via the hypothalamo-hypophysial portal blood system[1]. Somatostatin inhibits the release of growth hormone[2], whereas growth hormone-releasing factor (GRF) positively regulates both growth hormone synthesis[3,4] and secretion[5,6]. Two forms of human GRF, 40 and 44 amino acids long, have been characterized from extra-hypothalamic tumours[7,8] as well as from the hypothalamus[9]. Analysis of human GRF complementary DNA[10,11] and genomic[12] clones indicates that the GRF peptides are first synthesized as a 107- or 108-amino-acid precursor protein. To examine the physiological consequences of GRF expression, we have established strains of transgenic mice containing a fusion gene including the promoter/regulatory region of the mouse metallothionein-I (MT-I) gene[13] and the coding region of the human GRF gene[12]. We report that expression of the human GRF precursor protein in these animals results in measurable levels of human GRF and increased levels of mouse growth hormone in plasma and accelerated growth rates relative to control littermates. These results demonstrate a direct role for GRF in the positive regulation of somatic growth. Unexpectedly, female transgenic mice carrying the MT-GRF fusion gene are fertile, in contrast to female transgenic mice expressing human or rat growth hormone, which are generally infertile. These transgenic mouse strains should provide useful animal models for the study of several types of human growth disorders.

It was shown previously that the hormonal cascade controlling somatic growth could be manipulated by the germline introduction of foreign genes. Expression of either rat[14] or human[15] growth hormone (GH) in transgenic mice results in increased growth rates, giving rise to large animals. Because GRF is believed to be an important physiological regulator of growth hormone in animals, we sought to analyse further its role in growth control by creating strains of animals that express supraphysiological levels of GRF. Neither rat nor human growth hormone genes are themselves expressed in transgenic animals[16,17]; this deficiency has been overcome by fusing these structural genes to an expressed heterologous gene promoter.

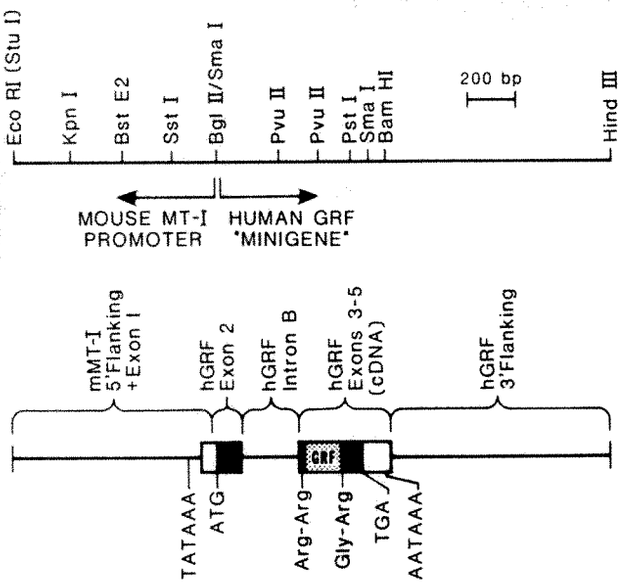


Fig. 1 Structure of the mouse metallothionein-I/human growth hormone-releasing factor fusion gene. The upper portion of the figure is a restriction map for common 6-bp cutters. *Bgl*II/*Sma*I indicates the site of the fusion between the mouse MT-I promoter and the human GRF minigene. The lower portion of the figure is a schematic representation of the 2.5-kb *Eco*RI–*Hin*dIII fragment of DNA used for microinjection. Open boxes represent 5′ and 3′ untranslated exon regions, and shaded boxes represent the coding exon regions. The derivations of various parts of the fusion gene are indicated. The fusion occurs in the 5′ untranslated regions of both genes. The large exon labelled 3–5 was constructed by replacing part of the human GRF gene with a human GRF cDNA, effectively eliminating intron C (2.4 kb) and intron D (3.0 kb) of the human GRF gene[12]. Consensus sequences involved in transcription initiation, translation initiation and termination, polyadenylation, and proteolytic processing of the GRF peptide from its precursor are shown.

The mouse metallothionein-I gene promoter offers several advantages for this type of approach; it is expressed at fairly high levels in most mouse tissues[18], it can be regulated by heavy metals[18], and both expression and regulation are retained in gene transfer experiments[19–21]. Because of the possibility that the human GRF gene, like the human growth hormone gene, might not be expressed in transgenic animals, we initially chose to use the heterologous mouse MT-I promoter to express human GRF.

The structure of the mouse MT-I/human GRF fusion gene construct used (referred to as MT-GRF) is shown in Fig. 1. A 770-base pair (bp) fragment of the mouse MT-I gene, including sequences responsible for metal-inducibility and transcription initiation, was fused to a human GRF minigene which includes the entire coding region of the GRF precursor protein. The human GRF minigene was created by combining cDNA and genomic clones such that the 10-kilobase (kb) human GRF gene, which normally includes five exons[12], has been reduced to <1 kb and retains a single intron of 230 bp (see Fig. 1). Analysis of human GRF cDNA clones suggests that the GRF precursor protein can consist of either 107 or 108 amino acids, differing in the presence or absence of serine 103 (ref. 11). DNA sequence analysis of the human GRF gene[12] indicates that this difference may be explained by alternative RNA processing. The cDNA used to construct the MT-GRF fusion gene should encode only the 108-amino-acid form of the GRF precursor protein[10]. To determine whether the MT-GRF fusion gene could be expressed and regulated correctly, it was introduced into cultured mouse fibroblast cells using $CaPO_4$-mediated DNA transfection[22] and co-selection for neomycin resistance conferred by the vector pSV2-neo[23]. Several stable cell lines that were generated in this manner express a MT-GRF fusion mRNA of the expected size whose abundance is increased by metal treatment, and accumulate radioassayable human GRF (hGRF) in the culture medium (K.E.M., unpublished results).

Table 1 Expression of the MT-GRF fusion gene in transgenic mice

Mouse	No. of gene copies per cell	Liver GRF mRNA (relative amount)	Plasma hGRF (ng ml^{-1})	Plasma mGH (ng ml^{-1})	Growth (ratio)
♂760-5	<1	3	24	1,051	1.35
♂762-3	2	0	<10	21	0.93
♀762-5	4	63	26	141	1.33
♀765-2	2	102	207	415	1.24
♂800-1	1	0	<10	149	1.12
♀800-8	1	3	14	402	1.05
♂801-3	3	0	<10	38	0.97
♀801-5	8	38	99	809	1.35
♂801-9	1	4	20	541	1.42
♀802-3	5	1	45	913	1.51
♀803-4	10	118	263	1,095	1.41
♀803-5	1	5	24	166	1.45
♂803-6	10	16	50	302	1.24
♀803-7	20	*	*	*	1.36

A 2.5-kb *Eco*RI–*Hin*dIII fragment containing the MT-GRF fusion gene was microinjected into the male pronucleus of fertilized F_2 hybrid mouse eggs and the eggs implanted into the oviducts of pseudopregnant recipients[24]. At weaning, DNA was isolated from a piece of tail and analysed for the fusion gene by dot-blotting using a human GRF cDNA as probe[10]. Positive animals were re-analysed by Southern DNA blotting and copy number determined by comparison with a standard curve generated from known amounts of MT-GRF plasmid DNA. Positive animals were maintained on water containing 25 mM $ZnSO_4$. RNA was isolated following partial hepatectomy and analysed by Northern blotting using a human GRF cDNA probe. Autoradiograms were densitometrically scanned to determine relative RNA amounts. Plasma GRF and GH were determined by radioimmunoassay of serum samples from animals at 9 weeks old. The GH radioimmunoassay was able to detect 16 ng ml^{-1} serum GH (two control animals had 16 and 46 ng ml^{-1} GH) and the GRF radioimmunoassay was able to detect 10 ng ml^{-1} serum GRF (two control animals had <10 ng ml^{-1} GRF). The growth ratio was determined at 9 weeks of age and represents a comparison with age- and sex-matched littermates.

* ♀803-7 died prematurely. Although we were able to measure liver GRF mRNA, the amount could not be quantitated because of RNA degradation in the postmortem liver. Plasma GRF and GH levels could not be determined accurately for the same reason.

The MT-GRF fusion gene (~1,600 molecules) was microinjected into the male pronucleus of 350 F_2 hybrid eggs (obtained by mating C57BL/6×SJL hybrid adults) and the eggs were transferred into the oviducts of pseudopregnant recipients as described elsewhere[24]. Fifty-nine animals developed from the microinjected eggs; at the time of weaning, DNA was isolated from a piece of tail taken from these animals. The presence of the foreign MT-GRF gene was detected by hybridization to a human GRF cDNA clone[10] using conditions in which cross-hybridization with the mouse GRF gene is negligible. Fourteen animals carried the MT-GRF fusion gene; these animals were maintained on water with 25 mM $ZnSO_4$ to enhance expression of the fusion gene[15]. Table 1 summarizes the expression of the MT-GRF fusion gene in these 14 transgenic mice; 11 of the 14 animals express the MT-GRF messenger RNA in the liver, a major site of metallothionein expression[18]. The amount of the fusion mRNA in the livers of these mice varies by as much as 100-fold, and does not seem to be strongly correlated with the number of copies of the fusion gene in each animal. Animals expressing the fusion gene have measurable levels of human GRF in their serum, and have increased levels of serum growth hormone (Table 1). Ten of the mice showed significant increases in growth at 9 weeks old, and were 25–50% larger than control littermates. At maturity, some of the MT-GRF animals were nearly twice the size of control animals. Examination of the data in Table 1 reveals that there is no strong correlation between GRF levels, GH levels and growth.

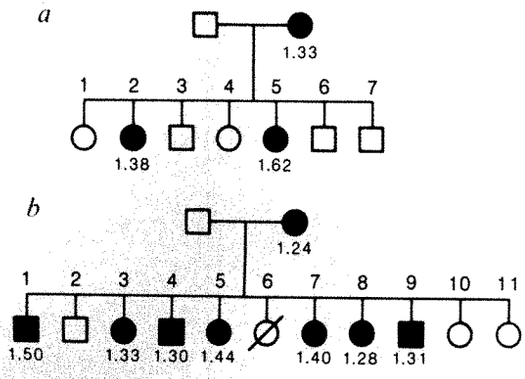


Fig. 2 Pedigree analysis of MT-GRF transgenic mice. Mice that carry the MT-GRF fusion gene (determined by DNA blotting of tail snips) are indicated by the solid symbols. Squares represent males and circles represent females. The animal number is given above each symbol; the numbers below the solid symbols indicate weight ratios at 9 weeks old compared with age- and sex-matched littermates. *a*, Pedigree of MT-GRF mouse ♀762-5; *b*, that of MT-GRF mouse ♀765-2. Mouse ♀765-2-6 died but was previously scored as a non-carrier.

To determine whether the MT-GRF fusion gene and the large size are heritable, two of the MT-GRF founder animals were bred with control animals; offspring from these matings were analysed for the presence of the MT-GRF fusion gene and for their rate of growth. Figure 2 shows two pedigrees demonstrating that both the fusion gene and the 'large' phenotype are inherited by ~50% of the total offspring. DNA analysis showed that all positive offspring carried the same number of copies of the fusion gene as the corresponding parent (data not shown). Four other females were also mated and produced litters. Thus, all six of the females tested were fertile (two were killed before breeding). In addition, male MT-GRF mice 760-5 and 801-9 (see Table 1) sired litters that included offspring carrying the fusion gene and displaying the large phenotype, indicating that both males and females carrying the MT-GRF fusion gene are fertile and transmit the gene. We now know that both the MT-GRF fusion gene and the large phenotype are inherited by ~50% of the offspring examined in the F_2 generation of animals.

To determine whether expression of the MT-GRF fusion gene in transgenic mice demonstrates a tissue specificity similar to that of the endogenous MT-I gene, we analysed both MT-I and MT-GRF RNAs in six tissues from either a control mouse or an MT-GRF mouse (female 765-2-3) (see F_1 in Fig. 2*b*); the results are presented in Fig. 3. The control animal expressed MT-I mRNA at high levels in liver, kidney, gut and pancreas, and at lower levels in brain and spleen. This agrees well with the known tissue distribution of MT-I mRNA[18]. The control animal did not express detectable hGRF mRNA in any tissue. Although the hGRF probe might detect endogenous mouse GRF mRNA in the brain, the low abundance of GRF mRNA even in the hypothalamus (<0.01%) makes detection unlikely. The transgenic mouse ♀765-2-3 showed a tissue distribution of MT-I mRNA much like that of the control mouse with one exception; the amount of liver MT-I mRNA was substantially reduced (Fig. 3). The reason for this is unclear, but it cannot be explained by RNA degradation and is a reproducible result in this animal. In the transgenic mouse, the MT-GRF fusion mRNA is expressed at high levels in the liver, gut and pancreas, at low levels in kidney and spleen, and was not detected in the brain (Fig. 3). Although the expression of the fusion gene in this animal demonstrates a tissue specificity similar to that of the mouse MT-I gene, there are clear and reproducible differences, most noticeably the low level of expression of MT-GRF mRNA in the kidney. We have recently examined the pattern of MT-GRF gene expression in a second pedigree (♂801-5-9) and have found that although the general pattern of tissue specificity parallels that of mouse MT-I, there are subtle differences particular to this animal and distinct from the MT-GRF animal ♀765-2-3 (data not shown). Similar results were obtained on examination of the tissue specificity of metallothionein/growth hormone fusion genes[15]. In this case, an extensive survey of multiple animals showed that expression varied among different tissues and different animals, suggesting that factors such as the site of integration probably influence expression of the foreign gene.

Although many tissues apparently express the MT-GRF mRNA and presumably synthesize the GRF precursor protein, we do not know which tissues are capable of proteolytically processing this precursor to generate the mature GRF peptide. The antibody used to detect plasma GRF would detect both the precursor and the mature peptide[10]. It seems likely that such processing must occur to some extent, as the free N-terminus of the GRF peptide is known to be required for biological activity[7,8]. We are presently using chromatographic techniques to determine what percentage of the immunoassayable GRF in the plasma is mature peptide as opposed to precursor.

Our results demonstrate that expression of supraphysiological levels of GRF in mice leads to increased somatic growth. Conversely, it has been shown that passive immunization of rats with antibodies against GRF can inhibit somatic growth[25]. Taken

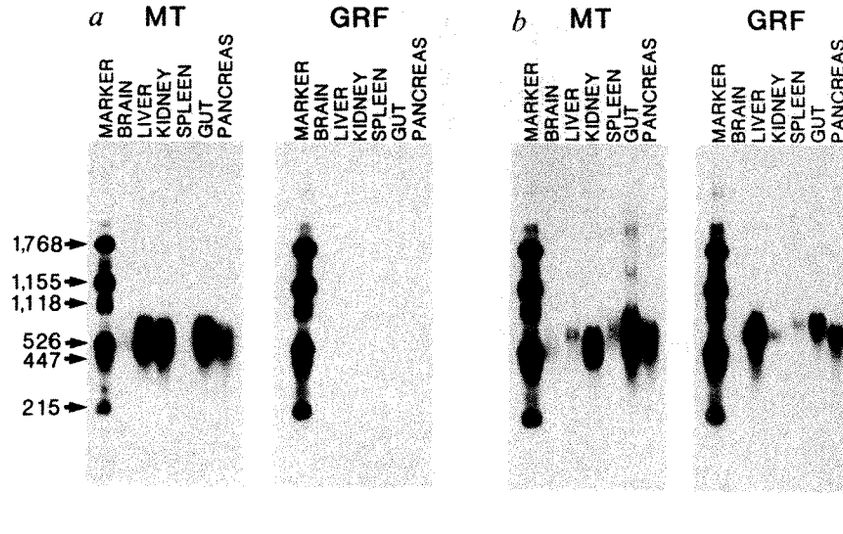


Fig. 3 Expression of human GRF mRNA in MT-GRF transgenic mice. *a*, Results from a control mouse; *b*, results from MT-GRF transgenic mouse ♀765-2-3. Each panel shows Northern blots of RNAs isolated from six different tissues. Duplicate blots from each animal were probed with either mouse MT-I (mMT-I) or hGRF probes as indicated. Size markers (in nucleotides) are denatured *Hin*dIII-cut simian virus 40 DNA.

Methods. Both animals were maintained on water with 76 mM $ZnSO_4$ for several weeks before killing. The indicated organs were removed and frozen at −70 °C until use. Total RNA was prepared by homogenization in guanidine isothiocyanate and centrifugation through caesium chloride as described[29]; 5 μg of each RNA was denatured and electrophoresed on formaldehyde/1.5% agarose gels[30] and the RNA transferred to nitrocellulose filters as described[31]. Filters were probed with either a mouse MT-I genomic clone, pSH[13], or a human GRF cDNA clone, phGRF-54 (ref. 10). Probes were labelled by nick-translation using [α-^{32}P]dCTP (NEN). Exposure time of the autoradiograms ~16 h.

together, these data suggest strongly that GRF has an important role in the promotion of growth. Transgenic mice carrying the MT-GRF fusion gene have elevated levels of plasma growth hormone and grow at a rate 25–50% greater than that of control littermates; this is significantly less than the increased growth observed in transgenic mice expressing growth hormone, many of which grow to twice the normal size[14,15]. However, in the transgenic animals expressing growth hormone, most body tissues express the gene and have the potential to make growth hormone, whereas in our experiments growth hormone is only made in the anterior pituitary and thus may be rate-limiting. An unexpected finding was that females expressing the MT-GRF fusion gene are fertile, although transgenic female mice expressing the growth hormone gene are generally infertile[16]; this suggests that the enhancement of growth in the MT-GRF females is more physiological, perhaps because the effect is mediated through endogenous somatotropes.

We examined the pituitaries of two MT-GRF transgenic animals that express the fusion gene at high levels (♀762-5 and ♀765-2-3). In both cases the pituitary was at least three times normal size and showed signs of marked hyperplasia. The condition of these animals is remarkably similar to the clinical findings in patients with certain types of human growth disorders characterized by elevated levels of growth hormone in serum and pituitary hyperplasia. Rather than pituitary adenoma, the more common cause of such symptoms, a tumour is found at a remote site, often a pancreatic or bronchial carcinoma[26–28]. These tumours do not produce growth hormone, yet on resection plasma GH levels fall. Such rare tumours are now recognized to be ectopic sources of GRF[7,8] and the MT-GRF transgenic mice seem to provide a good animal model for this class of growth disorders. The finding of pituitary hyperplasia suggests that GRF, in addition to regulating GH production, might have a role in somatotroph proliferation.

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Phagosome acidification blocked by intracellular *Toxoplasma gondii*

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***Toxoplasma gondii* belongs to a group of highly virulent intracellular parasites that reside in host cell vacuoles which resist typical phagosome–lysosome fusion[1]. Live *Toxoplasma* replicate prodigiously within modified phagocytic vacuoles formed during invagination of the host plasma membrane[2,3]. In contrast, heat-killed *Toxoplasma* or specific antibody (heat-inactivated)-coated live *Toxoplasma*-containing vacuoles readily undergo lysosome fusion and digestion in normal macrophages[2,4]. Of newly recognized significance to *Toxoplasma* survival is the microbicidal effect of phagosome acidification, which reportedly can occur independently of fusion with other acidic vesicles[5–8]. We report here that modified live *Toxoplasma*-containing vacuoles fail to acidify in normal macrophages, as indicated by the sensitive *p*H probe fluorescein. In contrast, when live *Toxoplasma* are coated with specific antibody (heat-inactivated), they trigger phagosome acidification when entering normal macrophages. A similar acidification is observed when normal phagocytes ingest dead *Toxoplasma*. Extracellular *Toxoplasma* are highly susceptible to acidic *p*H conditions, indicating that the acidification block in the modified vacuoles may be important for intracellular survival.**

The endogenous acidification of macrophage phagosomes may involve two independent mechanisms that operate before acidic vesicle fusion. First, NADPH-oxidase activity, associated with the phagocytic respiratory burst, forms superoxide radicals and delivers H^+ ions to the phagosome[5]. Second, phagosome acidification may involve the Mg-ATPase H^+-ion pump identified in pinosomes[9] and ligand-receptor endosomes[10–15] also formed by plasma membrane invagination. Given the potential for similar acidic changes in newly formed phagosomes[6] and the *p*H susceptibility of *Toxoplasma*[16,17], we investigated how the *in situ p*H of macrophage vacuoles containing *Toxoplasma* affected intracellular survival.

The fate of live compared with heat-killed or antibody-coated *Toxoplasma* in normal or activated mouse peritoneal macrophages was evaluated microscopically at intervals after infection *in vitro*. Live *Toxoplasma* here refers to extracellular parasites freshly collected from mouse ascites[18], representing the maximum viability obtainable. Acridine orange was used to prelabel acidic cell compartments, including lysosomes of macrophages cultured on coverslips[19]. One hour after infection, 80% of live *Toxoplasma*-containing vacuoles blocked fusion of acridine orange-labelled vesicles in normal macrophages. However, 86% of heat-killed (60 °C for 10 min) or antibody (heat-inactivated)-coated live *Toxoplasma* vacuoles fused with acridine orange-labelled vesicles within 1 h of phagocytosis by normal macrophages. In activated macrophages from immune mice[9] which were cultured *in vitro* for 8 h, 85% of live *Toxoplasma* vacuoles fused with acridine orange compartments, were digested, and thus resemble heat-killed or antibody-coated *Toxoplasma* vacuoles.

To evaluate the cidal effect of acidic *p*H, extracellular *Toxoplasma* were incubated in media at fixed *p*H values and then evaluated for their ability to survive in host cells. The viability of live *Toxoplasma* was reduced to $\leq$60% of control after incubation in media at *p*H 6.0, as evaluated by plaque formation on fibroblast monolayers[20] and fusion with acridine orange-labelled vesicles in normal macrophages (Fig. 1).

Having established the high susceptibility of extracellular *Toxoplasma* to acidic *p*H, we examined the *in situ p*H of modified

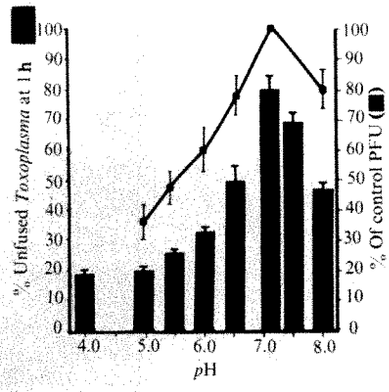


Fig. 1 Viability of *Toxoplasma* as evaluated by ability to block fusion of acidic vesicles (labelled with acridine orange) in macrophages (bars) and plaque formation on fibroblast monolayers (line) (expressed as plaque-forming units, PFU). Significant reduction in *Toxoplasma* viability was observed by both methods when extracellular *Toxoplasma* were exposed to media of *p*H 6.0.

Methods. RH strain *Toxoplasma* tachizoites (asexually replicating stages from acute infections in mice) were collected in Hank's balanced salt solution (HBSS) *p*H 7.2 (ref. 18). After 10 min incubation in isotonic HBSS adjusted to *p*H values from 4.0 to 8.0 (Orion Research millivolt *p*H meter 611), *Toxoplasma* were returned to HBSS at *p*H 7.2 and used for viability assays within 30 min. Normal mouse peritoneal macrophages were cultured in endotoxin-free ($\leq$0.01 ng ml^{-1}, Limulus Amebocyte Assay, Associates of Cap Cod Inc.) RPMI-1640 with 10% heat-inactivated fetal calf serum (CRMPI, Gibco) on 12-mm-diameter glass coverslips in 24-well plates for 18 h before use. The extent of fusion in normal macrophages labelled with acridine orange[19] was evaluated 1 h after *Toxoplasma* infection ($n = 3$)[25]. Plaque formation on human fibroblast monolayers[20] was evaluated after 7 days and expressed as mean % control (*p*H 7.2) from triplicate 25-cm^2 flasks.

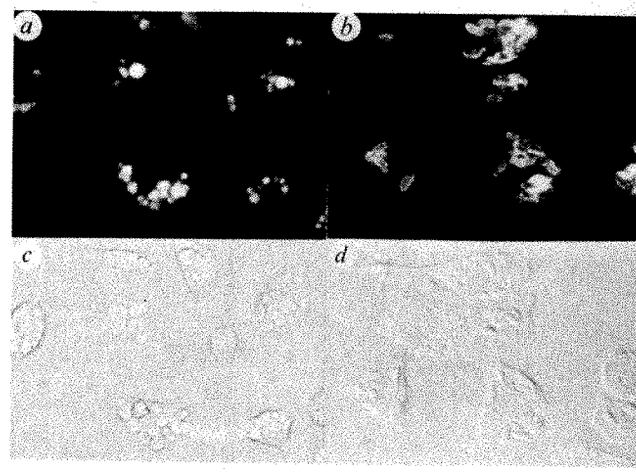


Fig. 2 Macrophage monolayers containing fluorescein-labelled *Toxoplasma*-containing vacuoles photographed on a Leitz Ortholux II epifluorescence microscope using a Nikon HFX 35-mm camera on Kodak Tri-X panchromatic film. *a*, Epifluorescence; *b*, phase contrast; *c*, epifluorescence; *d*, differential interference contrast. Monolayers were pulsed for 5 min with live or heat-killed *Toxoplasma* in CRPMI containing 2 mg ml^{-1} fluorescein dextran (relative molecular mass 20,000; Sigma), rinsed extensively in phosphate-buffered saline (PBS) and recultured. Approximately 80% of phagocytic vacuoles contained visible fluorescein dextran which did not affect block of acridine orange-labelled vesicle fusion or *Toxoplasma* replication in normal macrophages. Spectrofluorometry measurements indicated that pinocytosis of fluorescein dextran accounted for 10% of the total label internalized during *Toxoplasma* infection. *Toxoplasma* labelled with rabbit IgG and FITC-goat anti-rabbit (Sigma) were internalized by macrophage monolayers during 1 min infection challenge.

and heat-killed or antibody-coated *Toxoplasma* vacuoles in macrophage monolayers. *In situ* *p*H measurements were estimated from a standard curve relating fluorescence intensity (I_0) of intracellular fluorescein-labelled *Toxoplasma* to fixed *p*H values (s.e. = $\pm$0.15 unit; see Fig. 3*a* legend). The excitation spectrum of fluorescein is sensitive to *p*H changes between 4.0 and 8.0 and is independent of fluorochrome concentration when expressed as the I_0 (520 nm) ratio of excitation at 495/450 nm (ref. 21).

Modified live *Toxoplasma*-containing vacuoles were labelled using the fluid phase marker fluorescein dextran present in the media during infection with *Toxoplasma* (Fig. 2*a*, *b*). Normal macrophage monolayers containing modified vacuoles labelled with fluorescein dextran showed a slight decrease in I_0 during the first hour after infection (Fig. 3*b*). This slight acidification from *p*H 7.2 to 6.8 is probably due to the 20% of *Toxoplasma* which fuse with acidic cell compartments and the small amount of fluorescein dextran pinocytosed by macrophages during infection (see Fig. 2 legend). Most fluorescein dextran in the modified vacuoles did not undergo decreases in I_0, demonstrating that live *Toxoplasma* reside in neutral *p*H vacuoles.

The acidification of heat-killed or antibody-coated *Toxoplasma* vacuoles, which are destined to fuse with lysosomes, was evaluated in macrophages challenged with either heat-killed *Toxoplasma* in the presence of fluorescein dextran (Fig. 2*a*, *b*) or fluorescein isothiocyanate (FITC)-antibody-coated *Toxoplasma* (Fig. 2*c*, *d*). Normal macrophage vacuoles containing heat-killed *Toxoplasma* and fluorescein dextran underwent an extensive fall in *p*H from 7.2 to 5.2 after 1 h (Fig. 3*b*). Consistent with their intracellular fate, a similar rapid acidification of these vacuoles was observed with FITC-antibody-coated live *Toxoplasma* in normal macrophages (Fig. 3*b*). Of considerable interest, live *Toxoplasma* labelled with fluorescein dextran underwent a rapid acidification in activated macrophages (Fig. 3*b*). We did not detect the previously reported transient alkalinization to *p*H 7.4 at 2–3 min post-phagocytosis, although this may be because we used external media at *p*H 7.2 rather than 6.8 (ref. 6).

The kinetics of acidification of heat-killed or antibody-coated *Toxoplasma* vacuoles (Fig. 3*b*) indicates that a rapid shift to acidic *p*H is initiated within the first 3–5 min, starting from *p*H 7.2 and falling to *p*H 5.6 at 15 min post-phagocytosis. This rapid acidification rate appears similar to that of other reports on phagosomes of macrophages[6] and *Amoeba*[22]. During the initial 15 min post-phagocytosis, the rate of *p*H change in phagosomes is significantly higher than the rate of fusion with acidic compartments prelabelled with acridine orange (Fig. 4). At 15 min, when acidification is 70% complete, 65% of the vacuoles still show no evidence of acridine orange vesicle fusion (Fig. 4). This pattern is consistent with the model that phagocytic vacuole acidification precedes fusion with other acidic vesicles, including lysosomes[8].

Several mechanisms may be involved in the rapid acidification of compartments of heat-killed or antibody-coated *Toxoplasma* vacuoles. First, acidification may represent fusion of cytoplasmic vesicles which deliver proton-pumping ability to the phagosome, such as acidisomes described in *Paramecium*[23]. However, this explanation would only fit the data in Fig. 4 if the delivery vesicles are not active before vacuole fusion and thus not labelled with acridine orange. In this case, the failure of acidification of live *Toxoplasma* vacuoles represents a block of delivery-vesicle fusion. Alternatively, the observed differences in acidification may result from the degree of endogenous NADPH-oxidase and Mg-ATPase H^+-ion pump activity within the phagosome.

We have demonstrated here that live *Toxoplasma*-containing vacuoles show little capacity for acidification whereas both

antibody-coated and heat-killed *Toxoplasma* are internalized by normal macrophages into phagocytic vacuoles with an inherent ability to acidify. Activated macrophages challenged with live *Toxoplasma* also form phagocytic vacuoles with a marked ability to acidify. Similarly, the entry of live *Toxoplasma* into normal macrophages does not stimulate the oxygen radical production which is typical of phagocytosis of antibody-coated *Toxoplasma* by normal macrophages and of live *Toxoplasma* phagocytosed by activated macrophages[18]. These two observations suggest that differences in plasma membrane enzyme activation during formation of live as opposed to heat-killed or antibody-coated *Toxoplasma* vacuoles are responsible for their ultimate intracellular fate.

Although Fc and C3b receptors may trigger acidification during phagocytosis of antibody-coated *Toxoplasma*, acidification resulting from ingestion of heat-killed *Toxoplasma* by normal macrophages and live *Toxoplasma* by activated macrophages must be initiated by an alternative receptor, as antibody was not present in these preparations. Initiation is either not triggered when live *Toxoplasma* enter normal macrophages, or is inhibited, as demonstrated by the failure of modified live *Toxoplasma*-containing phagosomes to acidify. A similar pattern of variable extent of endosome acidification occurs in ligand-receptor endosomes, where *p*H values range from 6.4 to 5.0 depending on the receptor involved[12–15]. Although *Toxoplasma* show a specific attachment phase before entry[2–4], neither the receptor involved in this step nor the distribution of other cell-surface receptors within the phagosome have been characterized.

The significance of phagosome acidification to intracellular *Toxoplasma* is paramount to survival, judging from their high susceptibility to mild acidic conditions when they are extracellular. Thus, the failure of acidification exhibited by live *Toxoplasma*-containing vacuoles in normal macrophages enables *Toxoplasma* to survive within cells normally considered microbicidal. In support of this hypothesis, two other intracellular parasites which resist lysosome fusion have been shown to reside in neutral (*p*H = 6.9)[7] or weakly acidic (*p*H 6.1)[24] compartments in normal macrophages. Increased capacity for phagosome acidification in activated macrophages and in normal macrophages in the presence of specific antibody probably contributes to the destruction of intracellular *Toxoplasma*.

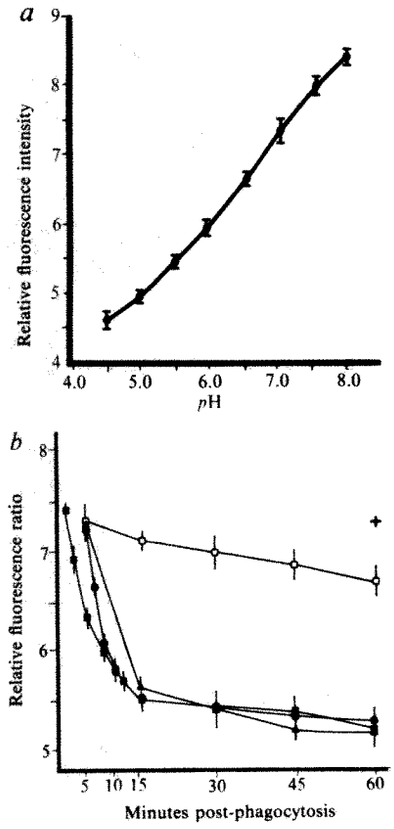


Fig. 3 *a*, *In situ* standard curve relating fluorescence intensity (I_0) ratio of intracellular FITC-labelled *Toxoplasma* to fixed *p*H values. *b*, Fluorescence intensity ratios from macrophage monolayers containing fluorescein-labelled *Toxoplasma* vacuoles at intervals after phagocytosis. A decrease in I_0 represents a shift in the intravacuolar *p*H to acidic conditions (*a*). A slight decline in I_0 is evident during the first hour after infection from modified live *Toxoplasma*-containing vacuoles in normal macrophages (□), probably due to the 20% of *Toxoplasma* in this preparation which fuse with acidic vesicles. In contrast, a rapid decrease in I_0 is observed from macrophage vacuoles containing heat-killed *Toxoplasma* and fluorescein dextran (▲), and FITC-antibody-labelled live *Toxoplasma* (■) in normal macrophages and from live *Toxoplasma* and fluorescein dextran (●) in activated macrophages.
Methods. *a*, Macrophage monolayers containing FITC-*Toxoplasma* (see Fig. 2*a*, *b*) were incubated in sodium citrate (*p*H 4.0, 4.5, 5.0) or PBS (*p*H 5.5, 6.0, 6.5, 7.0, 7.5, 8.0) containing 10 μm monensin to collapse cell *p*H gradients and clamp intracellular compartments at the fixed *p*H of the external buffer. Fluorescence intensity was recorded from macrophages attached to coverslips placed in 1-cm cuvettes using an Aminco-Bowman spectrofluorometer with 10-nm bandwidth excitation (altered between 450 and 495) and 5-nm bandwidth emission centred at 520 nm, $n = 6$ (ref. 21). Correction for background scatter and autofluorescence was made by subtraction of I_0 readings from a similar set of monolayers which did not contain fluorescein. *b*, Infected macrophages labelled with fluorescein (cell density 2×10^6 per coverslip; 2–5 *Toxoplasma* per host cell) were rinsed in PBS and I_0 recorded in PBS at 37 °C. Corrected values were obtained by subtracting background fluorescence readings from unlabelled infected monolayers. A separate set of three coverslips was used to determine mean I_0 ratio for each time point. Intracellular acidification of heat-killed or antibody-coated *Toxoplasma*-containing vacuoles was reversible by addition of 10 μM monensin in PBS at *p*H 7.2 (+), but was not affected by changes in external buffer *p*H in the absence of ionophore.

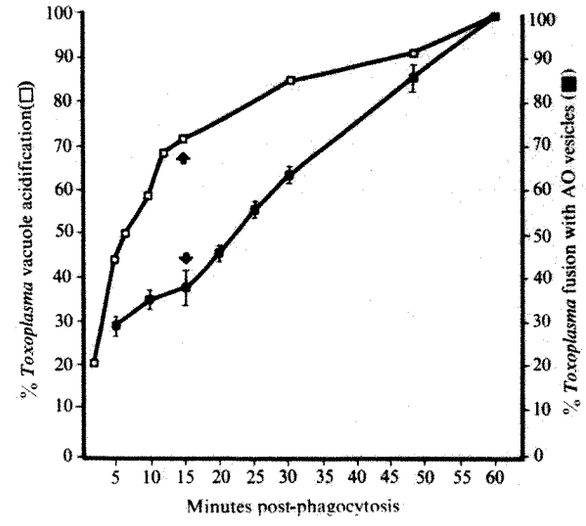


Fig. 4 Comparison of the rate of fusion of acidic vesicle (labelled with acridine orange, AO) (■) with *p*H changes (□) in normal macrophage containing heat-killed or antibody-coated *Toxoplasma*. Most acidification occurs before the fusion of acidic vesicles including lysosomes. The % of total acidification is calculated from I_0 data points in Fig. 3*b* representing FITC-labelled *Toxoplasma* ingested during a 1-min pulse. The extent of acidic vesicle fusion was obtained from normal macrophages labelled with acridine orange and challenged similarly with FITC-*Toxoplasma*. Three separate coverslips were used to score each time point. Similar kinetic curves for fusion rate were obtained with heat-killed *Toxoplasma* in normal macrophages and live *Toxoplasma* in activated macrophages.

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Mitogens trigger a calcium-independent signal for proliferation in phorbol-ester-treated lymphocytes

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The activation of T lymphocytes by mitogens requires at least two signals; the first, delivered to T cells by a mitogen in conjunction with accessory cells (monocytes/macrophages), leads to the generation of the second signal, interleukin-2 (IL-2). The first signal also induces the expression of IL-2 receptors on the surface of a subpopulation of T cells; binding of IL-2 to its receptor then initiates a cascade of events culminating in DNA synthesis by these cells[1-4]. Certain compounds act synergistically with mitogens in promoting T-cell proliferation by substituting for the activities of interacting cells or their products. For example, the phorbol ester 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) has been shown to restore the ability of macrophage-depleted T-cell populations to respond to mitogenic lectins[5-7]. Transmembrane fluxes of calcium, leading to increased free cytosolic calcium concentrations ($[Ca^{2+}]_i$), have been demonstrated following mitogen binding to lymphocytes and have been implicated in the initiation of cell proliferation[8-10]. We show here that the effect of TPA on lymphocyte proliferation occurs in the absence of extracellular Ca^{2+} or detectable changes in $[Ca^{2+}]_i$, but only in the presence of mitogens. This suggests that in cells which have been incubated with the phorbol ester, mitogens can induce proliferation by a calcium-independent signal.

Mitogenic lectins such as phytohaemagglutin (PHA) and concanavalin A, and non-mitogenic lectins such as wheat germ agglutinin, lead to a rapid rise in $[Ca^{2+}]_i$ even when there is no proliferative response[9], and this hampers attempts to evaluate the requirement for an increase in $[Ca^{2+}]_i$ in the initiation of cell proliferation. However, we have shown previously that, in contrast to PHA-induced activation of monocyte-depleted human T cells, the T-cell mitogen[11] protein A (SpA from *Staphylcoccus aureus*, Cowan I strain) does not produce changes in $[Ca^{2+}]_i$ or DNA synthesis in the absence of monocytes[12]. Monocytes preincubated with SpA trigger a rapid increase in $[Ca^{2+}]_i$ as well as a proliferative response in these isolated T cells. We therefore used this sytem to determine whether TPA can serve as a co-mitogen and whether the effect of TPA on SpA-induced proliferation of isolated human T cells (in the absence of monocytes) requires, or is accompanied by, changes in $[Ca^{2+}]_i$.

Table 1 illustrates the effects of PHA and SpA on $[Ca^{2+}]_i$ monitored by quin 2 fluorescence during the first 24 h and by the proliferative response measured using ^{3}H-thymidine incorporation at 72 h. Incubation of a mixed population of peripheral blood mononuclear cells (PBM) with PHA or SpA resulted in significant incorporation of ^{3}H-thymidine and increases in $[Ca^{2+}]_i$. The response to PHA was rapid, $[Ca^{2+}]_i$ more than doubling within 10 min, then falling progressively and reaching

Table 1 Mitogen-dependent effects on levels of cytoplasmic Ca^{2+} and lymphocyte proliferation

	$[Ca^{2+}]_i$ (nM)				^{3}H-thymidine incorporation (c.p.m.)
	0	10 min	3 h	24 h	
PBM + PHA	108 ± 2.0	235 ± 8.0	187 ± 7.4	114 ± 3.1	44,090 ± 2,670
PBM + SpA	110 ± 2.7	128 ± 12.4	180 ± 5.5	114 ± 3.8	22,165 ± 1,860
E^+ + PHA	106 ± 2.0	245 ± 7.8	186 ± 2.4	116 ± 3.5	62,210 ± 2,110
E^+ + SpA	106 ± 2.4	110 ± 5.3	113 ± 3.4	114 ± 3.0	4,520 ± 760

Peripheral blood mononuclear cells (PBM) were isolated following Ficoll-Hypaque gradient centrifugation. Erythrocyte-rosetting T cells (E^+) were obtained by rosette enrichment as described elsewhere[23]. Cells (200 μl, 5×10^5 ml^{-1}) were cultured with PHA (10 μg ml^{-1}, Difco) or SpA (100 μg ml^{-1}, Pharmacia for 72 h in RPMI-1640 containing 10% fetal calf serum. Four hours before the end of the culture, 1 μCi ^{3}H-thymidine (6.7 Ci mmol^{-1}) was added; labelled DNA was counted in a liquid scintillation counter. The mitogens were added at time 0 and $[Ca^{2+}]_i$ levels recorded at 10 min, 3 h and 24 h. For monitoring $[Ca^{2+}]_i$, 10×10^6 cells ml^{-1} were incubated with 10 μM acetoxymethyl quin-2 (generously provided by Dr T. J. Rink, and prepared as a 2 mM stock solution in dimethyl sulphoxide) as described previously[10]. The cells were incubated with quin-2 before each assay point. A Perkin-Elmer spectrofluorimeter (Model 650-40) was used for fluorescence measurements, for which the cells were suspended in HEPES-buffered saline containing 1 mM Ca^{2+} (ref. 10). Calculation of free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was as described previously[10,24]. Although reported to affect the response of lymphocytes to mitogen[9], the concentration of quin-2 used here was shown to be non-mitogenic and did not significantly interfere with mitogen-induced proliferation. Results represent the means ±s.e.m. of three separate experiments carried out in duplicate (Ca^{2+} determinations) or triplicate (^{3}H-thymidine incorporation). In the absence of mitogen, background c.p.m. were <1,000 and $[Ca^{2+}]_i$ was 106-112 nM.

Table 2 TPA modulation of mitogen-induced changes in levels of cytosolic free Ca^{2+} and lymphocyte proliferation

	$[Ca^{2+}]_i$ (nM)				^{3}H-thymidine incorporation (c.p.m.)
	0	10 min	3 h	24 h	
TPA (16 h)	112 ± 3.0	112 ± 3.2	110 ± 2.5	112 ± 2.5	3,100 ± 270
PHA	111 ± 2.3	236 ± 5.6	191 ± 5.3	—	40,530 ± 1,350
TPA (16 h) →PHA	108 ± 1.2	246 ± 7.6	204 ± 4.2	—	52,400 ± 5,790
PHA	114 ± 3.2	260 ± 6.2	201 ± 3.2	118 ± 4.6	34,870 ± 2,480
TPA (1 h) →PHA	108 ± 3.2	256 ± 8.2	202 ± 3.5	120 ± 5.2	47,980 ± 2,870
SpA	109 ± 2.4	112 ± 1.6	112 ± 4.0	—	4,040 ± 670
TPA (16 h) →SpA	112 ± 1.5	120 ± 5.6	115 ± 5.1	—	23,110 ± 3,160
SpA	105 ± 3.2	110 ± 6.4	116 ± 6.2	114 ± 5.2	2,250 ± 690
TPA (1 h) →SpA	116 ± 2.1	118 ± 2.6	120 ± 3.6	122 ± 5.2	20,570 ± 1,160

Human E-rosetting T cells (10×10^6 ml^{-1}) were incubated with TPA (10^{-9} M) (Sigma) for 1 or 16 h in RPMI-1640 plus 10% fetal calf serum. The cells were then washed three times in RPMI containing defatted bovine serum albumin (BSA, Sigma) and twice with RPMI to remove residual TPA[25]. Aliquots of the cells were then either loaded with quin-2 for $[Ca^{2+}]_i$ determinations or used in studies of lymphocyte proliferation (see Table 1). Results represent the means ±s.e.m. of three separate experiments.

Table 3 Mitogen-dependent changes observed in the presence of EGTA

Expt *a*

Initial incubation	Second incubation	^{3}H-thymidine incorporation (c.p.m.)
Medium	Medium	1,100 ± 200
Medium	TPA	2,100 ± 230
Medium	PHA	22,840 ± 3,240
Medium	TPA → PHA	25,110 ± 2,910
EGTA	TPA	2,190 ± 270
EGTA	PHA	4,220 ± 730
EGTA	TPA → PHA	24,790 ± 2,720
Medium	SpA	4,570 ± 660
Medium	TPA → SpA	16,200 ± 570
EGTA	SpA	5,870 ± 320
EGTA	TPA → SpA	18,500 ± 1,080

Expt *b*

	$[Ca^{2+}]_i$ (nM)		
Initial incubation	Second incubation		
Medium	106 ± 2.0	PHA	245 ± 7.8
Medium	112 ± 3.0	TPA	112 ± 3.2
TPA	108 ± 3.2	PHA	256 ± 8.2
EGTA	102 ± 6.6	TPA	103 ± 5.2
EGTA	105 ± 3.0	PHA	107 ± 2.8
EGTA → TPA	108 ± 2.6	PHA	108 ± 3.2

E-rosetting T cells were used in these studies. Expt *a*): Determination of ^{3}H-thymidine incorporation. In the initial incubation, 5×10^5 cells ml^{-1} were incubated with medium containing EGTA (2 mM) or medium (RPMI-1640) alone for 30 min at 37 °C. In the second incubation, TPA (10^{-9} M) was added where indicated for 1 h, followed by the addition of mitogen for 1 h at 37 °C. Only then were the cells washed three times with RPMI-containing defatted BSA and twice in RPMI. Aliquots (200 μl) of cells (5×10^5 ml^{-1}) were then incubated for 72 h in RPMI-1640 plus 10% fetal calf serum. Previous studies have shown that a 1-h incubation with PHA, followed by cell washing, results in 80–90% of the proliferative response observed when PHA is left in culture for the entire 72 h[16]. Results represent the means ±s.e.m. of three experiments carried out in triplicate. Expt *b*: Determination of $[Ca^{2+}]_i$ levels. Initial incubation: cells were first incubated with quin-2 (10 μM) for 60 min, washed, then incubated with medium containing EGTA (2 mM) or medium only (see Table 1) for 30 min at 37 °C. TPA (10^{-9} M) was then added for 1 h as indicated. Baseline levels of $[Ca^{2+}]_i$ were obtained. In the second incubation, PHA (or TPA) was added and $[Ca^{2+}]_i$ levels determined 10 min later. Results represent the means ±s.e.m. of three separate experiments carried out in duplicate.

baseline levels after 24 h. The response to SpA was somewhat slower, with a period of 2–3 h required to reach maximum $[Ca^{2+}]_i$ levels. This delay probably reflects the requirement for monocyte/T-cell interaction in the response to SpA[12]. Purified T cells (E^+) respond to PHA in a similar manner to the unfractionated PBM, with both a rapid increase in $[Ca^{2+}]_i$ and a greater proliferative response. In contrast, as previously reported[12], incubation of purified T cells with SpA resulted in a greatly reduced ^{3}H-thymidine incorporation and no significant change in $[Ca^{2+}]_i$.

TPA is the most potent of the group of phorbol diesters known to promote tumours or induce cellular differentiation[13]. By itself, TPA (10^{-9} M) is weakly mitogenic but does not lead to changes in $[Ca^{2+}]_i$ (ref. 8 and Table 2). We found that preincubation of cells with TPA for 1 or 16 h, followed by the addition of PHA, produced a greater proliferative response than observed with cells not preincubated with TPA. The increase in cytosolic free Ca^{2+} following addition of PHA was similar with or without TPA pretreatment.

When purified T cells were incubated with TPA for 1 or 16 h, they subsequently exhibited a proliferative response to SpA (Table 2); the magnitude of ^{3}H-thymidine incorporation was similar to that seen with PBM (Table 1). In the presence of SpA, $[Ca^{2+}]_i$ levels were only marginally higher in cells preincubated with TPA than in cells exposed to SpA alone; monitoring of $[Ca^{2+}]_i$ indicated little change in levels during the 24-h period following addition of SpA. These results indicate that preincubation enables T cells to proliferate in response to SpA without an accompanying rise in $[Ca^{2+}]_i$.

In monocyte-dependent systems, TPA can often restore the proliferative response to mitogens in monocyte-depleted cell populations[4–6]. Although TPA can augment the production of interleukin-1 (a monocyte-derived lymphokine)[14,15] the effects of TPA described in Table 2 are monocyte-independent and the drug seems to act directly on purified T cells. These effects of TPA on SpA-induced T-cell proliferation can be easily distinguished from those observed following addition of monocytes; in the presence of monocytes, $[Ca^{2+}]_i$ increased significantly (Table 1, PBM) whereas with TPA pretreatment of purified T cells there was little deviation from baseline $[Ca^{2+}]_i$ following the addition of SpA (Table 2).

We have shown previously that triggering of a PHA-induced proliferative response is dependent on the presence of extracellular Ca^{2+} (ref. 16). We therefore investigated whether TPA treatment would produce a PHA-induced proliferative response in the absence of raised $[Ca^{2+}]_i$. We found that, in contrast to cells in Ca^{2+}-containing medium, T lymphocytes treated with the Ca^{2+} chelator EGTA and then incubated with PHA for 1 h in EGTA-containing medium, failed to incorporate ^{3}H-thymidine when returned to normal (and lectin-free) medium for the remaining 72 h (Table 3, expt *a* and ref. 16). Cells treated similarly, except for preincubation with TPA, demonstrated a response to PHA (and SpA). As the TPA preincubation was carried out with EGTA-treated cells in EGTA-containing medium, this result suggests that the TPA effects do not require extracellular Ca^{2+}, in contrast to previous reports[17]. Cells pretreated with EGTA and incubated with TPA and PHA in EGTA-containing medium showed no increase in $[Ca^{2+}]_i$ (Table 3, expt *b*), but did show an increase in ^{3}H-thymidine incorporation (Table 3, expt *a*).

The importance of increases in Ca^{2+} as a signal for mitogen-induced proliferation has been demonstrated in several different systems (reviewed in ref. 18), although the nature of the Ca^{2+} signal and the target for its activity are unknown. On the other hand, TPA is known to be co-mitogenic for lymphocytes and may act by directly activating protein kinase C, leading to protein phosphorylation[19–22]. The present report, as well as previous studies[8], have shown that TPA does not mobilize Ca^{2+} as measured directly using the fluorescent indicator quin-2. We used two independent systems, one in which changes in $[Ca^{2+}]_i$ do not occur in response to mitogen alone and one in which changes in $[Ca^{2+}]_i$ were prevented by the presence of EGTA. In both systems, following treatment with TPA, T lymphocytes become able to proliferate in response to mitogens. As TPA, an activator of protein kinase C, is itself non-mitogenic, this suggests that another, calcium-independent, signal can be generated by the mitogen; the nature of this signal remains to be elucidated.

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Sequence of reovirus haemagglutinin predicts a coiled-coil structure

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The use of modern techniques has led to new insights into the molecular mechanisms of viral pathogenesis[1]. Although the infectious process is quite complex, it is clear that one critical stage, the interaction of viral attachment proteins with cell-surface receptors, often has a major role in determining the pattern of infection[2]. The mammalian reoviruses have served as useful models for understanding the molecular basis of viral pathogenesis[2]. The mammalian reovirus haemagglutinin (σ1 protein), which is an outer capsid protein, has been shown to be a major factor in determining virus–host cell interactions[2]. To further our understanding of the structure and function of the haemagglutinin, we have cloned a complementary DNA copy of the reovirus type 3 *S1* double-stranded RNA gene which encodes the virus haemagglutinin and have sequenced the DNA complementary to the *S1* gene. Analysis of the predicted amino-acid sequence of the virus haemagglutinin has allowed us to determine that the amino-terminal portion contains an α-helical coiled-coil structure and that the carboxy-terminal portion contains the receptor-interacting domains. Using this information, we propose here a model of how the reovirus haemagglutinin is attached to the virus particle.

Figure 1 shows the nucleotide sequence of the entire reovirus type 3 *S1* gene. The gene consists of 1,416 nucleotide bases and has two open reading frames. The first open reading frame begins at nucleotide 13 and terminates at nucleotide 1,377, encoding a protein of 455 amino acids and computed relative molecular mass of 49,071. This protein corresponds to the reovirus haemagglutinin (σ1 protein) as determined by the previously estimated sizes[3,4].

The predicted amino-acid sequence of the virus haemagglutinin contains three potential glycosylation sites (see Fig. 1). Although it has been reported that the haemagglutinin of reovirus grown in the L strain of mouse fibroblasts can incorporate radiolabelled amino-sugars at a low level[5], there has been no confirmation that the three potential sites are glycosylated in the haemagglutinin.

A second open reading frame in the *S1* gene begins at nucleotide 71 and terminates at nucleotide 430 and would encode a polypeptide of 120 amino acids. The presence of two open reading frames agrees with both ribosome binding studies[6] and *in vitro* translation studies[7] which conclude that the *S1* gene has two initiation sites. Recently, the 120-amino-acid protein has been detected in reovirus-infected cells[8].

Examinations of the amino-acid sequence of the reovirus type 3 haemagglutinin reveals certain features, in particular, a heptapeptide repeat pattern (*a–b–c–d–e–f–g*) between amino acids 28 and 158, representing approximately one-third of the molecule at the amino-terminal end. In this pattern, amino acids at positions *a* and *d* are primarily hydrophobic (Table 1). Another striking feature of the sequence is that there are two proline residues flanking this heptapeptide repeat at amino acid positions 3 and 176, but no prolines within the heptapeptide repeat pattern. The heptapeptide repeat, the position of the hydrophobic amino acid and the absence of both proline and aromatic amino acids from the heptapeptide repeat are typical of an α-helical coiled-coil structure where the hydrophobic residues at positions *a* and *d* form the interface between the α-helices[9,10]; this agrees with computer analysis of protein secondary-structure predictions[11] which shows this area to

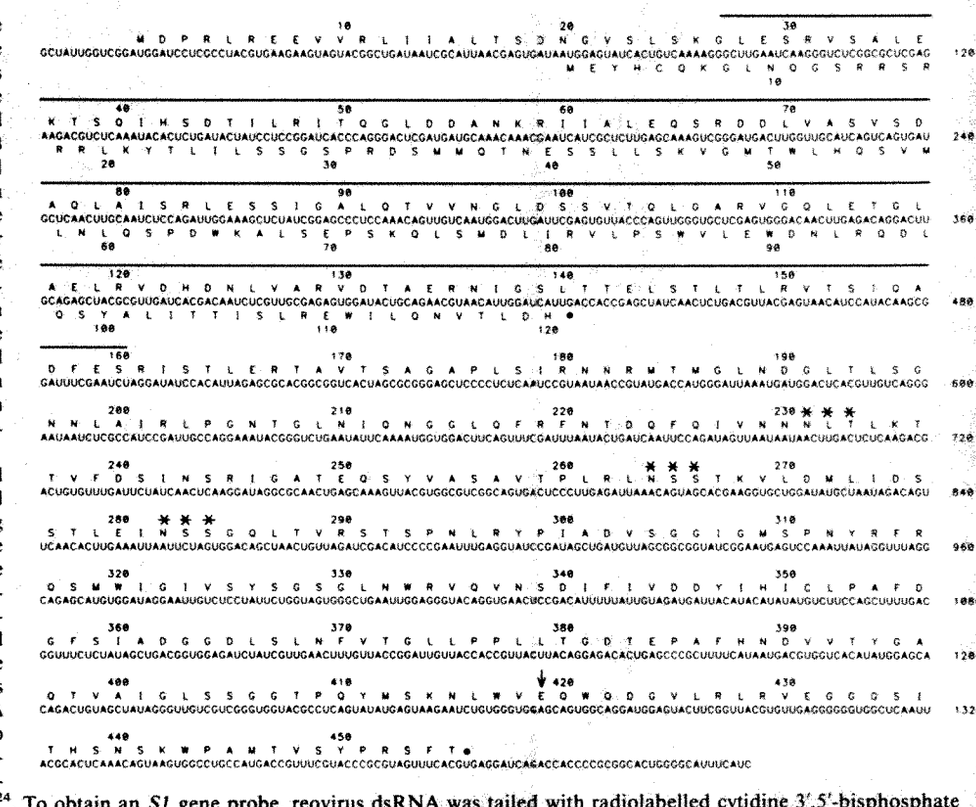
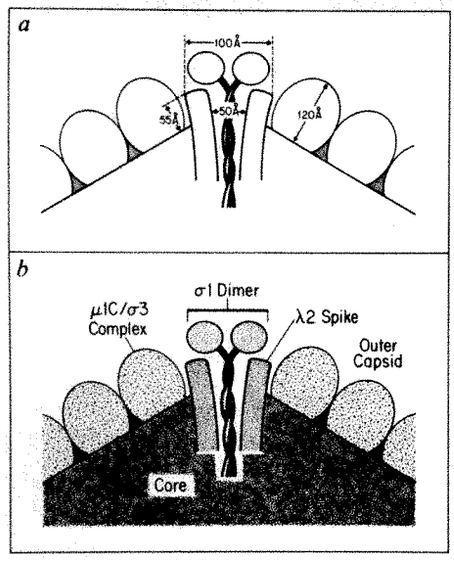


Fig. 1 The determined nucleotide sequence of reovirus type 3 *S1* gene and predicted amino-acid sequences for both open reading frames. The one-letter amino-acid notation used is recommenced by the IUPAC-IUB Commission on Biochemical Nomenclature[22]. The numbers given above the amino-acid sequence designate the residue positions for the first open reading frame. The numbers on the right show nucleotide positions. The α-helical region is indicated by a thick line above the amino-acid residues. The potential glycosylation sites are marked by an asterisk. The mutation site for an immune-selected variant (K) is indicated by an arrow.

Methods. Genomic double-stranded (ds) RNA was isolated from purified reovirus type 3 (Dearing) and, using the enzyme poly(A) polymerase (BRL), ~30 AMP molecules were added to the 3′ termini[23]. The polyadenylated RNA was used as a template to synthesize oligo(dT)-primed single-stranded (ss) cDNA[24]. The complementary ss cDNA fragments were hybridized to obtain ds cDNA fragments which were inserted into pBR322 by G-C tailing[25]. The recombinant plasmids were used to transform *Escherichia coli* MC1061 cells[24]. To obtain an *S1* gene probe, reovirus dsRNA was tailed with radiolabelled cytidine 3′,5′-bisphosphate using T_4 RNA ligase[26]. The radiolabelled *S1* dsRNA was then used to screen the cDNA library. A full-length cDNA clone of the *S1* gene was isolated. Restriction enzyme fragments for this cDNA clone were ligated into bacteriophage vector M13 mp9 RF-DNA[27] and sequenced by the dideoxy-chain terminator method[28].



c

LOCATION	AMINO-TERMINAL REGION	CARBOXY-TERMINAL REGION
STRUCTURE	α helix	Globular-like
Function	Anchoring	Receptor interactions
Features	α helical coiled-coil protein	Site of mutation of immune selected variant receptor site, bulky aromatic amino acids, 12 proline residues

Fig. 2 Schematic representation of the morphology of the outer capsid of reovirus type 3. *a*, Dimensions of the virion. *b*, Orientation of the $\sigma 1$ protein in the virus. The α-helical region is shown to extend through the $\lambda 2$ channel into the viral core. The globular-like structure sits on top of the $\lambda 2$ channel and interacts with host cell receptors. *c*, Summary of structure-function of the virus haemagglutinin.

consist of an α-helical structure. Similar predictions of a coiled-coil structure based on sequence data and computer analysis were reported for the influenza virus haemagglutinin[12]. This structure was subsequently verified by crystallography and shown to represent ~23% of the polypeptide that provides the major force stabilizing the trimeric subunit interactions[13].

By definition, the α-helices of a coiled-coil structure are wound around each other; this predicts at least a dimer form for the reovirus haemagglutinin. Studies show that there are 24 haemagglutinin molecules per virion which are located on the 12 projections/spikes on the outer capsid[14]. Thus, both the stoichiometry and the coiled-coil structure support a dimer form for the haemagglutinin.

In contrast to the amino-terminal portion, the carboxy-terminal portion of the virus haemagglutinin shows no dominant structural pattern. Computer analysis of protein secondary-structure predictions[11] show that this area is a mixture of short α-helices, β-sheets and random coils and that it contains many turns. This area also contains 12 proline residues and bulky aromatic amino acids, unlike the α-helical amino-terminal portion. All these features suggest a globular-like structure at the carboxy-terminal portion of the haemagglutinin protein.

Recent studies on a reovirus variant strain (designated variant K)[15] have localized the receptor-binding region to the carboxy terminus of the virus haemagglutinin (R.B.-D., unpublished data). In particlar, the *S1* gene of this immune-selected variant, in which the receptor-binding site is altered[16], is similar to the *S1* gene of reovirus type 3; the only nucleotide base change in the variant *S1* gene is a point mutation at nucleotide 1,267 (corresponding to a change in amino acid 419 from glutamic acid to lysine). Thus, the receptor-binding site is in the carboxy-terminal portion of the haemagglutinin protein.

Table 1 Heptapeptide repeat pattern for the amino-acid sequence of reovirus type 3 haemagglutinin

a	*b*	*c*	*d*	*e*	*f*	*g*	Amino-acid no.
			Leu	Glu	Ser	Arg	28–31
Val	Ser	Ala	Leu	Glu	Lys	Thr	32–38
Ser	Gln	Ile	His	Ser	Asp	Thr	39–45
Ile	Leu	Arg	Ile	Thr	Gln	Gly	46–52
Leu	Asp	Asp	Ala	Asn	Lys	Arg	53–59
Ile	Ile	Ala	Leu	Glu	Gln	Ser	60–66
Arg	Asp	Asp	Leu	Val	Ala	Ser	67–73
Val	Ser	Asp	Ala	Gln	Leu	Ala	74–80
Ile	Ser	Arg	Leu	Glu	Ser	Ser	81–87
Ile	Gly	Arg	Leu	Gln	Thr	Val	88–94
Val	Asn	Gly	Leu	Asp	Ser	Ser	95–101
Val	Thr	Gln	Leu	Gly	Ala	Arg	102–108
Val	Gly	Gln	Leu	Glu	Thr	Gly	109–115
Leu	Ala	Glu	Leu	Arg	Val	Asp	116–122
His	Asp	Asn	Leu	Leu	Ala	Arg	123–129
Val	Asp	Thr	Ala	Glu	Arg	Asn	130–136
Ile	Gly	Ser	Leu	Thr	Thr	Glu	137–143
Leu	Ser	Thr	Leu	Thr	Leu	Arg	144–150
Val	Thr	Ser	Ile	Gln	Ala	Asp	151–157
Phe							158
16/19	3/18	4/18	18/19	2/19	6/19	2/19	No. of hydrophobic amino acids
89	17	22	99	10	34	10	% Hydrophobic amino acids

The protein sequence of the reovirus haemagglutinin was compared with all the protein sequences available in the National Biomedical Research Foundation database, using the Goad–Kanehisa program for finding local homologies[17]. Significantly high scores (8 and 7 s.d.) were obtained with nematode myosin and rabbit tropomyosin. Optimal alignment of reovirus haemagglutinin (amino acids 27–100) and nematode myosin (amino acids 1,391–1,464) gives an average of 28% identical residues. When comparisons are based on grouping amino acids containing similar side chains, the extent of homology increases to an average of 51%. However, myosin[18] and tropomyosin[10] both consist of α-helical coiled-coil structures. To evaluate whether the observed homologies were due to evolutionary relatedness or structural relatedness, further computer analysis was performed on the homologies and revealed scores (4.5 and 4 s.d.) which indicated definite structural relatedness between reovirus haemagglutinin and myosin/tropomyosin. Because of the lack of any known functional similarities, the evolutionary relatedness of these proteins remains uncertain.

Figure 2 shows a model of the virus haemagglutinin and its orientation in the virus. Also shown are the known dimensions of the outer capsid, the location of the $\mu 1C/\sigma 3$ complex, the $\lambda 2$ projections/spikes and the channel within the $\lambda 2$ protein. Evidence that the haemagglutinin lies close to the $\lambda 2$ protein comes from analysis of extragenic suppressor mutations[19]. Temperature-sensitive (ts) mutants of reovirus containing defects in the $\lambda 2$ protein (tsB-352) revert to a non-ts phenotype with second mutations in the $\sigma 1$ protein. Such second-site mutations occur predominantly in proteins which share an intimate structural relationship. Further evidence for a close interaction between $\lambda 2$ and $\sigma 1$ proteins is the fact that although $\sigma 1$ protein is the major neutralization antigen, monoclonal antibodies against $\lambda 2$ protein also result in neutralization[20], presumably through steric hindrance of the $\sigma 1$ protein function.

The $\sigma 1$ protein has been drawn in the model as a dimer containing an extended α-helix whose length when fully extended is $\simeq$180 Å. Unlike the adenovirus fibre protein, which projects as an extended rod from the surface of the adenovirus core, no structure has been seen to project from the surface of

the reovirus particle. Therefore, the extended α-helix of the protein must be accommodated within the virion. The location of the haemagglutinin over the $\lambda 2$ channel makes it likely that the extended α-helical portion of the haemagglutinin extends into the channel, while the globular portion containing the receptor-interacting region is present on the outer surface of the virus particle. This model is consistent with the known channel diameter of the core spike (50 Å) and the radius of the outer capsid (120 Å)[21]. The location of the α-helix in the $\lambda 2$ channel would provide an anchor, attaching the $\sigma 1$ protein to the virus particle. Recent work in this laboratory on the morphology of the reovirion supports the proposed model (M. Nibert, unpublished data). The present studies should now make it possible to analyse, on a molecular level, the structure of the reovirus attachment protein and its interaction with its host cell receptor.

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Note added in proof: Since the submission of this manuscript, two other laboratories have published the sequence of the reovirus type 3 *S1* gene[30,31].

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An immunoglobulin promoter displays cell-type specificity independently of the enhancer

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Recent studies in which cloned immunoglobulin genes were introduced into cultured cells have produced two significant findings. First, the genes are expressed after transfection into lymphoid cells but not non-lymphoid cells[1-3]. Second, transcription of an immunoglobulin gene requires, in addition to the promoter region, an enhancer element located downstream of the transcription start site[4-10]. These findings raise the question of whether it is the promoter or the enhancer region that is responsible for the observed cell-type specificity. It has, in fact, been shown that immunoglobulin enhancers function only in lymphoid cells[6,8,9]. We show here that the promoter for an immunoglobulin κ light-chain gene also is strongly specific for lymphoid cells. Our result re-emphasizes the importance of promoters relative to enhancers in determining which cells express which genes.

Because the κ light-chain enhancer is already known to be cell-type specific, any study of the specificity of the κ-chain promoter must separate the promoter from the enhancer. For this purpose, we constructed the plasmid pLX40 (Fig. 1*a*), which contains three important elements: (1) part of the MOPC 41 κ light-chain gene[11] extending from ~1.1 kilobases (kb) before the transcription initiation site to 2.1 kb after the initiation site; (2) the simian virus 40 (SV40) early promoter attached to the bacterial *neo* gene[12]; and (3) the polyoma virus large-T antigen gene and origin of replication. The part of the κ-chain gene used excludes the enhancer, which has been mapped[5] to a region 1.6 kb further downstream. The κ-chain gene polyadenylation signal, also deleted in this construction, is replaced by an SV40 poly(A) signal to allow processing of κ-chain transcripts into stable messenger RNA[4,5]. The role of the SV40 promoter is to provide an internal control for transcription from the κ-chain promoter. The polyoma segment allows the plasmid to replicate to a high level in most mouse lymphoid and non-lymphoid cell lines (ref. 13 and our unpublished data), thus increasing the amount of RNA synthesized by increasing the amount of DNA template[4,5,7].

The κ-chain promoter cannot function without an enhancer, and so, to study transcription from the promoter, we inserted three different enhancers into pLX40 at an upstream *Eco*RI site. A 238-base pair (bp) fragment of polyoma (nucleotides 5,028-5,265)[14] including the enhancer region[15] was inserted in both orientations to form the plasmids pLX40P.1 and pLX40P.2. A 216-bp fragment of the Moloney murine sarcoma virus (MSV) long terminal repeat (nucleotides 210-426)[16], containing the 73/72-bp repeat unit with full enhancer activity[16], was inserted to form the plasmid pLX40M.1. For purposes of comparison, a 473-bp fragment of the κ-chain gene (nucleotides 3,689-4,161)[17] containing the κ enhancer itself[5,6] was also inserted in both orientations to form the plasmids pLX40K.1 and pLX40K.2. Although pLX40 already contains the SV40 enhancer as part of the SV40 origin (Fig. 1*a*), we inserted the enhancers from murine viruses to obtain maximal activation of the κ-chain promoter in mouse cells[18,19]. In fact, pLX40 also contains the polyoma enhancer as part of the polyoma replicon, but the enhancer does not stimulate the κ-chain promoter in this configuration[4,5].

This set of six plasmids was transfected into antibody-secreting MPC 11 mouse myeloma cells and three lines of non-lymphoid mouse cells. Forty-eight hours after transfection, total RNA was extracted from the cells and assayed by the S_1 nuclease method[20,21] in conditions of probe excess. For each RNA sample, one aliquot was tested with a 5′ end-labelled, double-stranded probe specific for κ-chain transcripts and an equal aliquot with such a probe specific for *neo* transcripts (Fig. 1*b*). The two hybridizations were carried out at temperatures optimal for the respective probes, and the two S_1 nuclease-treated reactions combined before loading on a gel.

Figure 2 shows the results of a typical S_1 experiment after transfection of the plasmids into MPC 11 cells. Bands of the intact *neo* and κ probes were present (Fig. 2), because of some re-annealing of the probes during the hybridizations and to some upstream transcription from pBR322 sequences (data not shown). In addition, a strong band was present beneath the *neo* probe. The size of this fragment corresponds to transcripts

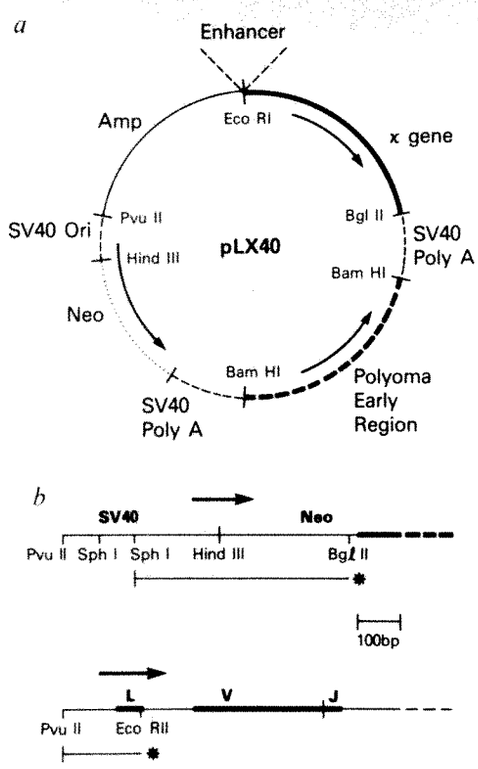


Fig. 1 *a*, Schematic diagram of the plasmid pLX40. Sources of DNA fragments are indicated as follows: pBR322, solid line; SV40, dashed line; *neo* gene, dotted line; polyomavirus, heavy dashed line; κ gene, heavy line. The structure of the pBR322-*neo*-SV40 segment is as described elsewhere[12]; the κ segment extends from the upstream *Eco*RI site to the *Bgl*II site in the intron[11,17]; the SV40 segment after the κ-chain gene is the same as the SV40 segment after the *neo* gene, and the polyoma segment is the same as used previously[4]. Arrows indicate the approximate initiation sites and directions of transcription for the SV40 early promoter, the polyoma early promoter and the κ promoter. Enhancers were inserted at the *Eco*RI site by means of linkers to form other plasmids. *b*, Strategy for S_1 nuclease assays. The *neo*-specific and κ-specific probes are shown underneath the respective genes, with the ^{32}P-labelled ends denoted by asterisks. Initiation sites and direction of SV40 early and κ transcription are shown by arrows, and translated regions of DNA are drawn in heavy line. L, leader segment; V, variable region; J, joining segment. The plasmid pLX40 and derivatives were constructed by standard methods; details are available on request to the authors. The *neo* and κ probes were prepared as described previously[2] with specific activities of $\sim 2 \times 10^6$ d.p.m. per pmol fragment.

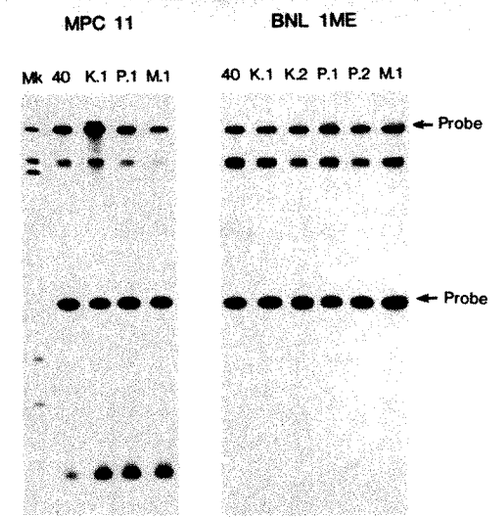


Fig. 2 S_1 nuclease assay of RNA from transfected cells. Cells of strains MPC 11 or BNL 1ME A.7R.1 were transfected with DNA of the indicated plasmids. Total RNA was extracted from the cells after 48 h and an equal aliquot of each RNA sample was hybridized at 53 °C to a *neo*-specific probe and at 48 °C to a κ-specific probe. After S_1 digestion, the reactions were combined and analysed on a polyacrylamide gel. The upper and lower bands marked probe co-migrated respectively with undigested *neo* and κ probe. The markers are from a labelled, denatured *Hin*fI digest of the plasmid pSV2-neo[12] and have lengths in nucleotides of 517, 396, 362, 134, 109 and 83. Mk, markers; 40, plasmid pLX40; K.1, pLX40K.1; K.2, pLX40K.2; P.1, pLX40P.1; P.2, pLX40P.2; M.1, pLX40M.1. Transfection of cells, extraction of RNA and S_1 assays were performed as described previously[2].

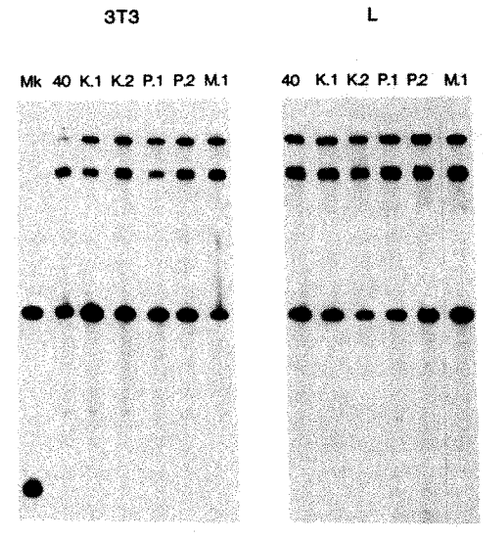


Fig. 3 S_1 nuclease assay of RNA from transfected cells. The assay was performed as in Fig. 2. Lane Mk is an S_1 analysis with the κ probe of RNA from MPC 11 cells transfected with pLX40P.1, performed in parallel with the other reactions. The cells were transfected as before[2], with the addition of a dimethyl sulphoxide shock step as described elsewhere[26].

initiating from the SV40 promoter; the band runs as a doublet because of heterogeneity in the SV40 early transcription start site[22]. Another strongly protected fragment was present in all the samples below the κ probe. This fragment is characteristic of transcripts initiated by the κ-chain promoter 25 bp before the first codon of the gene[4,7]. Neither the *neo*-specific nor κ-specific fragment were protected by RNA extracted from untransfected cells[2]. The transcription from the κ-chain promoter on pLX40 presumably resulted from the effect of the SV40 enhancer. κ-chain transcription was stimulated ~fivefold more by any of the murine enhancers (Fig. 2).

The set of plasmids was also transfected into a transformed line of liver cells BNL 1ME A.7R.1 (ATTC TIB 75) as a representative non-lymphoid cell line. Strong *neo*-specific bands were detected after S_1 analysis, showing that the plasmids were successfully transfected into the liver cells and could be transcribed. Indeed, more *neo* RNA was produced in the liver cells than in the myeloma cells, probably because of a higher transfection efficiency. Nonetheless, κ-chain transcripts were almost undetectable in the liver cell RNAs, although longer photographic exposures did reveal a small amount of κ-chain specific transcript from pLX40P.1, P.2 and M.1. Very similar results were obtained when the plasmids were transfected into mouse L and 3T3 cells (Fig. 3): the SV40 promoter transcribed the *neo* gene strongly on all the plasmids, but κ-chain transcription was very weak from pLX40P.1, P.2 and M.1 and undetectable from the other plasmids. The MSV enhancer is known to function in both L and 3T3 cells[16,18] and the polyoma enhancer functions in a wide range of cells, including 3T6 cells[19], and apparently in 3T3 cells where polyomavirus can replicate[14]. Hence, the very low level of κ-chain transcripts detected from pLX40P.1, P.2 and M.1 suggests that it is the κ-chain promoter itself which is intrinsically inactive in the non-lymphoid cells.

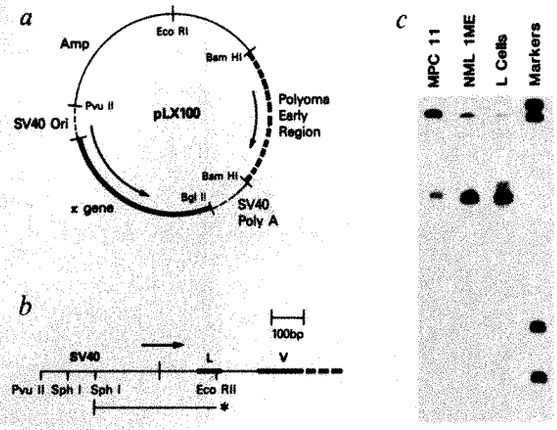


Fig. 4 *a*, Schematic diagram of the plasmid pLX100. Transcripts and sources of the DNA fragments are indicated as in Fig. 1. To construct the plasmid, a fragment of the plasmid pLX40 extending from the *Pvu*II site upstream of the κ gene (Fig. 1*b*) to the *Bam*HI site after the gene was inserted between the unique *Hin*dIII and *Bam*HI sites of the plasmid pSV2-neo[12]; the *Bam*HI polyoma fragment of pLX40 was then inserted at the *Bam*HI site of the new plasmid. *b*, Probe used for S_1 nuclease assays of RNA from pLX100. The probe is shown beneath the region of pLX100 where the SV40 origin and the κ gene are joined, with the ^{32}P-labelled end denoted by an asterisk. The arrow indicates transcription from the SV40 early promoter. *c*, S_1 nuclease assay of RNA from transfected cells. Cells of the indicated strains were transfected with pLX100 DNA, and extracted RNA was analysed at 53 °C with the probe shown in *b*. The topmost band in each lane co-migrated with undigested probe. The markers are part of a *Hin*fI digest of pSV2-neo DNA and have sizes in nucleotides of 396, 362, 134 and 109.

However, an alternative explanation is that κ-chain transcripts are synthesized at a higher level but are unstable in non-lymphoid cells. To eliminate this possibility, we constructed the plasmid pLX100 (Fig. 4*a*) in which the SV40 early promoter was attached to the same deleted κ-chain gene used in the pLX40 plasmids. The new plasmid was transfected into MPC 11, BNL 1ME and L cells, and extracted RNA was assayed by the S_1 method, using a suitable probe (Fig. 4*b*). In contrast to transcripts of the κ-chain gene initiating from its own promoter on the pLX40 plasmids (Figs 2, 3), transcripts of the κ-chain gene initiating from the SV40 promoter gave a strong signal (Fig. 4*c*). In fact, these transcripts were detected at a higher level in the non-lymphoid cells than in the myeloma cells, probably because of the higher transfection efficiency of the non-lymphoid cells seen above. In any case, the results show clearly that transcripts of this κ-chain gene are stable in non-lymphoid cells, and we therefore conclude that the κ-chain promoter initiates transcription very weakly in those cells.

We also transfected the pLX40 plasmids into lines of pre-B, B and T cells. We found that both the promoter and enhancer function in these cells, but not as efficiently as in myeloma cells, the activities in T cells being especially weak (manuscript in preparation).

The precise sequences responsible for the observed cell-type specificity of the κ-chain promoter may *a priori* be at a considerable distance from the actual transcription start site. However, we have shown that sequences more than 100 bp upstream of the start site are not required for high-level transcription in myeloma cells (C. Stauber and C.Q., unpublished data), and sequences downstream of the variable segment are also not required[7]. Hence, it seems likely that the actual sequences inducing high-level transcription specifically in B-lymphoid cells are fairly close to the transcription start site. Excellent candidates for these sequences would be two short regions of homology found in all light-chain genes examined, about 50–100 bp upstream of the start site[23,24]. Similar conclusions have recently been reached by others[25].

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Identical V_β T-cell receptor genes used in alloreactive cytotoxic and antigen plus I–A specific helper T cells

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T lymphocytes involved in the cellular immune response carry cell-surface receptors responsible for antigen and self recognition. This T-cell receptor molecule is a heterodimeric protein consisting of disulphide-linked α- and β-chains with variable (V) and constant (C) regions[1–5]. Several complementary DNA and genomic DNA clones have been isolated and characterized[6–12]. These analyses showed that the genomic arrangement and rearrangement of T-cell receptor genes using V_T, diversity (D_T), joining (J_T) and C_T gene segments is very similar to the structure of the known immunoglobulin genes (see ref. 13 for review). We have isolated two cDNA clones from an allospecific cytotoxic T cell, one of which shows a productive V_β–J_β–C_β1 rearrangement without an intervening D_β segment. This V_β gene segment is identical to the V_β gene expressed in a helper T-cell clone specific for chicken red blood cells and H-2I^b (ref. 14). The other clone carries the C_β2 gene of the T-cell receptor, but the C_β2 sequence is preceded by a DNA sequence that does not show any similarity to V_β or J_β sequences.

An allospecific cytotoxic T-cell clone 3F9 of BALB/c origin with specificity for H-2D^b has been established[15]. To isolate cDNA clones bearing sequences for the different chains (α and β) of the T-cell receptor molecule(s), we constructed a cDNA library in the bacteriophage λgt11. A synthetic oligonucleotide complementary to part of the C region of the β-chain was synthesized and used to screen the λgt11-3F9 cDNA library for cross-hybridizing cDNA clones. Twenty such clones were isolated from ~100,000 recombinant phages. Southern blot analysis of cloned phage DNA indicated two groups of cDNA clones, probably corresponding to C_β1 and C_β2 sequences. One clone bearing the longest DNA insert of each group was chosen, and the cDNA was subcloned into the *Eco*RI site of pEMBL8 (−). The restriction map and sequencing strategy of the two clones is outlined in Fig. 1. Sequence analysis of p3F9-β1 confirmed that this clone was derived from a C_β1-bearing transcript

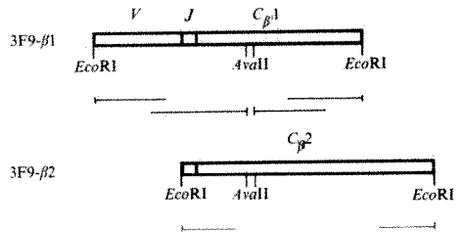


Fig. 1 Restriction site map and sequencing strategy. The 3F9 cDNA library and the cDNA clones p3F9-β1 and p3F9-β2 were produced according to standard procedures[18]. Briefly, poly(A) RNA was isolated from 3F9 cells and reverse transcribed into cDNA. Double-stranded cDNA was generated with reverse transcriptase and *Escherichia coli* polymerase I by self priming of second-strand synthesis. The DNA was methylated with *Eco*RI methylase, treated with S_1 nuclease, ligated to *Eco*RI linkers, size selected and cloned into λgt11 (ref. 19). An oligonucleotide complementary to the C region of the β-chain (position 211–239) was synthesized using an Applied Biosystem 380A DNA synthesizer, ^{32}P-labelled with [γ-^{32}P]ATP and T_4 polynucleotide kinase and used to screen the 3F9 cDNA library for cross-hybridizing phage clones. The cDNA inserts were subcloned into the *Eco*RI site of pEMBL8 (−) (ref. 20) and subjected to DNA sequence analysis [21].

(sequence not shown), whereas p3F9-β2 was derived from a $C_\beta 2$-bearing transcript (Fig. 3*b*).

Figure 2 shows the DNA sequence of the 5′ half of p3F9-β1. We found a DNA sequence encoding the $J_\beta 1.1$ gene segment upstream of the $C_\beta 1$ sequence. This $J_\beta 1.1$ sequence is preceded by a DNA sequence with an open reading frame. With two exceptions this sequence is identical to the known V_β gene segment of the T-helper cell line LB2, specific for chicken red blood cells and H-2I^b restricted[14]. The p3F9-β1 clone does not contain the 5′ end of the corresponding messenger RNA because its sequence lacks the first seven amino acids of the mature β-chain and the hydrophobic leader sequence when compared with the LB2 cDNA sequence. Towards the 3′ end of the V_β region, the p3F9-β1 sequence shows two base differences from the LB2 sequence. Both of these nucleotide changes alter the corresponding amino acids (Ala to Thr, and Leu to Ile); it is possible that these are either allelic differences between 3F9 (BALB/c) and LB2 (C57BL/6) or somatic mutations that have arisen during T-cell development. The V_β gene in LB2 is expressed with $J_\beta 2.3$ and $C_\beta 2$ and it is likely that a *D* gene segment has been inserted between V_β and $J_\beta 2.3$. A DNA sequence corresponding to the *D* gene segment of LB2 is missing in p3F9-β1. This implies that the V_β gene in 3F9 has joined directly to the $J_\beta 1.1$ gene segment or that the D_β gene segment has been deleted during a V_β to D_β-J_β joining event.

To confirm the absence of a *D* gene segment in 3F9, we determined the 3′ end of the V_β gene by comparing it with a germline V_β gene. A genomic unrearranged V_β gene had previously been cloned from B10.A liver DNA[8]. This germline V_β gene is homologous at its 3′ end with the V_β gene found in 3F9 and LB2 (Fig. 2). The last 17 nucleotides of the 2B4.71 germline *V*-region gene are identical to the sequence immediately preceding the $J_\beta 1.1$ gene segment in p3F9-β1. The 2B4.71 coding sequence ends with CTG (coding for Leu) and is followed by the conserved heptamer CACAGCA thought to be involved in *V*-(*D*)-*J* joining.

Comparison of the 3′ end of the 2B4.71 germline gene with the cDNA sequence of p3F9-β1 and LB2 implies that the 3′ end of the V_β gene in p3F9-β1 is represented by the triplet CTA (coding for Leu) and in LB2 by the triplet ATA (coding for Ile). From this we conclude that the same V_β gene can be used by T cells with different antigen specificities and different effector functions. In the allospecific killer T cells 3F9, the V_β gene is rearranged and transcribed as a V_β-$J_\beta 1.1$-$C_\beta 1$ unit without a *D* gene segment between the V_β and $J_\beta 1.1$ gene segment. In the chicken red blood cell-specific T-helper cell LB2, the same V_β gene has been rearranged and is transcribed as a V_β-*D*-$J_\beta 2.3$-$C_\beta 2$ unit[14]. Together with an earlier report[16] of a human cDNA molecule lacking a *D* gene segment inserted between *V* and *J* gene segments, this confirms the notion that diversity of the β-chain of the T-cell receptor can be created by insertion of none, one or more *D* gene segment(s) between the V_β and a J_β gene segment of the $J_\beta 1$ or $J_\beta 2$ gene cluster.

Our work shows that cytotoxic and helper T cells draw V_β genes from the same gene pool, although cytotoxic T cells are restricted by class I antigens of the major histocompatibility complex (MHC), whereas helper T cells recognize MHC class II molecules. If the V_α gene pool is also shared between cytotoxic and helper T cells, it becomes difficult to explain strict MHC class I versus class II restricted recognition of antigen by the $\alpha\beta$ heterodimer alone. In this light it is interesting that a third T-cell-specific molecule (the γ-chain) has been identified[17]. This γ-chain is predominantly expressed in cytotoxic T cells and it is thought to be part of a second receptor in cytotoxic T lymphocytes involved in recognition of MHC class I molecules. Alternatively, the distinction between class I and class II molecules could be made by the α-chain of a single receptor. In this case, V_α gene of cytotoxic and helper T cells should be derived from two different V_α gene pools.

Figure 3 shows part of the DNA sequence of p3F9-β2. The sequence corresponding to the 3′ end of the cDNA molecule is identical to the 3′ untranslated sequence of the $C_\beta 2$ gene so far sequenced. The 5′ end of the molcule has an open reading frame of 42 nucleotides (as far as the cDNA molecule extends) which leads into the start of the $C_\beta 2$ gene. The nucleotide sequence upstream of the $C_\beta 2$ gene is not the same as one of the $J_\beta 2$ gene segments normally found in this position. However, it is almost the same as a sequence detected upstream of the $D_\beta 2.1$ gene segment (at the 5′ end of the $J_\beta 2$ gene cluster)[12].

Figure 3 shows the sequence comparison of the 5′ end of p3F9-β2 and the genomic DNA sequence found 50–90 nucleotides upstream of the $D_\beta 2.1$ gene segment. The end of this homologous stretch of DNA is characterized by a consensus sequence of a donor site for RNA splicing. The p3F9-β2 cDNA is derived from a mRNA that may have arisen by splicing a RNA transcript from this genomic DNA sequence directly to the $C_\beta 2$ coding sequence. This means that the normal donor sites for RNA splicing which are present at the 3′ ends of the various $J_\beta 2$ gene segments have been omitted. The reasons for this aberrant RNA splicing are not clear; however, Southern blot analysis of genomic 3F9 DNA indicates that DNA deletions have occurred near the $J_\beta 2$ region (M. Schilham, R. Lang and

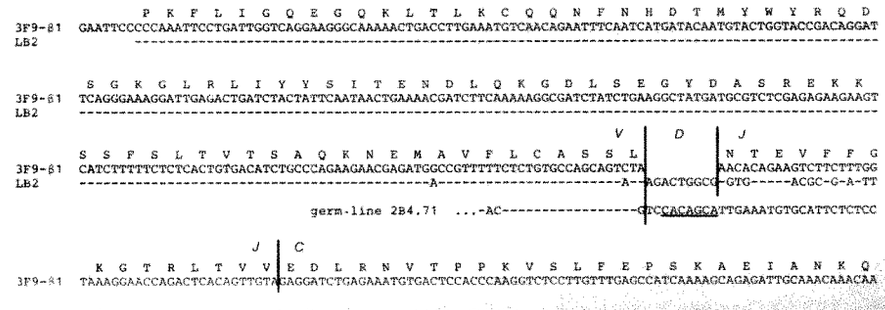


Fig. 2 Sequence of p3F9-β1. The V_β, $J_\beta 1.1$ and the beginning of the $C_\beta 1$ sequence of p3F9-β1 is shown and compared with the corresponding sequence of LB2 (ref. 14). The first seven nucleotides are derived from the *Eco*RI linker. Identical nucleotides are indicated by dashes. The germline (liver) gene 2B4.71 is shown to determine the *V*/*D* boundary of p3F9-β1 (ref. 8). The conserved heptamer following the coding region of the germline V_β gene is underlined.

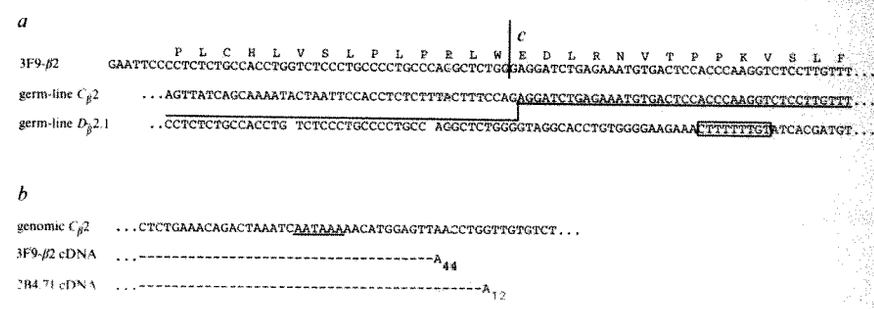


Fig. 3 Sequence of p3F9-β2. *a*, The 5′ terminal sequence and the beginning of the $C_\beta 2$ sequence are shown and compared with the germline sequence[10,11] of $C_\beta 2$ and a DNA sequence 5′ to the $D_\beta 2.1$ gene segment[12]. The conserved nonamer preceding the $D_\beta 2.1$ gene segment is boxed. The sequence found in the cDNA plasmid p3F9-β2 is indicated by a line. *b*, The 3′ terminal sequence of two cDNAs and the corresponding genomic sequence is shown. The polyadenylation signal ATAAA is underlined.

R.J., unpublished observations). Therefore, it is possible that the $J_\beta 2$ gene cluster has been deleted and a RNA transcript starting upstream of this deletion is now differently spliced. This finding implies that a promoter activity should be present 5′ of the J_β gene region. Similar cDNA molecules have been isolated from other T cells, but the cDNA molecules from other T cells contain J_β sequences 5′ of their respective C_β sequences[12,16].

p3F9-β2 contains a long tail of A residues at its 3′ end. Therefore, we could determine the position of poly(A) addition in the primary transcript. Figure 3*b* compares the 3′ ends of two different cDNA molecules both derived from a β2 mRNA with the corresponding genomic $C_\beta 2$ gene[10,11]. Although both transcripts are identical in the heteropolymeric region of the 3′ end, the site where polyadenylation begins varies by four to six nucleotides. If this does not result from allelic differences in the 3′ untranslated regions of 3F9 (BALB/c) and 2B4.71 (B10.A), then this comparison indicates that the exact position of polyadenylation is not determined on different transcripts even of the same gene.

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Detection and sequence of mutations in the factor VIII gene of haemophiliacs

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The most common inherited bleeding disorder in man, haemophilia A, is caused by defect in factor VIII, a component in the blood coagulation pathway. The X-chromosome-linked disease almost certainly stems from a heterogeneous collection of genetic lesions. Because, without proper treatment, haemophilia can be a fatal disease, new mutations are necessary to account for its constant frequency in the population[1]. In addition, haemophilia A displays a wide range of severity, and some 15% of haemophiliacs generate high levels of antibodies against factor VIII ('inhibitor patients'). The present work elucidates the molecular genetic basis of haemophilia in some individuals. Using the recently cloned factor VIII gene as a probe[2,3], we have identified two different nonsense point mutations in the factor VIII gene of haemophiliacs, as well as two different partial deletions of the gene. Our survey of 92 haemophiliacs indicates no firm correlation between antibody (inhibitor) production and gross gene defects.

In an initial study involving 36 haemophilia patients, DNA samples were digested and analysed by Southern blot hybridization to the cloned factor VIII gene. Each DNA sample was digested with 4–11 restriction enzymes to search for variant hybridization patterns. One result of this endeavour has been the discovery of a common restriction fragment length polymorphism with *Bcl*I which allows the tracking of specific X chromosomes in many families. The use of this factor VIII DNA polymorphism in gene localization and prenatal diagnosis is reported elsewhere[4]. The restriction enzyme *Taq*I proved particularly useful for revealing differences in the DNA of individual haemophiliacs. This result is not surprising, because the recognition sequence for *Taq*I contains the CpG dimer, which is the major site of methylation of human DNA. CpG dimers are probably mutation hot spots because of subsequent deamination of methylcytosine to thymidine, resulting in C to T transitions[5]. The location of the affected restriction enzyme sites and the sequence of the factor VIII gene[2] alerted us to the possibility of in-frame stop codon mutations generated by C to T transitions in the *Taq*I sequence. Five of the seven *Taq*I sites in exons of the factor VIII gene can be converted to nonsense mutations by such a mechanism. We therefore concentrated on the screening of 92 haemophilic DNA samples with *Taq*I. (The restriction enzyme *Msp*I also contains CpG in its recognition sequence, but no variant hybridizing bands were found in 20 samples.) Case-specific *Taq*I site mutations were discovered in the DNA of four severe haemophiliacs (none of these four patients has any detectable factor VIII activity).

Figure 1 shows a representative Southern blot of 11 DNA samples digested with *Taq*I and probed with the 9-kilobase (kb) cloned factor VIII complementary DNA. Distinct variant fragments can be seen in haemophilic patients H2 and H6. The *Taq*I site change in patient H2 was mapped to exon 24 of the 26 exons comprising the human factor VIII gene[3]. A hybridiz-

ation probe containing only exons 21–26 simplifies the band pattern and was subsequently used in the analysis of DNA from haemophiliac H2 and members of his family (Fig. 2*a*). Absence of the *Taq*I site in exon 24 produces a 4.2-kb hybridizing band found only in patient H2. His mother as well as his father, sister and two brothers display the normal 1.4- and 2.8-kb fragments, but not the 4.2-kb fragment, suggesting that this new mutation occurred in the X chromosome of individual H2, a result consistent with the lack of previous haemophilia in this family.

A region of H2 DNA which includes this mutation was cloned into bacteriophage λ, and exon 24 was sequenced. The DNA sequence was identical to that of normal DNA analysed previously[2], except that the *Taq*I site had changed from TCGA to TTGA in the H2 DNA. This mutation generates an in-frame stop codon in the factor VIII gene 124 amino acids before the normal chain termination. Because this mutation was found in only 1 case out of 92 haemophilic and 80 non-haemophilic X chromosomes examined, and because it first appeared in the only affected member of this family, we propose that this nonsense mutation is likely to be the cause of haemophilia in this individual. In addition to having a new haemophilia mutation,

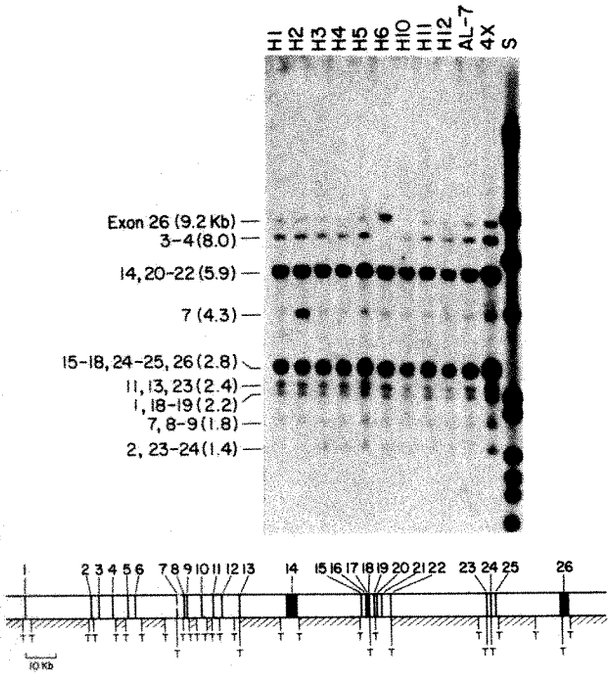


Fig. 1 Southern blot of haemophilia DNA samples showing two variations with the enzyme *Taq*I. The blot was hybridized to the full-length factor VIII probe (9.0-kb ClaI/*Sst*II fragment of pSAT.8cl; ref. 2) which contains all coding sequences and the 3′-untranslated region. Each hybridizing band corresponds to at least one exon; band sizes in kb are shown in parentheses. The lanes containing the haemophiliac samples are indicated by a number preceded by the letter H. The control lanes labelled AL-7 and 4X contain DNA prepared from cell lines which served as a source of the factor VIII mRNA and factor VIII genomic clones, respectively[2,3]. Lane S contains ^{32}P-end-labelled *Hin*dIII-digested λ DNA and *Hae*III-digested ΦX174 DNA as standards. The diagram shows the location of *Taq*I sites (T) in exons (indicated by a lower T) and introns of the factor VIII gene. The intron areas indicated by hatchmarks are not mapped with respect to *Taq*I sites as they are not detected by the factor VIII cDNA probe. Bands predicted to correspond to exons 5–6 and 12 were not detected in the blot shown; the size of the fragment corresponding to exon 10 is uncertain.

Methods. Genomic DNA (5 μg), prepared either from peripheral blood lymphocytes of haemophiliacs or from cell lines, was electrophoresed and blotted onto a nitrocellulose filter. The filter was hybridized to calf thymus primed ^{32}P-labelled probe in 50% formamide at 42 °C overnight and washed at 60 °C in 0.2XSSC before autoradiography[11,12].

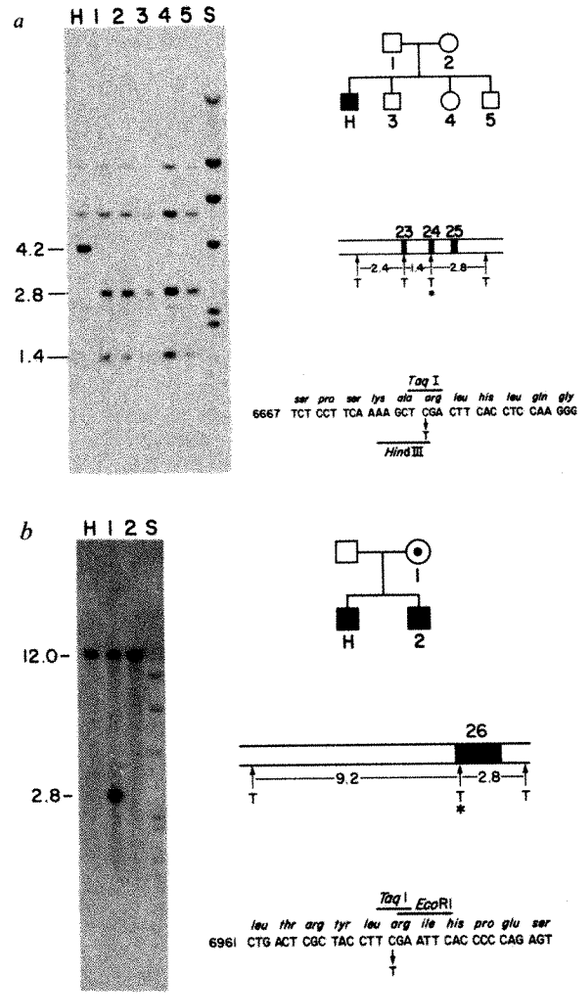


Fig. 2 Analysis of the factor VIII gene of patient H2 and patient H22. ***a***, A Southern blot of DNA from patient H2 and his family members was probed with a cDNA 783-bp *Apa*I/*Eco*RI fragment that includes all of exons 22–25 and parts of exons 21 and 26. Lanes H (haemophiliac), 1, 2, 3, 4 and 5 correspond to family members in the accompanying pedigree [□, normal male; ■, haemophiliac male; ○, normal female; ⊙, carrier female (not present on this particular pedigree)]. The diagram below the pedigree indicates with an asterisk the location of the missing *Taq*I site in exon 24. Below this is shown the sequence of normal DNA[2] and the changed base in H2 DNA that results in the loss of a *Taq*I site, the generation of a *Hin*dIII site (verified by a Southern blot, data not shown), and the occurrence of an in-frame stop codon TGA. The nucleotide number to the left follows ref. 2. ***b***, A Southern blot of *Taq*I-digested DNA from patient H22, his mother and brother was probed with a 1.9-kb *Eco*RI cDNA fragment (from clone λ10.3) that includes almost all of exon 26 (ref. 2). The mother (lane 1) has the normal 2.8-kb *Taq*I allele as well as the variant 12.0-kb allele, which she shares with her two haemophilic sons. The *Taq*I site that is missing in H22 DNA, and the normal DNA sequence and the altered nucleotide in H22 are indicated.

Methods. Blot hybridization was performed as described in Fig. 1 legend. For sequence analysis, DNA from patient H2 was preparatively digested with *Bam*HI. A 15-kb region, known to contain exon 24, was gel isolated and cloned into bacteriophage vector Charon 30 (ref. 11). An exon 24-containing 600-bp *Hae*III fragment of the resulting λ clone was subcloned into M13 and sequenced by dideoxy-chain termination procedures. The C to T transition is the only difference found between the normal and H2 sequence. Similarly, for patient H22, a 5.3-kb *Bam*HI fragment was cloned into vector Charon 30. A 1.6-kb *Sst*I/*Bam*HI fragment from this cloned DNA was then subcloned into M13 for sequencing from the *Sst*I site for ~600 bp to read through the *Taq*I and *Eco*RI sites.

H2 is also a significant case because the patient, a severe haemophiliac with <1% factor VIII activity, has developed an extremely high titre of anti-factor VIII antibody (8,000 Bethesda units).

A different premature termination mutation was found in patient H22. The *Taq*I site normally found in exon 26 has been lost, leading to the replacement of the normal 2.8-kb hybridizing band with a 12-kb band (Fig. 2*b*). Haemophiliac H22 and his haemophilic brother (Fig. 2*b*, lanes H and 2, respectively) display the 12-kb fragment, while DNA from their mother (lane 1), who is a carrier of haemophilia, contains the affected X chromosome with the 12-kb fragment, and a normal X chromosome with the 2.8-kb fragment. Cloning of H22 DNA and sequence analysis demonstrated a TCGA to TTGA transition of the *Taq*I site in exon 26, again generating an in-frame stop codon which we propose to be the basis of this haemophilia. Although this sequence predicts the synthesis of a protein only 26 amino acids shorter than normal factor VIII, such a truncated protein may well be inactive. The absent portion of the molecule is contained in one of the active subunits (relative molecular mass 80,000)[2,6,7] of the mature protein and includes a cysteine residue which is conserved in the duplicated C domains[3,7] and which may be important for the stability or active conformation of the protein. (Although patient H22 is a severe haemophiliac, he does not produce antibodies against factor VIII.)

In the case of patient H6, the missing *Taq*I site is located within an intron, about 400 base pairs (bp) 3′ to exon 2 and 2,000 bp 5′ to exon 3. Figure 3*a* illustrates the inheritance of this mutation in the H6 family. The normal *Taq*I fragments of 1.5 and 8.0 kb detected using a subgenic probe are replaced by a 9.5-kb fragment in H6 DNA. The haemophiliac's mother (Fig. 3*a*, lane 1) is an obligate carrier of haemophilia because her father (and four uncles) was a haemophiliac. The carrier status of the haemophiliac's sister and her two daughters (Fig. 3*a*, lanes 4–6) was unknown before this experiment. The results from this Southern blot indicate that the sister and one niece are carriers of haemophilia, whereas the other niece is not. We believe this to be the first carrier detection of haemophilia A made by DNA diagnosis using a probe within the factor VIII gene. The H6 DNA surrounding the missing *Taq*I site was cloned and sequenced and, again, a TCGA to TTGA transition in the *Taq*I site was found. This mutation does not create a sequence resembling consensus splice donor or acceptor sites[3,8], and is not located near the intron–exon borders. Thus, it is not apparent whether this sequence change leads to aberrant RNA splicing, and therefore whether it is directly responsible for the disease in this family.

The final variant involves a new *Taq*I site in haemophiliac H40 DNA. The site of mutation is in the last intron of the factor VIII gene, ~20 kb 3′ of exon 25 and 1.7 kb 5′ of exon 26. A TCAA to TCGA change creates the new *Taq*I site. Sequence analysis of this intron also revealed a second difference when compared with the sequence of our previously isolated genomic clones[3]. A T to C transition appears 22 nucleotides 5′ to the *Taq*I site mutation. We do not know whether this particular T-C variation is common among individuals. However, both of these sequence changes occur in a region homologous to the *Alu* family of interspersed repetitive DNA[9]. Furthermore, there is no obvious connection between these sequence anomalies in the H40 family and the disease; no consensus splice sites are created by these changes.

To summarize the *Taq*I survey, we have identified four independent *Taq*I site mutations in the factor VIII genes of haemophiliacs. Three of these involve the loss of a *Taq*I site (two of these changes are in exons) and one involves the introduction of a *Taq*I site in an intron. In addition to the 92 haemophilic DNA samples examined with *Taq*I, 80 normal (non-haemophilic) chromosomes have been screened by hybridization of Southern blots of *Taq*I-digested DNA with a full-length factor VIII probe. Of those 172 chromosomes, only the 4 *Taq*I site mutations have been observed, suggesting that each one is a rare variant (≤0.6%).

We propose that the in-frame stop codons found in exon 24 of the factor VIII gene in patient H2 and in exon 26 in H22 are the cause of haemophilia A in these families. Additional mutations cannot be ruled out, however. The phenotype could be due to inactivity or instability of the truncated protein or an effect of premature termination on the stability of the messenger

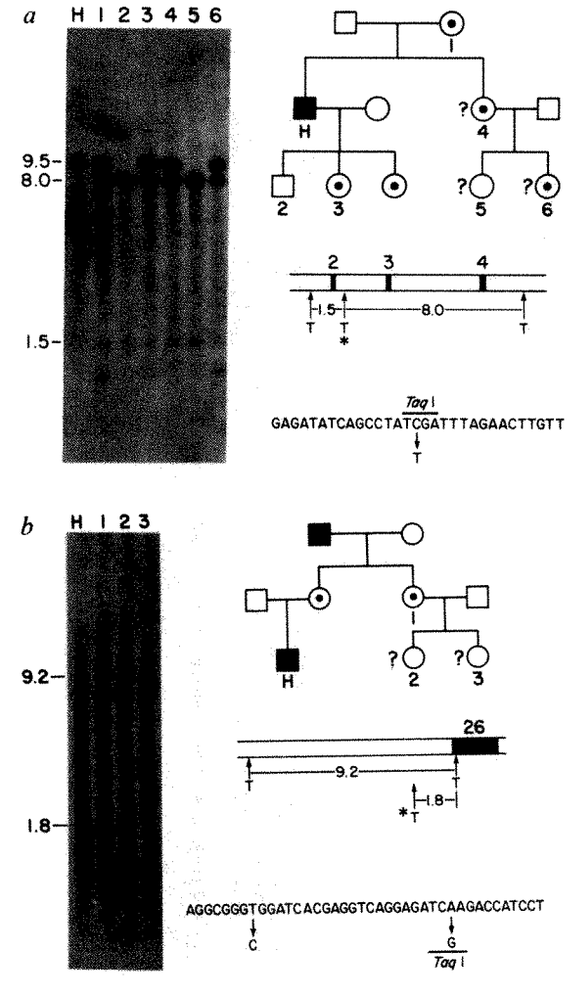


Fig. 3 Analysis of the factor VIII gene of patients H6 DNA and H40. ***a***, A Southern blot of *Taq*I-digested DNAs from patient H6 (H) and his family members was probed with 347-bp *Eco*RI cDNA fragment that includes only exons 2, 3 and 4 (ref. 2). The question marks on the pedigree indicate that before this experiment the carrier status of 4, 5 and 6 was unknown. The diagram below the pedigree shows the location of the *Taq*I site (T*) 3′ of exon 2 that is absent in the DNA of patient H6. Also shown is the sequence of normal DNA and the C to T change in the sequenced H6 DNA. ***b***, A Southern blot of *Taq*I-digested DNA from patient H40, his aunt and two cousins was hybridized to a ^{32}P-labelled 500-bp *Sst*I/*Eco*RI genomic fragment prepared from cosmid clone p613 (ref. 3). The unusual *Taq*I site in H40 DNA was mapped to an intron 1.7 kb 5′ of exon 26, as shown by T* in the schematic diagram. The 1.8-kb *Taq*I fragment is present in DNA samples from the haemophiliac and the aunt, who also has one normal allele, but is not found in DNA from the female cousins.

Methods. From the H6 DNA, the appropriate 7.0-kb *Bcl*I fragment was prepared and cloned into *Bam*HI arms of the Charon 30 vector. A 600-bp *Eco*RI/*Kpn*I fragment from this H6 λ clone, as well as the corresponding normal fragment from cosmid clone p624 (ref. 3), were subcloned into M13 and sequenced by dideoxy-chain termination methods. The C to T change in the *Taq*I site was the only difference in sequence between these two samples. From patient H40, *Bam*HI-digested DNA was preparatively run on a gel, and a 5.3-kb region, known to contain the new *Taq*I site, was isolated and cloned into Charon 30 vector. From this λ clone, a 1.0-kb *Sst*I/*Sma*I fragment was isolated, and along with the analogous normal fragment, a 1.0-kb *Sst*I/*Sma*I fragment from cosmid clone p613 (ref. 3), was cloned into M13 and sequenced.

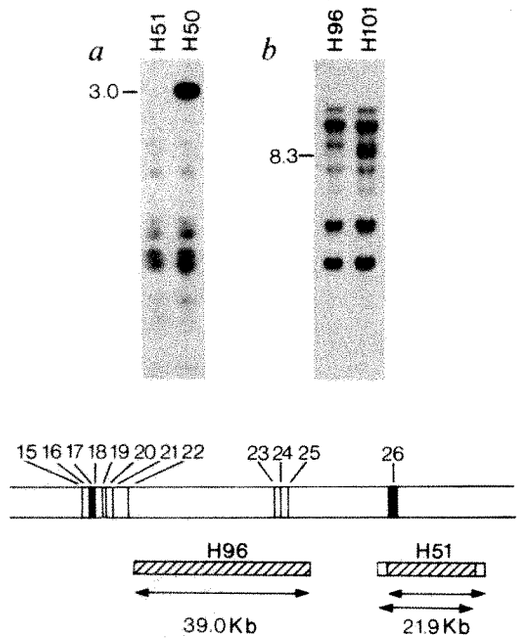


Fig. 4 Map of the deletions in the factor VIII genes of patients H51 and H96. ***a***, A Southern blot of *Rsa*I-digested genomic DNA from a number of haemophiliacs, including H51, was hybridized with the full-length factor VIII probe. A 3.0-kb *Rsa*I fragment present in all other haemophilic samples tested is absent in H51 DNA (only one non-deletion DNA, H50, is shown). To map the H51 deletion, three single-copy genomic fragments were prepared from a cosmid clone p625, which extends 37 kb beyond the 3′ end of the factor VIII gene. In addition, two genomic fragments 5′ of exon 26 were used. The extent of the 21.9-kb deletion in H51 DNA is shown below the diagram of the 3′ portion of the factor VIII gene. Due to the presence of repetitive DNA near the deletion boundaries, there is an uncertainty of ~2 kb in the precise deletion end points. ***b***, A Southern blot of *Sst*I-digested haemophilic DNAs similarly revealed that H96 DNA lacks an 8.3-kb fragment that is present in every other sample tested. The 39.0-kb deletion in H96 was mapped using one cDNA and three cloned genomic fragments from λ114, λ482 and p542 (refs 2, 3).

RNA. Neither patient H2 or H22 possesses detectable levels of factor VIII activity. (It has not been possible to measure truncated protein immunologically, as patient H2 produces high levels of anti-factor VIII antibodies, and patient H22 receives frequent infusions of factor VIII for treatment.) Eventual expression of these mutant genes in a heterologous cell system[2] may resolve these questions.

Finally, two cases of partial deletions of the factor VIII gene were detected in our survey. A Southern blot (Fig. 4) demonstrates that the DNA of patient H51 (a severe haemophiliac but not an inhibitor patient) lacks a 3.0-kb *Rsa*I fragment, which contains exon 26. Similarly, a Southern blot of *Sst*I-digested haemophiliac DNAs indicates that the DNA from the severe haemophiliac H96, who produces antibodies (10–50 Bethesda units), lacks an 8.3-kb fragment that includes exons 23–25. The extents of the H51 and H96 deletions were mapped by hybridizing genomic Southern blots with a series of cloned genomic probe fragments at the 3′ end of the factor VIII gene. In H96 DNA, the section missing from the factor VIII gene extends from 1 kb 3′ of exon 22 to 17.5 kb 5′ of exon 26, a total deletion of 39.0 kb. The deletion in H51 DNA, totalling 21.9 kb, extends roughly from 1 kb 5′ of exon 26 to 19 kb 3′ of the end of the transcribed factor VIII gene.

The 92 haemophilia A patients in this study can be categorized on the basis of their factor VIII activity as follows: 73 severe (<1% activity), 7 moderate (1–5% activity) and 12 mild (>5% activity). Fourteen of the severe haemophiliacs also produce inhibiting anti-factor VIII antibodies. All of the 92 DNA samples have been analysed with at least 2 restriction enzymes, while all 14 inhibitor patients were extensively examined with at least 6 enzymes to ensure resolution of all 26 exons of the factor VIII gene. In contrast to the findings of Giannelli *et al.*[10], who reported deletions in the factor IX gene of four out of five haemophilia B inhibitor patients, we observed only one case of a deletion (H96) in the factor VIII genes of the 14 haemophilia A inhibitor patients tested. The only other patient with a deletion found in this survey, H51, does not produce antibodies. Therefore, the suggestion that there is a correlation between the presence of antibodies and a deletion of part of the gene[10] is not borne out in the case of factor VIII.

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A role for branchpoints in splicing *in vivo*

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The nucleotides immediately surrounding intron/exon junctions of genes transcribed by RNA polymerase B can be derived from 'consensus' sequences for donor and acceptor splice sites by only a few base changes[1–3]. Studies *in vivo* have underlined the importance of these junction nucleotides for splicing[4–7]. In higher eukaryotes, no evidence has been found for specific internal intron sequences involved in splicing[8–10]. However, the recent discovery that, *in vitro*, introns are excised in a lariat form where the 5′ end of the intron is joined via a 2′-5′-phosphodiester linkage to an A residue (branchpoint acceptor) close to the 3′ end of the intron, suggests that internal intron sequences may nonetheless be important for splicing[11–13]. Indeed, in yeast nuclear genes, the internal sequence 5′-TACTAAC-3′ (or close homologue) is essential for splicing *in vivo*[14,15]. A proposed consensus sequence for branchpoints in mammalian introns is 5′-CT(A/G)A(C/T)-3′ (refs 11, 16, 17). This sequence resembles the essential yeast internal sequence. Are branchpoints involved in the splicing of introns of higher eukaryotes *in vivo*? We show here that a branchpoint sequence from a human globin gene (5′-CTGACTCTCTCTG-3′)[11] greatly enhances the efficiency of splicing of a 'synthetic' intron in HeLa cells. A mutated branchpoint sequence, 5′-CTCCTCTCTCTG-3′, in which the branchpoint acceptor nucleotide A has been deleted and the neighbouring purine G mutated to a C, does not exhibit this enhancing capability. We conclude that branchpoints have an important function in the splicing process *in vivo*.

We have described elsewhere the construction of synthetic splice sites designed to conform to the consensus sequences for

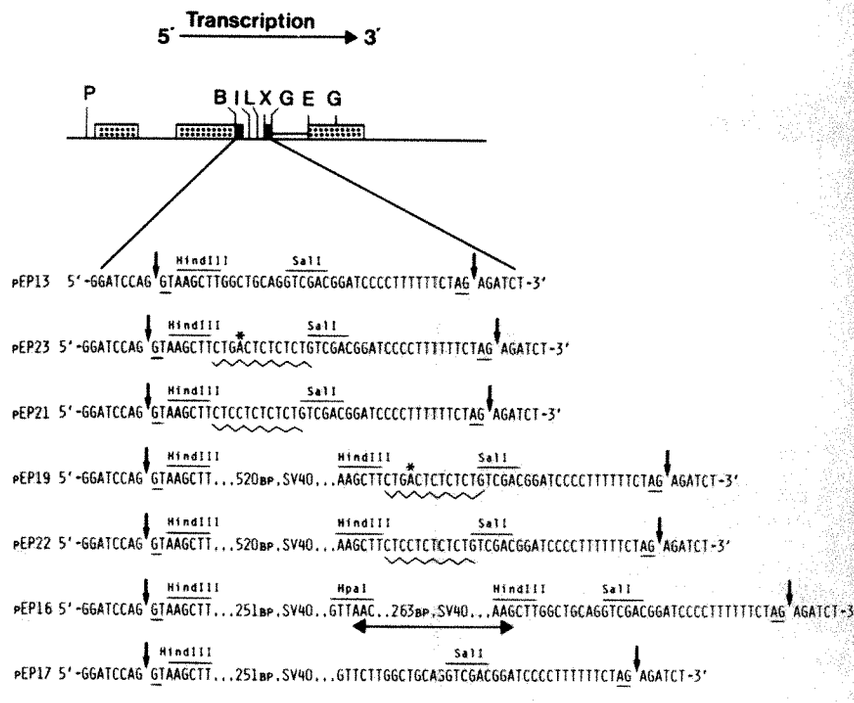


Fig. 1 Transcription units used to study splicing of synthetic introns. Dotted boxes represent rabbit β-globin gene exons and solid boxes represent exon sequences of the splicing oligonucleotide as described elsewhere[7]. Thus, the 5′ solid box represents the sequence 5′-GGATCCAG-3′ and the 3′ solid box the sequence 5′-AGATCT-3′. The *Bgl*II/*Eco*RI fragment shown as a double line represents a ~100-bp fragment of pBR328 (see ref. 7 for a full discussion). P, *Pst*I; B, *Bam*HI; I, *Hin*dIII; L, *Sal*I; X, *Xba*I; G, *Bgl*II; E, *Eco*RI. Sequences of synthetic introns described in the text are shown. Splice points are marked by arrows, and the invariant dinucleotides GT and AG present at the 5′ and 3′ ends of introns, respectively, are underlined. Construction of pEP13, pEP16 and pEP17 has been described elsewhere[7]. The *Hpa*I/*Alu*I fragment of the internal intron sequence of pEP16 deleted to make pEP17 is shown by horizontal arrows below the pEP16 sequence. *Hin*dIII and *Sal*I sites used to introduce the globin branchpoint sequence[11] 5′-CTGACTCTCTCTG-3′ or its mutated counterpart 5′-CTCCTCTCTCTG-3′ are shown (wavy underlining). The branchpoint acceptor nucleotide A used to make a 2′-5′-phosphodiester linkage with the G residue at the 5′ end of the intron is shown by an asterisk in the pEP23 and pEP19 sequences.

Methods. To introduce the branchpoint sequence, chemically synthesized[18] oligonucleotides 5′-AGCTTCTGACTCTCTCTG-3′ and 5′-TCGACAGAGAGAGTCAGA-3′ (phosphorylated at their 5′ ends by treatment with ATP and polynucleotide kinase) were mixed in a 1:1 ratio and ligated to a phosphatase-treated *Hin*dIII + *Sal*I digest of pEP13. pEP23 generated in this manner was linearized with *Hin*dIII and ligated to a *Hin*dIII fragment of SV40 DNA (bp 3,476–4,002, BBB numbering system[19] to make pEP19. The same *Hin*dIII fragment is present in the same orientation in pEP16, pEP 19 and pEP22. It has been represented in a different fashion in pEP16 only to show the deletion used to generate pEP17. A similar strategy was used to construct pEP21 and pEP22. Drawings are not to scale.

donor and acceptor splice sites and their expression *in vivo* as RNA from a β-globin gene vector[7]. This vector (pEP1, Fig. 2) contains a rabbit β-globin gene linked to simian virus (SV40) enhancer. In plasmids designed to express the synthetic splice sites, the *Bam*HI/*Eco*RI fragment of pEP1 carrying the globin large intron and flanking exon/intron junctions was replaced by a *Bam*HI/*Eco*RI fragment carrying the synthetic donor (5′-GGATCCAG↓GTAAGCTT-3′) and acceptor (5′-CTTTTTTCTAG↓AGATCT-3′) splice sites separated by various synthetic introns. In pEP13, the synthetic splice sites are separated by an internal intron sequence derived from the bacterial plasmid pUC8 (Fig. 1). In pEP16, a 526-base pair (bp) *Hin*dIII fragment from SV40 genomic DNA has been added to the internal intron sequences of pEP13 (Fig. 1). When introduced into HeLa cells by the calcium phosphate co-precipitation technique, both of these plasmid constructions lead to a very small amount of cytoplasmic RNA in which the synthetic donor splice site has been correctly spliced to the synthetic acceptor splice site[7].

Deletion of some internal intron sequences from pEP16 (arrows in Fig. 1) generated pEP17 (Fig. 1). Much greater amounts of correctly spliced RNA were obtained from cells transfected with pEP17 than with either pEP13 or pEP16 (see Fig. 10 of ref. 7 and below). It seemed possible that pEP13 and pEP16 lacked a sequence capable of functioning efficiently as a branchpoint. The deletion used to generate pEP17 might have fortuitously positioned a branchpoint sequence at a suitable distance from the synthetic acceptor, thereby greatly increasing splicing efficiency. A possible candidate for such a sequence is the pentanucleotide 5′-TTAAA-3′ positioned 50 nucleotides away from the acceptor splice site in pEP17 (within the 251-bp SV40 DNA segment (Fig. 1), but over 270 nucleotides away from the acceptor splice site in pEP16 (the sequences 5′-TTAAC-3′ and 5′-TTAAT-3′ have been proposed as branchpoints[16]). To test the hypothesis that pEP13 and pEP16 lack an efficient branchpoint sequence, we decided to introduce a known branchpoint sequence into these plasmids and test its effect on splicing.

The *Hin*dIII/*Sal*I fragments in the internal intron sequences of pEP13 and pEP16 were replaced by synthetic *Hin*dIII/*Sal*I fragments carrying the identified branchpoint sequence (5′-CTGACTCTCTCTG-3′) of a human globin gene[11] to generate pEP23 and pEP19, respectively (Fig. 1; pEP16 and pEP19 contain the same *Hin*dIII fragment in the same orientation). Each of these four plasmids and pEP17 was introduced by the calcium phosphate co-precipitation technique into HeLa cells, together with pEP1 (to monitor the efficiency of the transfection). Cytoplasmic RNA was collected 72 h later and used for S_1 nuclease mapping with a mixture of two 5′-end-labelled single-stranded DNA probes. One of these probes (probe A, Fig. 2) detects correctly spliced globin RNA derived from pEP1; this RNA will protect 54 nucleotides of probe A from S_1 nuclease digestion. The second probe (probe D, Fig. 2) is designed to detect RNA derived from pEP13, pEP16, pEP17, pEP19 or pEP23 in which both the small globin intron and the synthetic intron have been correctly removed by splicing. Such spliced RNA will protect 325 nucleotides of probe D from S_1 nuclease digestion (Fig. 2).

Figure 3 shows that much less spliced RNA is obtained with pEP13 and pEP16 than with pEP17, confirming our previous observations[7]. (We term spliced RNA, here and below, as RNA in which the synthetic donor site has been spliced to the synthetic acceptor site. We have shown previously[7] that when the synthetic acceptor is not used for splicing to the synthetic donor, splicing of the latter to a cryptic splice site in the *Eco*RI/*Bgl*II globin exon fragment occurs. This RNA cannot be detected using probe D.) Of the plasmids containing the globin branchpoint sequence, one (pEP23) yielded low levels of spliced RNA similar to those observed with pEP13 and pEP16, while the other (pEP19) yielded much higher levels of spliced RNA, similar to those observed with pEP17. It is highly unlikely that these results are due to different transfection efficiencies, as in all cases, roughly similar amounts of globin RNA were produced from the co-transfected pEP1 (as evidenced by the intensities of the ~54-nucleotide protected fragment in each lane of Fig. 3).

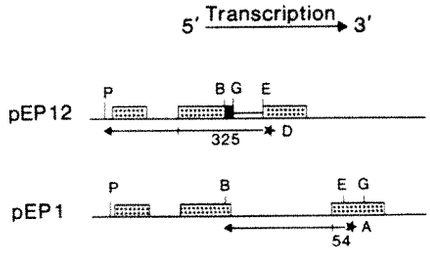


Fig. 2 Probes used for S_1 nuclease mapping. Symbols are as described in Fig. 1 legend. Construction of pEP1 and pEP12 has been described elswhere[7]. Briefly, pEP1 contains a rabbit β-globin gene linked to an SV40 enhancer. pEP12 represents a cDNA version of pEP13 from which the synthetic intron sequence has been deleted using the enzymes *Bam*HI and *Xba*I. For preparation of probe D, a *Pst*I/*Eco*RI fragment of pEP12 was 5′-end labelled with [γ-^{32}P]ATP and polynucleotide kinase, and strand-separated as described previously[7]. The strand labelled at the *Eco*RI terminus was isolated. RNA extracted from HeLa cells transfected with any of the plasmids pEP13–pEP23 in which both the small globin intron and the synthetic intron have been removed correctly will protect 325 nucleotides of probe D from nuclease S_1 digestion as shown (see ref. 7 for a more detailed discussion). For preparation of probe A, a *Bam*HI/*Eco*RI fragment of pEP1 was 5′-end-labelled and strand-separated. The strand labelled at the *Eco*RI terminus was isolated. Globin RNA from pEP1 will protect 54 nucleotides of this probe from nuclease S_1 digestion as shown. Drawings are not to scale. Cuts on the arrows indicate extents of probes protected by correctly spliced RNA.

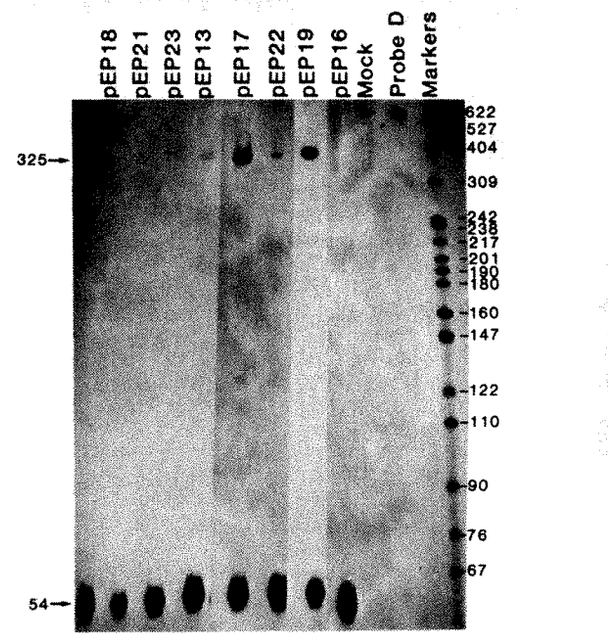


Fig. 3 Effect of a globin branchpoint sequence on splicing of synthetic introns. Plasmids shown in Fig. 1 were mixed in a 4:1 ratio with pEP1 and introduced into HeLa cells by the calcium phosphate co-precipitation procedure. Cytoplasmic RNA was isolated 72 h later and 50 μg used for nuclease S_1 mapping with a mixture of probes A and D (Fig. 2). Protected fragments of probe were electrophoresed on an 8% polyacrylamide 7 M urea gel. Size markers are from an *Msp*II digest of pBR322. Fragments of probe A protected by RNA from pEP1 are indicated by their size, ~54 nucleotides. Fragments of probe D protected by RNA from pEP13–pEP23 are also indicated by their size, ~325 nucleotides. Mock, mock-transfected HeLa cells. All experimental conditions were as described previously[7].

Introduction of a globin branchpoint sequence into the synthetic intron of pEP16 thus leads to a large increase in splicing efficiency. A similar effect is not seen for pEP23, perhaps because in this plasmid the branchpoint acceptor nucleotide A of the sequence 5′-CTGACTCTCTCTG-3′ is too close to the 5′ end of the intron (only 11 nucleotides away). However, there are clearly other differences between pEP19 and pEP23 (for instance intron size) which could explain the reduced splicing efficiency observed with the latter construction. We also show in Fig. 3 results obtained with pEP18, a version of pEP23 containing a branchpoint of the form 5′-CTGATCTCTCTG-3′ and thus carrying a single-nucleotide deletion relative to the pEP23 branchpoint. A low level of spliced RNA, similar to that seen with pEP23, was obtained with pEP18. If the significantly higher efficiency of splicing observed with pEP19 as compared with pEP16 is due to the presence of an efficient branchpoint sequence in the former, elimination of the branchpoint acceptor nucleotide A from the sequence 5′-CTGACTCTCTCTG-3′ present in pEP19 should greatly decrease splicing efficiency. The plasmid pEP22 contains the mutated branchpoint sequence 5′-CTCCTCTCTCTG-3′ (Fig. 1). For the mutated branchpoint sequence, we chose to delete the branchpoint acceptor nucleotide A and to mutate the neighbouring G residue to a C, as, although the branchpoint acceptor nucleotides identified to date are all A, it has not been demonstrated that a G cannot function as a branchpoint acceptor. We hypothesized that a pyrimidine would be less likely to function in this way, and therefore mutated the G to a C. Levels of spliced RNA obtained with pEP22 were indeed low (Fig. 3), and comparable to those obtained with pEP13. As expected, pEP21, a version of pEP23 with the mutated branchpoint sequence, also gave rise to the low level of spliced RNA apparently characteristic of constructions lacking an efficient branchpoint sequence.

We have shown that introduction of a globin branchpoint sequence into a synthetic intron can greatly enhance splicing efficiency, thus demonstrating the importance of branchpoint sequences for splicing *in vivo*. We assume that the low levels of spliced RNA obtained with constructions such as pEP13 and pEP16 reflect the use of an inefficient branchpoint, although it has not been shown that a branchpoint is essential for splicing. The sequence features defining a branchpoint and its optimal position relative to an acceptor splice site are unknown. Plasmids such as pEP16 which lack an efficient branchpoint sequence should prove useful in this respect.

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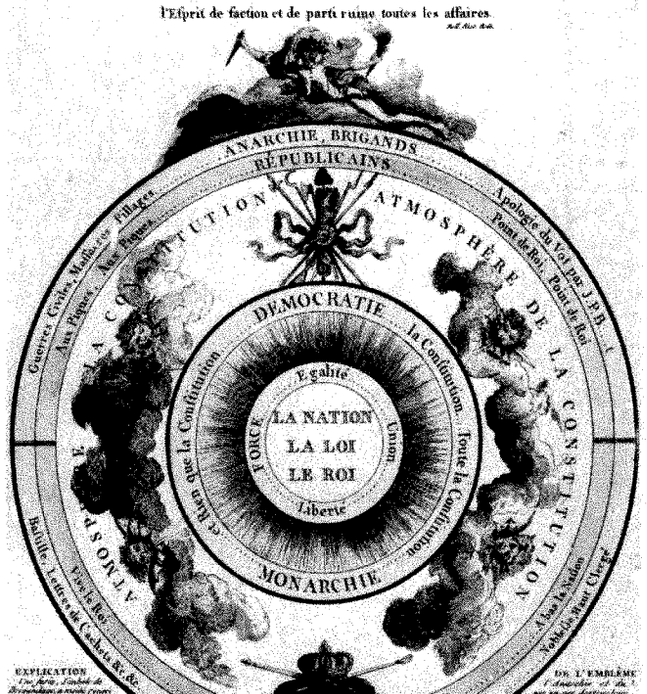
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1687 and all that

A. Rupert Hall

Revolution in Science. By I. Bernard Cohen.
Harvard University Press: 1985. Pp.656. $25, £22.75.

FOR a considerable period of time historians and philosophers, but not to any extent working scientists, have debated the question: has the development of modern science been steadily continuous, or has it occurred by leaps and bounds with essentially level passages in between? The anonymous features that can be counted — numbers of scientists or journals, expenditure on research — show a reasonably smooth (and mutually comparable) exponential increase; but at the other extreme, selecting individuals, there is a universal belief that some have achieved far more than others. We speak of bosons and fermions, of amps and volts, of Newtonian mechanics and Mendelian genetics, and so in the ordinary discourse of science honour one name in a generation while forgetting all the others. Few doubt that despite the regularity of statistics the scientific papers of a Freud or an Einstein have meant more than the publications of many competent professional scientists. Moreover, the sense of singularity attaches to movements and institutions as well as to individuals: to the group of theoretical astronomers beginning with Copernicus and ending with Newton; to the Cavendish laboratory in the early part of this century; to the group of French chemists headed by Lavoisier who produced the *Méthode de Nomenclature Chymique* of 1787. There are networks of people who have collectively, so to speak, brought a given piece of science to the boil, and institutions that have maintained ebullition for a generation.



Astronomy as metaphor — political structures represented as a cosmos in the "Astronomical System of the French Revolution" (c. 1790). Nation, law and king replace the Sun at the centre of the system. (Reproduced from Revolution in Science.*)*

Boiling is clearly a different phenomenon from the slow and steady rise of temperature preceding it. Twenty odd years ago Thomas S. Kuhn generalized an alternation of steady and revolutionary states in the history of the different branches of science into a normal, indeed perpetual feature of science, at least for the "modern" epoch. Now his neighbour in Cambridge, Massachusetts, I. Bernard Cohen, has perfected a spacious study of the whole idea of revolution. Revolutions of the hours, of the planets, of states, of politics, of philosophy, are all gathered into his net but not (perhaps because the vehicles of expression are different) revolutions in painting and music. A whole chapter is given to the German equivalents of the romance noun "revolution" and another to the common (though erroneous) dictum that Immanuel Kant qualified his revolution in metaphysics as "Copernican" (Kant incidentally used the word "revolution" on 57 occasions and a German synonym such as "Umlauf" on 108!). The history of the phrase "industrial revolution" and its appropriateness in historiography are discussed in one chapter, the question of whether Alfred Wegener effected a revolution in the earth sciences in another. It is clear, even from these few examples, that the various chapters of the book are not on all fours with one another: some seem to be much concerned with *language* (as employed in exposition or apologia, in history or in philosophy), others with *judgement* (contemporary or later) of the significance of particular ideas or investigations.

It is hardly surprising that some great scientists (Einstein is a prominent example) have preferred the evolutionary to the revolutionary view of scientific progress, as Newton did too in his phrase about standing on the shoulders of giants, or that the idea of successions of revolutions in the sciences has commended itself more to historians, philosophers, sociologists and journalists than to the more modest of the innovators themselves. Again, Cohen documents learnedly the truth of the fairly obvious expectation that *talk* of revolutions in science has tended to become more usual as the *fact* of political revolution has become more common and traces the increasing frequency of political metaphor in the discourse of science itself. (The vivid case of the great cell pathologist and political activist Rudolf Virchow has been recently analysed by Renato Mazzolini.) Any suggestion, however, of a direct connection between sociopolitical revolution and revolution in science (considering, for example, the simultaneity of the special theory of relativity with the abortive Russian revolution in 1905) is surely to be regarded with scepticism: the Soviet Union, no mean exporter of political revolution, has tended towards conservatism in the wider issues of science, following the lead of Lenin.

Bernard Cohen's monumental scholarship makes evident in overwhelming detail the currency, since the late seventeenth century, of the notion that certain discoveries or theories have "revolutionized" science. His choice of examples is wide both in time and in topic: in sheer variety of riches this book has few parallels. From John Freind's affirming Harveian influence upon the treatment of amputations to the astronomical system of the French Revolution, it embraces a wealth of intriguing curiosities. Broadly, however, biology suffers in comparison with the physical sciences: the names of Schleiden and Schwann occur only in a quotation from Friedrich Engels. The late-nineteenth and twentieth century revolution in the medical sciences is silently passed over.

Not that one needs more examples, for it might be said that Cohen has both opened and wrapped up the history of scientific revolutions. Of course, one may well dispute his assessment of some well-known historical episodes, about which Cohen himself seems not always fully consistent.

Most curious is his endorsement of the view (first stated by Derek Price in 1957) that Copernicus was no revolutionary. It seems odd to minimize the cosmological and epistemological significance of Copernicus; if the Pythagoreans had long preceded him, Newton (with less occasion, we may think) allowed as much with respect to the theory of gravitation, for he thought that the Pythagoreans must have understood this also. No greater tribute could be paid to Copernicus than that implied in Newton's conclusion to the first Scholium of *Principia Mathematica* (1687): I wrote the following treatise, he declared, with the object of elucidating true motions and causes in nature from apparent causes and effects, and conversely of elucidating causes and effects from both true and apparent motions. If Copernicus had not proposed this problem, who did?

The Newtonian system without Copernicus as the first of the "giants" is indeed inconceivable. Bernard Cohen's difficulty arises from the perhaps inavoidable confusion between the idea of revolution as the work of an individual (Lenin, Lavoisier) and the idea of revolution as the fruition of a movement (bolshevism, pneumatic chemistry). Sometimes Cohen seems virtually to be defending the "great man" concept of change, or even (in his discussion of Wegener) the "neglected genius". More frequently, he seems to mark the great men on a scale of revolutionary activity or fervour: thus Bacon's great contribution to methodology "did not constitute a revolution in *science*"; Gilbert "did not establish a new science", unlike Galileo, "a heroic figure of the Scientific Revolution", although even "Galileo's revolution was not complete". Descartes's revolution "did not last" and "it seems clear there was no Keplerian revolution in science before 1687". One remembers that Alfred was a Good King. What is historically important is an understanding of how the separate, even disparate, endeavours of individuals contemporaneous with each other or in quick succession, culminate in some particularly noteworthy and well-founded shift in our vision of nature, for few historians believe that scientific revolutions are literally effected single-handed by a unique scientist. And so to me, for example, it makes good sense to speak of a "revolution in geology" between about 1790 and 1840, whereas talk of a "Lyellian revolution" would be unjustifiable. Or again, it is more constructive to consider the triumvirate Faraday–Maxwell–Hertz as a movement, than to try to weigh out the praise for innovation due to each.

Like many large historical works, Bernard Cohen's book excels as a quarry, rather than as a coherent argument. He is likely to have the last word for a long time to come. □

A. Rupert Hall is Emeritus Professor of the History of Science and Technology at the University of London.

Agents of change

S. Venitt

Chemical Carcinogens, 2nd Edn. ACS Monograph 182.
Edited by Charles E. Searle.
American Chemical Society: 1984. Pp. 1,373. $129.95 (North America), $155.95 (elsewhere).

UNTIL recently, the study of carcinogenesis has been largely correlative and phenomenological. What does chemical A do to biological systems X, Y and Z? Does B do it better and is it metabolized to C? What happens when you apply A before B, or B before A, or mix A and B together? And so forth. This approach has been very fruitful: we now know, for example, that most carcinogens are genotoxic and that most non-carcinogens are not; that many carcinogens are themselves electrophiles or are metabolized to electrophiles; that some substances "promote" tumours but do not initiate them; that many carcinogens are organotropic; and that carcinogenesis is a multi-step process. Much of this information was gathered together in the first edition of *Chemical Carcinogens*, published in 1976.

The study of the mechanism of carcinogenesis was transformed and became at once both analytical and synthetic when two groups independently showed that NIH-3T3 cells could be transformed *in vitro* by transfection with DNA from a human bladder tumour, and that a single base change within a proto-oncogene was in some cases sufficient to activate the oncogene. There has followed a flood of research (which shows no sign of abating) which stresses the importance of both point mutation and chromosomal rearrangement in the activation of proto-oncogenes. The idea that DNA is the critical target for carcinogens has thus gained support from two different streams: the older, painstaking, sometimes stumbling correlative approach, and the rather more breezy and with-it analytical methods of cell biology and recombinant DNA technology.

Nevertheless, "descriptive chemical carcinogenesis" will not go away. We still need to know whether chemicals, new or already in use, are likely to be carcinogenic. In this light appears the second edition of the American Chemical Society's *Chemical Carcinogens*, which offers an expanded view of the descriptive approach embodied in the first edition.

ACS *Chemical Monographs* are intended to serve two main functions: (i) "to make available to chemists a thorough treatment of a selected area in form usable by persons working in more or less unrelated fields to the end that they may correlate their own work with a larger area of physical science"; and (ii) "to stimulate further research in the specific field treated". The first edition filled these roles admirably, not only for chemists, but also for a wider audience interested in chemical carcinogenesis. This second edition, in two well-indexed and richly-referenced volumes, is a valuable update and expansion: there are now 22 chapters (all by leading specialists in each field) instead of 16.

Included are completely new chapters on cancer epidemiology, DNA–carcinogen–metabolite interactions, halogenated organic compounds, inorganic carcinogens, environmental *N*-nitroso carcinogens, triazenes, hydrazines, azo and azoxy compounds, aflatoxins, fusarial mycotoxins, carcinogenic medicines, and the inhibition of chemical carcinogenesis. Other topics which were covered in separate contributions in the first edition (for example, metabolism of carcinogens, carcinogens in plants and microorganisms) are now spread over several. Although much of the core of the first edition remains (polynuclear aromatic hydrocarbons; soots, tars and oils; aromatic amines and their epidemiology; laboratory hazards; alkylating agents; mineral fibres; *N*-nitroso compounds; bracken carcinogens; carcinogens in food; bioassays and short-term tests) several important topics are omitted, such as tumour promotion and co-carcinogenesis, endocrine aspects of carcinogenesis and respiratory carcinogenesis. This is a pity, and it is fair to assume that the vagaries of deadlines and non-availability of authors dictated the final content.

Much of the material consists of detailed and very helpful tabulations of carcinogens and descriptions of their metabolism and biological activity, with much emphasis on structure–activity relationships. In a text of this size and scope there are bound to be some contributions which are more thorough or more readable than others and it is tempting to give each of them marks out of ten. Although I have resisted the urge I must nevertheless single out Lawley's contribution on carcinogenesis by alkylating agents as the chapter which gives the deepest and broadest view of chemical carcinogenesis as it stood in 1981–1982. Viewing alkylating agents as "archetypal carcinogens", he provides us with a masterly, wide-ranging review, which includes discussions of chemical reactivity, dose–response relationships, mechanisms of mutagenicity, short-term tests, DNA repair, models of carcinogenesis, alkylating agents and human cancer — and, as a final flourish, squeezes in (in a note added in proof) a short account of the first reports of activation of proto-oncogenes by point mutation.

Orchestrating such an encyclopaedic text must have been an awesome task. The editor, Dr C.E. Searle, must be congratulated for producing such a useful revision of an already indispensable work. □

S. Venitt is a member of the Section of Chemical Carcinogenesis, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surrey.

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TISSUE CULTURE AND RETICULOENDOTHELIAL SYSTEM

Edited by P. Röhlich and E. Bácsy

Proceedings of the Joint Congress of the European Tissue Culture Society and the European Reticuloendothelial Society, Budapest, 9–13 May 1983.

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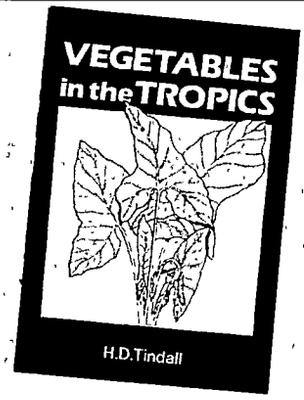
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Faulting the heavens

David W. Hughes

Under Newton's Shadow: Astronomical Practices in the Seventeenth Century.
By Lesley Murdin.
Adam Hilger: 1985. Pp. 152. £16.75, $26.

WHAT was it like being an astronomer in the Newtonian age (1643–1727)? There is no easy answer to the question as there was in fact only *one* full-time professional and even he, the Reverend John Flamsteed, diverted himself with his parish at Burstow, instrument-making and tide tables. The remainder were scientific generalists who saw little reason to specialize and flitted from subject to subject apparently as the whim took them. Even Edmond Halley, probably next in line for the astronomer appelation, spent only a quarter of his time studying lunar motion, planetary transits, occultations, eclipses, southern stars, proper motion and comets. For the rest he enjoyed the pleasures of meteorology, geomagnetism, physics, pure mathematics, and the editing and translating of ancient mathematical texts, not to mention his duties at various times as a captain in the Royal Navy, a diplomat and the deputy comptroller of the Chester Mint.

The Newtonian age was a time of transition. The pre-Civil-War concept of a magical and mystical world was tackled by men with a desire to avoid speculation and a preference for observation and the accumulation of data. Aristotelian views of a changeless, perfect (and extremely boring) heaven crumbled with the invention and development of telescopic instruments. Astronomers were provided with a goal, the discovery of an easy method of finding the longitude of a ship at sea, and the government were even prepared to spend money to find an answer. In the new Royal Society the scientific generalists found an excellent means of exchanging ideas, meeting like-minded friends and publishing their results. The gap between amateur and professional astronomer was only just beginning to open up. All that was needed was a grammar school education, enthusiasm, a cheap refracting telescope, an altitude measurer and a clock.

Lesley Murdin has tried to introduce us to the sort of men who found astronomy fascinating. The list is mercifully short — Flamsteed, a dozen or so university dons (for example Gregory, Halley, Hooke, Newton, Wallis, Ward, Whiston and Wren) who spent a fraction of their time with stars and planets, another dozen interested amateurs (among them Towneley, Derham, Pound, Gray and Moore) who had also to work for a living, and a final dozen "observatory technicians" (Crosthwait, Denton, Leigh and Sharp, for instance). From this narrow base Murdin has drawn a series of sweeping conclusions. "The number of astronomers who never married seems high and might suggest a general tendency to put work before relationships." Astronomers were independent thinkers and creative but generally had "an interest in objects rather than people" and "found difficulty in cooperating with others". They exhibited "a tendency to solitariness".

At the end of the book I was left with a very sketchy idea of the motivation of astronomers as such, and of their specific problems of learning, observing, calculating, interrelating, publishing, teaching and instrument-making. Little is said about their standing in the community. Murdin has provided us with a readable gallop through the astronomical seventeenth century but one which makes clear that much more work needs to be done in this field. Additionally, the lack of references is frustrating, often leaving the reader with no idea of the provenance of a quotation. An index that only lists proper names also hints at a job half done. □

David W. Hughes is Senior Lecturer in Astronomy and Physics at the University of Sheffield.

Diverse questions

Elvin A. Kabat

The Antibody Enigma.
By Thomas J. Kindt and J. Donald Capra.
Plenum: 1984. Pp. 270. $35, £33.25.

A PERSON wishing to learn about the structural and genetic basis of antibody diversity and antibody specificity might well be overwhelmed by the literature. *The Antibody Enigma* is a good place to start to get to grips with the subject.

The authors do not follow a strictly historical treatment but attempt to present the subject in the light of the working hypotheses considered and discarded as new experimental data became available. As they state in the preface, "*The Antibody Enigma* is a somewhat personal view of the antibody diversity problem from two investigators who have spent the past 18 months trying to penetrate the enigma". Presumably, much of this was done in face-to-face discussion, to formulate points of agreement and resolve differences. I wish I could have listened in. It would have been difficult not to interrupt, however, for despite enormous progress this is an area which is still filled with differences of opinion, of interpretation, and of judgement about the relative weight to be given to the various mechanisms contributing to the vast repertoire of antibody combining sites.

After defining the problem, the authors set down the serologists' and biochemists' approaches to it and their unique features. In subsequent chapters, they analyse "polar", specifically the germ-line versus somatic mutation hypotheses, and the "maverick" solutions (those based on data not in accord with either). This is followed by discussion of the contributions of molecular biology and a final chapter entitled "Antibody Diversity: A Contemporary Solution". Many key references are provided, but the authors often mention individuals by name with no reference and at many points I found it frustrating not to be able to pin down the source of some of the statements.

In the preface, Kindt and Capra apologize if they have "offended anyone by omitting their own contribution. "; given the vastness of the literature they may well have. In line with the general trend among molecular biologists who have established so many ways of generating antibody diversity that they no longer consider how much of this diversity is related to antibody complementarity, the authors do not deal with this question. More understandably, there is no hint of the ferment of activity consequent upon the cloning and sequencing of the T-cell receptor (the book was in press when all the excitement began). But clearly this is a volume that should be read by individuals wishing to enter the field. □

Elvin A. Kabat is Higgins Professor of Microbiology and Professor of Human Genetics and Development at Columbia University.

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Growing analysis

Bernard Wood

Size and Scaling in Primate Biology.
Edited by William L. Jungers.
Plenum: 1985. Pp.491. $69.50, £66.

THIS book is about allometry, which, in both the literal and the metaphorical sense, is a growth area. Literally, because it is the study of the effects of an increase in the size of organisms on their shape. Metaphorically, because in recent years allometry has taken over from multivariate morphometrics as one of the main analytical preoccupations of primatologists and hominid palaeontologists.

A system is allometric when a variable (or variables) measuring overall size has more than a random effect on another measure, be it tooth size, gut area or limb length to name but a few of the associations discussed in the book. Various contributors try to classify allometric studies, but they can be broadly divided into three categories. The first, which I shall call "organism-based", and which includes ontogenetic and intraspecific allometry, deals with the effect of size on the shape of an organism while holding functional and ecological variables partly, or wholly, constant. The second category is "system-based". In this approach groups of organisms are used as tools to investigate the influence of size on function and ecological context. Such studies are usually broad and interspecific, and include the analysis of grade shifts; that is, changes in the intercept as well as differences in the slopes. Research of the third category makes use of the empirical observation that variables are correlated to predict one variable from another. Often this stands on its head the notion of a "dependent" and an "independent" variable, for what is usually regarded as independent (body size, for example) is often predicted from dependent variables such as tooth size. This final method is applied most often in palaeontological studies which are perforce based on fragmentary material and may use ontogenetic, intraspecific or interspecific associations.

The twin aims of the book are to assess past progress in allometric work and to review the future prospects. Several of the contributors discuss the role of allometry in evolutionary change. Is evolution simply the transposition of the derived form to a position up or down the ontogenetic slope (Shea; Cochard), or is it more than one process — perhaps "short term", involving relatively simple adjustments of growth, and "long term", involving adjustments of function and grade, and perhaps dictated by general metabolic constraints (Martin and Harvey; Ford and Corruccini)? Although not always in agreement with him, I especially enjoyed Shea's contribution. His work on ontogenetic allometry is stimulating, but even though my prejudices about the pattern of hominid evolution are reinforced by the findings of his excursion into that minefield, I remain unconvinced that ontogenetic allometry "explains" all evolutionary change. Lande provides a much needed genetic perspective on allometry, and Leutenegger and Cheverud's account of the effect of size on sexual dimorphism in primates is both an analytically ingenious and intellectually rigorous attempt to understand the mechanism of the strong positive allometry that is seen in sexually dimorphic species.

A number of the chapters provide overviews of particular systems — Martin *et al.* on the gut, Jungers on the skeleton, Larson on organ weights, and Gingerich and Smith on dentition. Hidden within the latter is an admirably concise discussion of the vexed question of line-fitting. This is a point which is addressed by several of the contributors (Martin *et al.*; Wolpoff; Steudel and Ford; Corruccini). Wolpoff takes an admirable, but misguided, swim against the tide of consensus. The majority vote of the contributors is against him. Least squares regression is a technique best reserved for prediction, with association being best studied by the major axis technique, or by one of its variations. Smith and Steudel each address the problem of using apparent empirical allometric associations between variables to predict body size in fossil taxa, with Smith tackling *Gigantopithecus* and Steudel the fossil hominids. They both advocate what Smith has described as the "narrow" allometric approach, but differ in their attitude towards the advisability of averaging the results of several attempts to estimate size. I side with Smith on this; the average of three poor estimates is not likely to provide a good one.

The remaining contributions include investigations of the influence of size on limb design (Preuschoft and Demes; McNeill Alexander), energetics (Heglund) and polygyny (in which Clutton-Brock rightly criticizes others for offering circular arguments to explain sexual dimorphism and polygyny, and then proceeds to offer another himself!). Bookstein contributes a paper on how to measure shape change and Fleagle furnishes a general review of allometry. Finally, Armstrong provides an excellent review of the relationships, causal or otherwise, between body size and brain size and structure.

Jungers is to be congratulated on his editorial efforts. The 20 contributions necessarily vary in scope, quality and novelty, but are uniform in style and layout, and remarkably free of typographical errors. Altogether this is a suitable volume to mark the coming of age of primate allometry, and it is only three years late to mark the golden jubilee of Huxley's seminal book on relative growth. □

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Clinical exchange

Robert Walgate

Harrap's French and English Science Dictionary.
Harrap: 1985. Pp.302. £17.50.

IN 1982, when socialism was the rage again in France, the French became passionate about protecting their language "as a language of science". The then minister of research even established a priority action, a *programme mobilisateur*, to that end, and launched an ambitious plan for a great new French-language encyclopaedia to embrace all knowledge, as the encyclopaedists of the eighteenth century had done before him. Heady days! The programme and the encyclopaedia project still exist, but somehow the passion has gone. The encyclopaedia has had difficulty finding a publisher. French scientists speak English at international conferences in France, against a ministerial directive. And the preferred language of publication, for anyone wishing to be heard outside France, is still English.

Nevertheless a considerable amount of good science still appears only in French — for example in the *Comptes Rendus* — and the publication of a new French–English (and English–French) science dictionary is an event of some interest.

However, the readership of the dictionary may be more limited than Harrap's expect. The book includes relatively few technical terms in the physical as opposed to the medical and biological sciences (with their naming of parts, and organisms and maladies), and since the translations of the physical terms are often self-evident (astronomy — *astronomie*), this dictionary will prove of real help mostly to biologists and clinical scientists. Picking a page at random confirms this impression: out of 50 definitions nearly half are in biology, zoology or medicine; 15 per cent in physics, chemistry and astronomy; 15 per cent in geology and oceanography; and the rest are general terms. As further examples, the book offers no translation of "hadron" or "lepton", and classifies "neutrino" as a biological term; includes "geode" but not "geoid"; and seems not to have heard of the "magnetosphere" or a "white dwarf". Nevertheless with much French clinical science in particular still published in French, this dictionary will find a role in easing the two-way traffic between English-speaking biologists and clinical scientists and their French counterparts. □

Robert Walgate is European correspondent of Nature.

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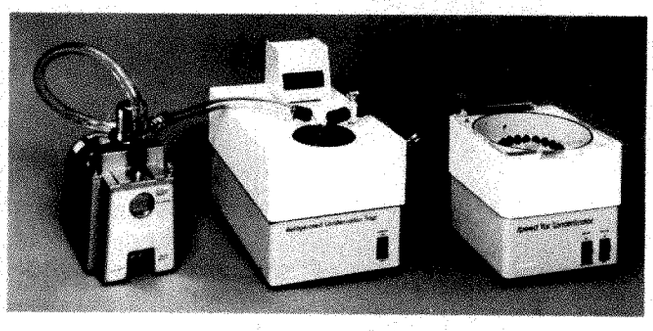
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TITLES IN AGING

MOLECULAR BASIS OF AGING

Edited by A. K. Roy and B. Chatterjee
1984, 416pp., $29.50/£24.00 (UK only), ISBN: 0.12.601060.9

Recent advances in molecular biology have led to a new understanding of the molecular basis of aging at the level of gene expression and gene organization. This volume, contributed to by noted experts in the field, presents state-of-the-art developments in this rapidly expanding area of biochemical gerontological research.

In chapters in which various aspects of the molecular basis of aging are discussed, background information is presented, followed by reviews of the results of recent investigations and the significance of these results in the development of new concepts.

The 1984 Sandoz Lectures in Gerontology

THRESHOLDS IN AGING

Edited by: Professor M. Bergener, Dr. M. Ermini, Dr. H. B. Stahelin
1985, 322pp., $25.00/£22.50 (UK only), ISBN: 0.12.090160.9

This volume provides a record of an international meeting convened in Basle, Switzerland, in February 1984, at which leading scientists presented current work on biological, neurological and epidemiological aspects of the aging process. The contributions to this book reflect the exciting, multidisiplinary approach of modern gerontology.

BIOLOGY OF MENOPAUSE

The Causes and Consequences of Ovarian Ageing

R. G. Gosden
1985, 188pp., $37.50/£29.00 (UK only), ISBN: 0.12.291850.9

All the diverse aspects of ovarian ageing in our own species and in animal models have been brought together for the first time in this concise volume. The physiological and biochemical processes responsible for the deterioration of ovarian function, the consequential loss of fertility, and somatic effects of postmenopause are fully explained for biologists interested in reproduction and gerontology and for clinicians concerned with fertility in middle age and hormone replacement therapy.

TITLES IN HUMAN PHYSIOLOGY

Monographs of the Physiological Society No. 42

MEMOIR ON THE PANCREAS

And on the Role of Pancreatic Juice in Digestive Processes Particularly in the Digestion of Neutral Fat

Written by C. Bernard
Translated by J. Henderson
1985, 131pp., $48.00/£36.00 (UK only), ISBN: 0.12.092880.9

This volume, the first English translation, details these studies and follows Bernard's line of hypothesis through many experiments. Claude Bernard describes the comparative anatomy of the gland (beautifully illustrated by colour and black and white engravings) and demonstrates both the collection and the chemistry of the pancreatic juice. The Memoir also includes descriptions of human pancreatic disease, and a good deal of speculation on the role of the pancreas in humans and the rest of the animal kingdom, while at the same time it criticises severely a large number of his contemporaries who disagree with him.

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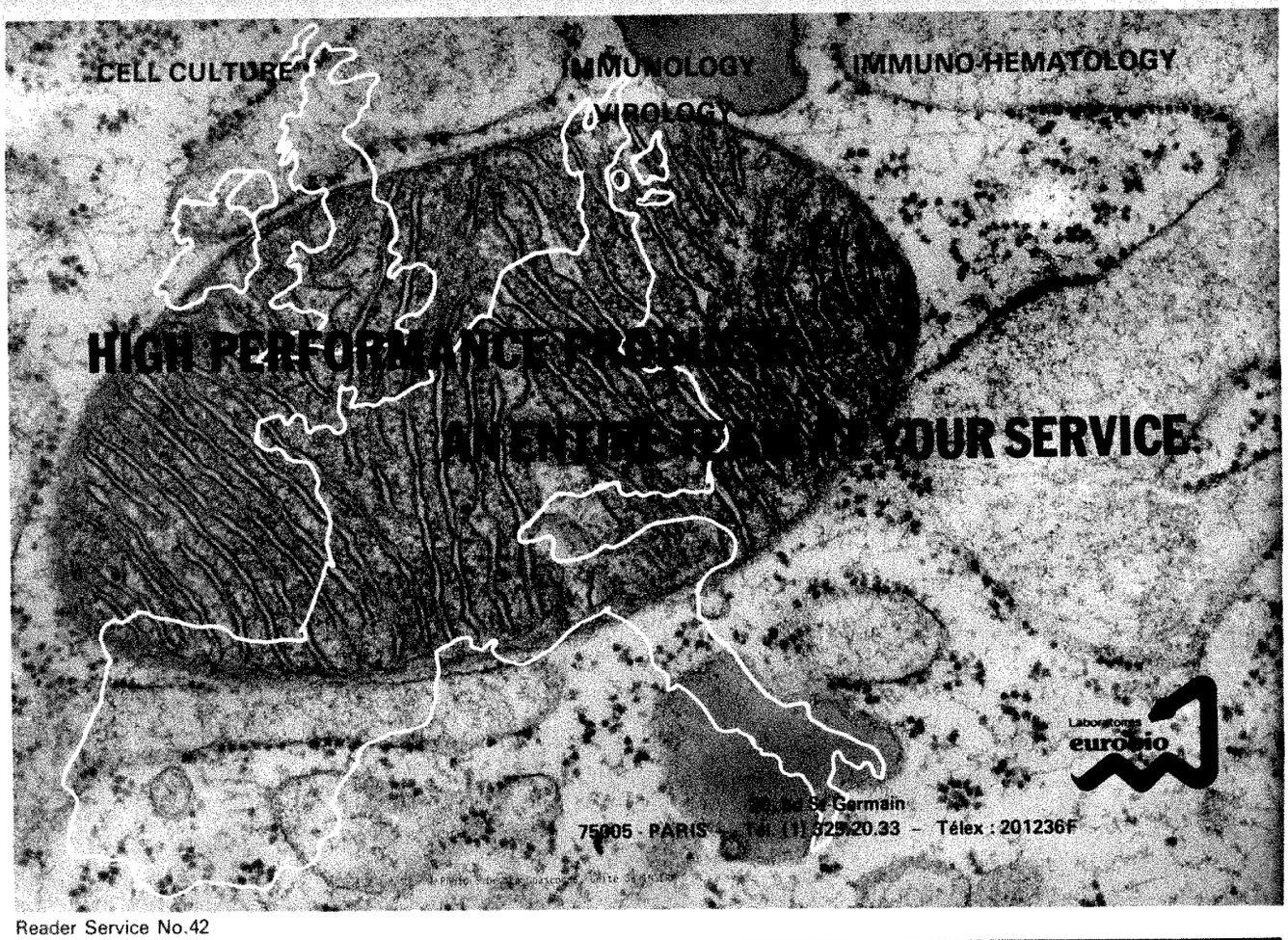
J. M. Tanner, R. H. Whitehouse, N. Cameron, W. A. Marshall, M. J. R. Healey and H. Goldstein
1984, 116pp., $49.50/£33.00 (UK only), ISBN: 0.12.683360.5

Accurate assessment of 'bone age' in children is essential in diagnosing and monitoring treatment of children with growth disorders. In this second edition, new data from very tall, short and delayed children have allowed revised formulae for predicting adult height from bone age whose greater accuracy renders those of the first edition obsolete. An essential tool for those working in paediatric medicine.

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Reader Service No.18

Tissue culture equipment

New items available for the tissue culture laboratory include carbon dioxide incubators, novel bioreactors and media.

● Imperial Laboratories of Salisbury, working with the Wessex Regional Cytogenetics Unit at Salisbury General Hospital, have developed a new medium for use in cytogenetics procedures. This medium, based on Iscove's modificaiton of Dulbecco's MEM, has the folic acid level reduced from the normal 4.00 mg to 0.01 mg per litre. The folate-deficient Iscove's medium has proved successful in producing mitotic indices, with well-spread elongated chromosomes suitable for high resolution banded analysis, and is now used routinely at the Salisbury hospital for all lymphocyte cultures and also for bone marrow specimens from patients with leukaemia.
Reader Service No. 100.

● A new cell culture medium, Ventrex PC-1, is now available from Tissue Culture Services to complement the Ventrex HL-1 medium. Like HL-1, Ventrex PC-1 is a low-protein, serum-free medium which has been specially formulated using defined components for optimal cell growth, while maintaining the lowest possible protein content (less than 750 μg per ml). The PC-1 medium is intended for the culture of primary cells and cell lines and has been successfully tested on many widely used cell lines. Ventrex PC-1 is available as a sterile liquid base medium, with a sterile frozen supplement, in 1 × 100 ml and 2 × 500 ml packs. When stored under suitable conditions, the components are stable for 120 days; when the components are combined to form the complete medium, it remains stable for 45 days at 4°C. The medium is also available in a 10-litre component package for users who prefer to prepare their own media.
Reader Service No. 101.

● Opti-MEM I from Gibco is a multi-use reduced serum medium which supports optimal growth with reduced fetal bovine serum for a wide variety of mammalian cells. Most cells routinely cultured in serum-supplemented medium may be directly transferred into Opti-MEM I with a minimum of 50% reduction in serum. Opti-MEM I is performance-tested for cloning efficiency and growth promotion using various mammalian cell types, including murine myelomas, hybridomas and adherent cultures. Opti-MEM I has a shelf life of six months from date of manufacture, and is easily prepared in only three steps: add 2-mercaptoethanol (included); mix thoroughly; and add fetal bovine serum.

These notes are based on information provided by the manufacturers. Use the reader service card bound inside the journal to obtain further information.

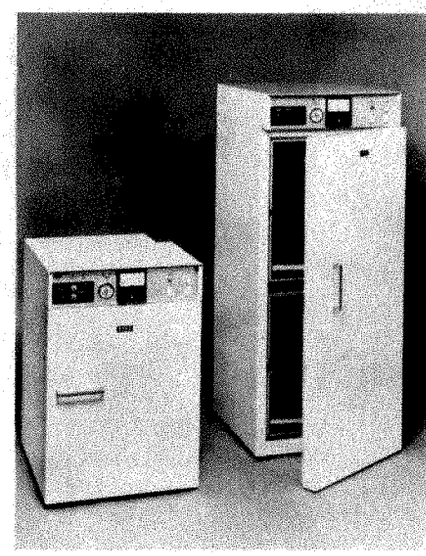


Clandon's new cell counter.

● The range of Diffcount II microprocessor-controlled differential cell counters has been extended with the introduction of Comp-U-Diff, a direct differential data entry terminal. Manufactured by Modulus Data Systems, and available in the UK from Clandon Scientific, the Diffcount II/Comp-U-Diff allows efficient, direct, computer entry of the differential counts, RBC morphology, MLSO ID code, specimen identification and general comments. Straightforward instructions and suggested software enable Comp-U-Diff to be readily interfaced with an existing laboratory computer.
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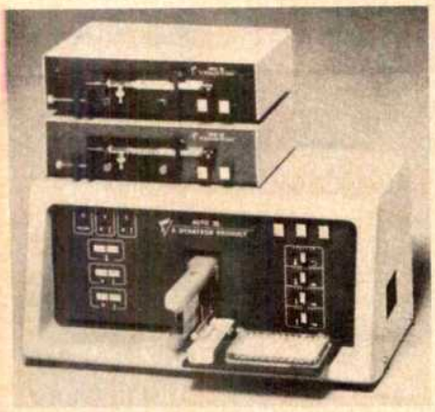
● MBR Bioreactor AG has introduced a membrane bioreactor for the cultivation of mammalian, plant, hybrid cells and microorganisms. The Membroferm consists of a medium chamber, a cell chamber and a product chamber which are fitted with a special net support which acts as a carrier to fix the cells as well as supporting the membranes. This membrane stack is held in position by stainless steel endplates which are fitted with connections to the conditioning vessel where the medium can be continuously treated to maintain the correct environment for growth of the cells. Cells are introduced into the cell chamber which is separated from the medium chamber by a membrane on one side and the product chamber with a different membrane on the other side. This allows the cells to receive nutrients and gases via the medium chamber and to remove the products of metabolism or toxins via the product chamber. The technique has achieved very high cell densities with mammalian cells.
Reader Service No. 104.

● Plant diseases can cause crop losses ranging from slight yield depression to complete crop failure. To counter this Twyford Plant Laboratories offers 'disease indexing' services to plant breeders, propagators, seed producers and growers. Using this service it is possible to 'clean-up' a plant or crop, for further use as a mother stock for further propagation, or as parent plants for high quality seed production, for instance. TPL's indexing service is based on the use of plant tissue culture. Viruses are identified by a range of techniques including ELISA, PAGE and occasionally electron microsopy. Fungal diseases are detected using selective nutrient media. For more details on TPL's range of techniques based on an expertise in plant tissue culture use the reader service card.
Reader Service No. 105.



Tissue culture incubators from LEEC.

● In the tissue culture laboratory, CO_2 incubators with high humidity levels are constantly subjected to contamination by yeasts, moulds and all forms of airborne bacteria. The new LEEC range of automatic CO_2 incubators incorporates a self-sterilizing facility which by-passes the normal heater controls and holds the temperature at 90°C. On completion of a cycle the control is transferred back to the normal controllers and the incubator rapidly re-stabilizes at pre-set temperatures. The range includes models of 150 litres and 320 litres capacity, each featuring dual proportional voltage controllers, safety overides, heated inner glass door, solid state CO_2 detection and a self-sterilizing facility. The LEEC range of incubators is manufactured with high-grade stainless steel throughout.
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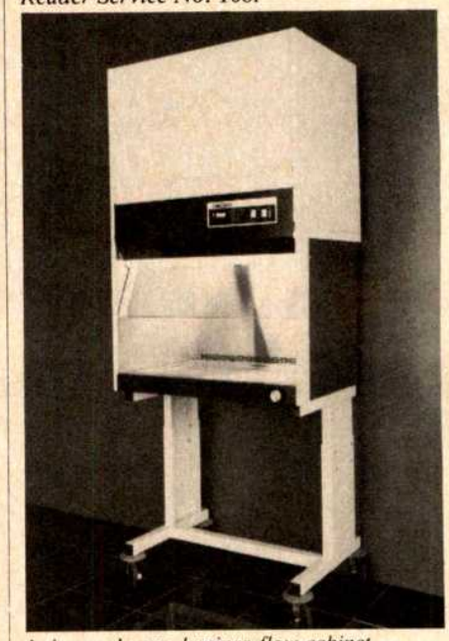
The Dynatech Autodiluter with features aimed at tissue culture.

● Through the use of modular, solid-state logic controls, the Dynatech Autodiluter III can be programmed to carry out such diverse procedures as ELISA washing and aspirating, haemagglutination, complement fixation, microtitration and a variety of tissue culture applications. The Autodiluter III can be linked to Dynatech's Dynadrop multi-reagent dispenser, for simultaneous addition of up to 12 different reagents to a row of microtitre plate wells. Aspirator dwell time can be controlled between 2 and 6 s and the downward travel of the head limited, a feature necessary in tissue culture where the Microdiluter and aspirator tips must not touch the bottom of wells.

Reader Service No. 107.

● Dystonia is a neurologic disease consisting of sustained twisting movements and postures. It may be inherited, sporadic, or secondary to other nervous system diseases. The Dystonia Clinical Research Center at Columbia University has established a Fibroblast Tissue Resource Facility. Fibroblasts from patients with genetic and sporadic dystonia (as well as controls) are cultured and stored in liquid nitrogen; samples are available to investigators with established probes for biochemical markers. The Columbia centre invites scientists to utilize the tissue bank without charge. If interested, investigators should send a brief description of assays to be carried out to: Stanley Fahn, Director, Dystonia Clinical Research Center, Neurological Institute, 710 West 168th Street, New York, New York 10032, USA.

Reader Service No. 108.



Labconco's new laminar flow cabinet.

● Labconco's new range of laminar flow cabinets — the Purifier Class II — is well suited to tissue culture manipulations including inoculation of viruses. A stainless steel working area and coved and dished work surface contribute to operator protection. Other features include a variable speed motor, easy-to-read differential pressure gauge monitor, Hepa filters readily accessible for replacement, adjustable height and position sash for comfort, remote controlled gas fixture with recessed nozzle and safety valve, and adjustable height stand on heavy duty rubber-tyred castors. All lights are recessed and accessible from the cabinet exterior. The air movement and positioning of the filters places virtually all the cabinet under negative pressure, those areas of positive pressure are surrounded by areas of negative pressure ensuring complete containment in the event of a leakage. The Purifier meets all standards for Class II operation and is supplied with full performance certificates.

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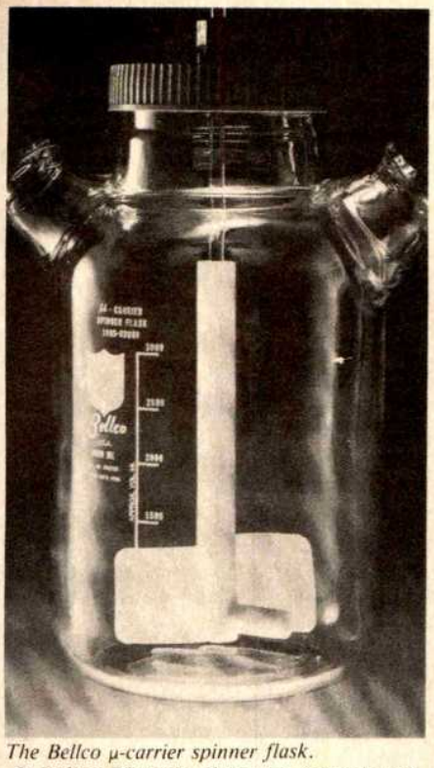
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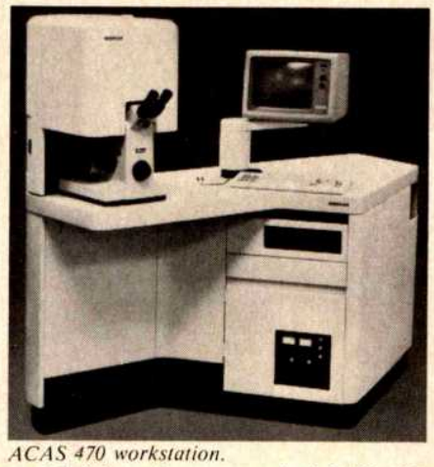
The Bellco μ-carrier spinner flask.

● Bellco Biotechnology has introduced a new line of non-heating, precision magnetic stirrers. The Sci/ERA range is offered in one-, two- and four-position units, each featuring a port to accept computer-generated commands for external control. Stirring speeds are set with a precision ten-turn control, and are ramped up and down to minimize disruption of cell attachment. Two LED displays are incorporated; one indicates stirrer r.p.m., and the other reads out cycle and countdown to show exactly where the system is in relation to internally-controlled cycles. Electronic on/off cycle timers permit precise repetitive cycling. Stepper motors are controlled by pulses generated with such precision that repeatability of settings is ±0.1 r.p.m., 12-volt signal level assures operation at selected speed if the system switches to back-up battery power. In addition, the new Bellco system offers a new stand-by Sci/ERA Power Pack to protect stirrers and valuable cell cultures in the event of failure of the main power supply.
Reader Service No. 110.

● The new 50-watt Model MS-50 Microson miniature ultrasonic cell disruptor is intended primarily for cell disruption, homogenization, and emulsification in the laboratory and classroom. The Microson, from Heat Systems-Ultrasonics, Inc., can also degas liquids, accelerate chemical and physical reactions, and deagglomerate and disaggregate. The unit features a new on/off momentary control switch, and micro cup horn for disruption in total isolation and a micro-Pulsar cycle timer for limiting temperature build up in heat-labile materials.
Reader Service No. 111.

● An eight-page brochure from Beckman describes two compact Microfuge centrifuges used for spinning down small samples fast. The Microfuge 11 and 12 provide a choice of three rotors. The Microfuge 11 has a fixed horizontal rotor; and the Microfuge 12 a bowl rotor. Both can spin the 13.2 fixed angle rotor and the capillary tube rotor.
Reader Service No. 112.

● A modification kit is now available to convert the standard Marubishi fermenter for the growth of cells in continuous culture. The modification is suited to both bacterial and cell cultivation units. All components are interchangeable and do not require additional expense or modifications when different size vessels are used. The standard bench-top fermentor is equipped with temperature, agitation and aeration controls. The transfer of medium is accomplished by pumps which automatically remove medium to be processed and add back medium from a reservoir.
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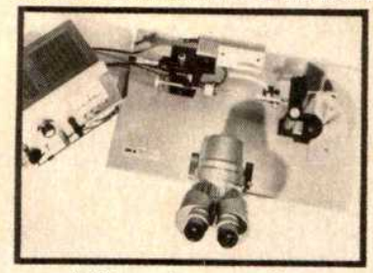


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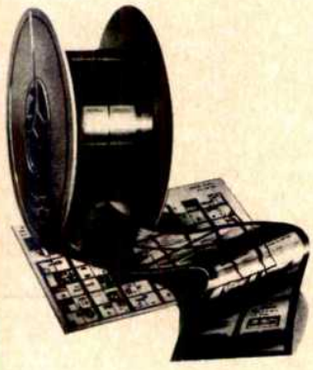
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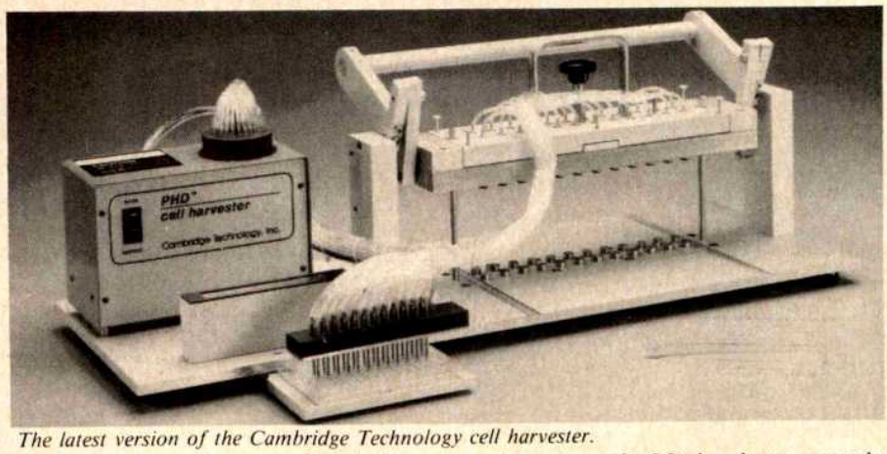
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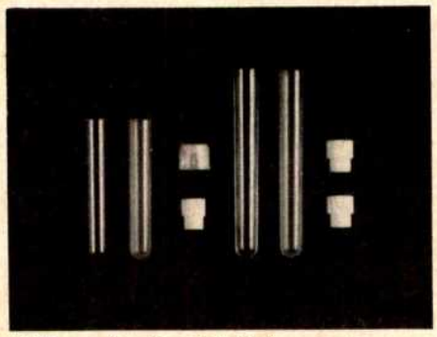
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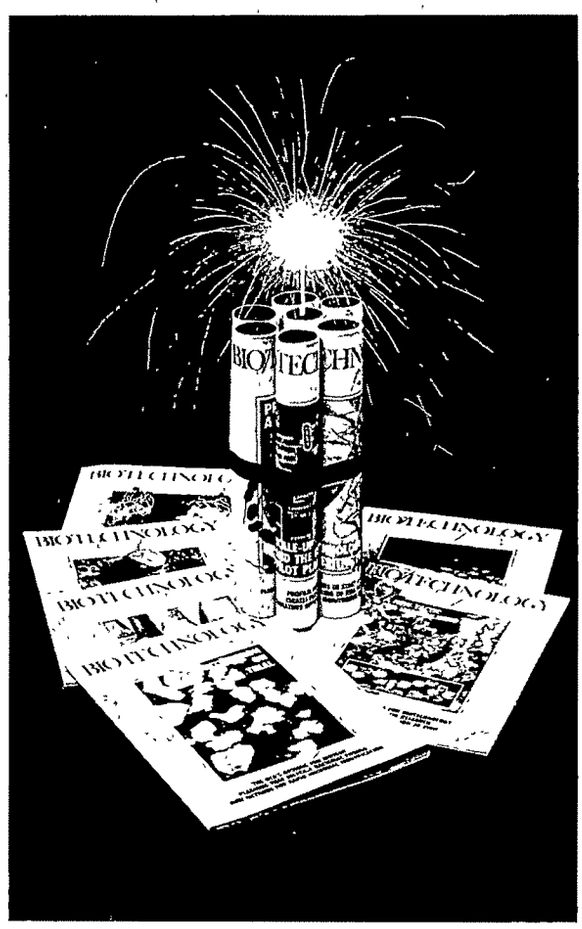
● The typing or classification of a monoclonal antibody's immunoglobulin class or subclass is best done when the antibody is in culture supernatant. Conventional methods of typing (such as traditional Ouchterlony diffusion) cannot be performed on unconcentrated supernatants, but now the new Miles monoclonal typing kits make it possible to utilize the easy-to-use Ouchterlony technique for the determination of the immunoglobin class of mouse or rat monoclonal antibodies in tissue culture supernatants. The kits allow detection of monoclonal antibodies at down to 0.1mg per dl.
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Further particulars can be obtained from the Secretary and Registrar, UC NW, Bangor, Gwynedd, LL57 2DG.

Ten copies of the application (one from overseas applicants), together with the names and addresses of three referees, should be sent to reach the Secretary and Registrar by 1st July 1985. (6669)A

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Scientific enquiries to Dr A Johnstone (01-672 1255 ext 4756) and further particulars and application forms from the Personnel Officer, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE (ext 4873) Closing date 27 June 1985. (6628)A

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Applications and nominations are invited for a Director, Center for Atmospheric and Biospheric Effects of Combustion, located in the Applied Science Division. The scope of the Center is interdisciplinary and includes four major areas: Combustion and Abatement, Atmospheric Pathways and Chemistry, Radiative Balance, and Ecosystem Pathways and Effects. The Director will coordinate the efforts of a number of scientists, including chemists, physicists, engineers, biologists, geologists, meteorologists, and energy analysts to carry out the objectives of the Center; will be responsible for obtaining research support from governmental and non-governmental organizations; will represent the Laboratory at various related national and international meetings; and will also carry out research within the Center. This position requires a Ph.D., and a record of distinguished leadership, outstanding research accomplishments and an international reputation in one or more of the above areas. Experience and background in program planning, development, implementation, administration and evaluation is required. The incumbent should also have experience in obtaining research funds and have a familiarity with organizations that provide these research funds. Salary will be negotiable. The successful candidate will be appointed a Senior Staff Scientist at the Lawrence Berkeley Laboratory.

Applications and/or nominations should be forwarded to: Lawrence Berkeley Laboratory, One Cyclotron Road, Employment Office 90-1042, Berkeley, CA 94720. Please specify Job A/3330. An Equal Opportunity Employer M/F/H. (NW1777)A

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To lead research teams and supervise research students and develop research projects in areas of interest to the NEBC. The major strengths of the Centre are in Fermentation, Microbial Physiology and Downstream Processing of biological products

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Industrial experience would be preferable and membership of a professional institution an advantage

Salary: £13,095 — £16,467 per annum.

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It is expected that the successful candidate will also undertake research projects, especially in aspects of downstream process design

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Closing date for applications in respect of all posts: 24 June 1985

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THE UNIVERSITY OF ALBERTA

invites applications for the

CANADIAN PACIFIC — ALBERTA AGRICULTURE CHAIR IN PLANT BIOTECHNOLOGY

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The successful candidate will be a member of the Department of Plant Science, in the Faculty of Agriculture and Forestry. The Faculty of Agriculture and Forestry has ninety academic members housed in a modern, well-equipped research complex. In addition, within the Facilty of Science, members of the Departments of Botany, Genetics, and Microbiology are currently involved in basic and applied plant research The University of Alberta (approximately 25,000 students) is located in the capital city of Alberta **Please send curriculum vitae, names and addresses of at least three referees, and an outline of research interests, including reprints of relevant publications, to.**

Dr. Mary Spencer
Department of Plant Science
Faculty of Agriculture and Forestry
University of Alberta
Edmonton, Alberta T6G 2P5
Canada

Closing date for receipt of applications is August 31, 1985. Salary will be by negotiation. Appointment will commence on January 1, 1986, or as soon as possible thereafter

The University of Alberta is an equal opportunity employer but, in accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada. (NW1794)A

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Application including C.V , a short summary of previous scientific and educational experience, proposal for future research, list of publications as well as reprints or copies of publications or manuscripts (four copies of each) should be received at Karolinska Institutes Registratorskontor, Solnavagen 1,S-104 01 Stockholm, no later than Thursday June 13th, 1985. Applicants who wish to have a later deadline may submit material so that it reaches the above address no later than July 4th, 1985, provided that they, no later than June 13th, make clear to the Karolinska Institute (same address as above) that they wish to apply for the position, and that they state what material they will submit later

Further information can be obtained from Professor Jan-Oke Gustafsson (Tel. No. 8/7463351) and Dr. Rune Toftgord (Tel. No. 8/7463361). (W1897)A

Tenure track Assistant Professor positions are available in the Department of Pharmacology, University of Iowa, College of medicine. Applications are invited from well-trained individuals who possess the Ph.D. and/or M.D. degree and who are active in an area suitable for development of a vigorous research program related to molecular and cellular pharmacology or more traditional pharmacological disciplines. The University of Iowa is an equal opportunity/affirmative action employer. Submit curriculum vitae and names of three references to:

Search Committee
Department of Pharmacology
Bowen Science Building
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Iowa City, IA 52242

(NW1762)A

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For further details and an application form, please contact Personnel Officer at the address below or telephone (0752) 264639.

Closing date for applications: 21 June 1985.

(6660)A

Plymouth Polytechnic
Drake Circus, Plymouth
Devon PL4 8AA

Bureau of Mineral Resources, Geology & Geophysics

Canberra

MARINE RESEARCH GEOPHYSICISTS

The Division of Marine Geosciences and Petroleum Geology in the Australian Bureau of Mineral Resources, Canberra, has vacancies for two Marine geophysicists, one being at senior level.

The principal research aims of the Marine Division are to define the regional geological framework, petroleum prospectivity and evolution of the sedimentary basins of Australia's extensive continental shelf and margins; further to develop new scientific concepts of basin structures and evolution of passive margins and also the active margins of the South Pacific. The main objective is the stimulation of ongoing programs of offshore petroleum exploration. The Division has recently acquired a research vessel with full multichannel seismic capability and seismic processing centre.

SENIOR MARINE GEOPHYSICIST:

to initiate and lead research and resource assessment programs on the Australian Continental Margins and in Australian Territorial waters.

The successful candidate should have a strong background of research experience and an international reputation. He/she should be experienced in multichannel seismic operations, seismic structural and stratigraphic interpretation and in aspects of geohistory analysis as well as have associated experience in other fields of marine geophysics and petroleum. **(Salary at SPRS/PRS level depending on qualifications and experience.)**

MARINE GEOPHYSICIST:

to undertake research and resource assessment programs on the Australian Continental Margins and in Australian Territorial Waters.

The successful candidates should have a history of research, be experienced in multichannel seismic operations, in seismic structural and stratigraphic interpretation and in at least some aspects of geohistory analysis. Associated experience in other fields of marine geophysics and petroleum would be valuable. **(Salary at SRS/RS level depending on qualifications and experience.)**

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Applications together with full personal and professional details and the names of at least three referees should be sent to:

The Director,
Bureau of Mineral Resources,
GPO Box 378, Canberra ACT
2601, AUSTRALIA.

Applications close 21 June, 1985.

AUSTRALIA

(W1903)A

Cell Biologist (PhD)

elf bio recherches

Elf Bio Recherches, a subsidiary of Elf Aquitaine and Sanofi, specialises in biotechnology. It is looking for a Cell Biologist for its laboratory of animal cell physiology and fermentation. This laboratory (2 scientists and 3 technicians) forms part of the recombinant animal cell technology unit. The position is to investigate the role of physiological factors involved in the expression of genes cloned into animal cells. Candidates should have a profound knowledge of animal cell physiology. Experience in medium or large-scale tissue culture will be an advantage. Knowledge of French would be appreciated. Elf Bio Recherches R&D facilities are located in the Toulouse area, in Southern France. Curriculum vitae and list of publications, together with a handwritten letter of application, should be sent to M. Fabre quoting reference **B/T 2018N.**

PA

(W1900)A

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Tel: Toulouse 63.70.63.

NATIONAL OPTICAL ASTRONOMY OBSERVATORIES

Associate Director-NOAO and Director-Cerro Tololo Inter-American Observatory — Applications and nominations are invited for the position of Director, Cerro Tololo Inter-American Observatory. The position reports to the Director NOAO and carries the added designation of Associate Director, NOAO. The incumbent will be a key member of a management team who plans and guides the development of NOAO as a program characterized by authoritative in-house science and imaginative and effective support to the user community. The position will be located in La Serena, Chile, and will carry explicit responsibilities for the planning, implementation, and oversight of AURA's program in Chile. The post offers excitement, challenge, and the promise of deep personal satisfaction derived from direct participation in the growth of an institution dedicated to the highest standards of in-house research, service to the astronomical community, and innovative approaches to astonomy. Applicants must have an earned doctorate in astronomy, or a closely related physical science, and a distinguished record of achievement in astronomy; evidence of leadership ability of a high order will also be sought. Interested individuals who feel that their background and temperament match the opportunities and inherent challenge of this position are invited to forward applications, with names of at least three people from whom a confidential evaluation can be sought to The Chair, Search Committee for Director CTIO, National Optical Astronomy Observatories, 1002 North Warren Avenue, Tucson, AZ 85719. Closing date for applications is July 31, 1985.

Further information can be obtained from Dr. John T. Jefferies, Director NOAO, at 602/881-1960. An EEO/AA Employer.
(NW1786)A

LUDWIG INSTITUTE FOR CANCER RESEARCH
UNIVERSITY OF SYDNEY

Post Doctoral Research Fellow

Applications are invited from Post Doctoral Scientists for a Research Fellow to join a group studying in vivo growth regulation of solid tumours, microenvironment and the effects of cytotoxic drugs Techniques currently in use employ tissue culture, syngeneic and xenogeneic animal tumours, and clinical material, with an excellent flow cytometry facility.

Applicants should possess a good background in cellular biology, and some experience in tumour growth kinetics would be an advantage

An attractive salary within the range A$22,614-A$25,246 p a will be negotiated.

Further information from Dr D. Hedley, (02) 692 3675/76.

Applilcations should be addressed to:

The Administrator,
LUDWIG INSTITUTE FOR CANCER RESEARCH,
Blackburn Building,
University of Sydney, NSW 2006 Australia

(W1896)A

MRC
MEDICAL RESEARCH COUNCIL
CLINICAL PHARMACOLOGY UNIT
UNIVERSITY DEPARTMENT OF CLINICAL PHARMACOLOGY — OXFORD

NEUROCHEMIST

Applications are invited for a MRC SHORT-TERM NON-CLINICAL SCIENTIFIC post in this MRC Unit, tenable for 3 years in the first instance. Candidates with some post-doctoral experience will be preferred.

The successful candidate will be required to study the neuro-chemical mechanisms by which neurotransmitter systems respond to the short- and long-term effects of psychotropic drugs. Knowledge and experience of the biochemical mechanisms of neuronal membrane function, neurotransmitter synthesis and release, and techniques of brain slice and synaptosome incubation and stimulation would be advantageous This work has strong links with an active clinical psychopharmacology section.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from Professor D.G. Grahame-Smith, University Department and MRC Unit of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE (Oxford (0865) 241091), with whom applications, including a full curriculum vitae and the names and addresses of two professional referees, should be lodged by 24th June 1985.

(6613)A

AN ACTIVE FERTILITY AND IN VITRO PROGRAMME IS DUE TO OPEN AT THIS HOSPITAL ON 1st AUGUST 1985

Applications are invited for the following position, for which date of commencement will be by mutual agreement

TECHNICAL ASSISTANT/EMBRYOLOGIST

The successful applicant will be expected to undertake both routine laboratory and research work. Research is currently undertaken in collaboration with MRC (Carshalton) and the Institute of Animal Physiology (Cambridge)

Experience in mammalian embryology would be desirable and a PhD or MSc in reproductive physiology or embryology would be an advantage, but is not essential

The Hospital offers a generous salary and excellent benefit package.

Applicants should forward a Curriculum vitae, including the names of three referees to: The Personnel Department, Humana Hospital Wellington, Wellington Place, London NW8

Closing Date 22nd June 1985. (6599)A

Humana Hospital Wellington

Wellington Place London NW8 9LE

UNIVERSITY OF WISCONSIN-MADISON AQUATIC BOTANIST

RESEARCH ASSOCIATE (postdoctoral) position available by 1 June, 1985, for laboratory and lake studies on integrated control of Eurasian watermilfoil and impact of control strategies on water quality. Additional experience in water chemistry, limnology, or mycology desirable. Funding for two years. Salary competitive and commensurate with qualifications.

Send résumé, including the names of three referees, to: Dr. John H. Andrews, Department of Plant Pathology, 1630 Linden Drive, Madison, Wisconsin 53706.

An affirmative action-equal opportunity employer.

(NW1781)A

POST DOCTORAL RESEARCH

position is available to study the molecular biology of the enzymes involved in connective tissue degradation. A research background in biochemistry or molecular biology is advantageous. Applicants should send curriculum vitae and three references to Gregory I. Goldberg, PhD, Washington University School of Medicine, Box 8123, St Louis, Missouri 63110

Equal Opportunity/Affirmative Action Employer.

(NW1788)A

MOLECULAR IMMUNOLOGY POST-DOCTORAL SCIENTIST

Salary from £9,000

Celltech, a leader in the field of biotechnology, has achieved its present success in the space of five years. Pioneers in several areas, our achievements are based on the foundations of scientific excellence, innovation and commercial expertise.

Celltech's Molecular Immunology group is expanding its activities in the molecular cloning and expression of immunological targets, principally antibodies. We are looking for a Molecular Biologist/Immunologist, either a post-doc or a graduate expecting soon to finish a PhD. Experience in molecular or cellular immunology would be desirable.

We are an ambitious and forward looking company, which places as much emphasis on its people as its products. You will work in a small friendly team, gaining invaluable broad-based experience, with plenty of opportunity for advancement.

Please send a detailed c.v. to Jane Walley, Celltech Ltd., 244–250 Bath Road, Slough, Berks SL1 4DY, quoting Ref. 268.

CELLTECH

Express Dairy UK Ltd., one of the country's largest Dairy Food organisations has a vacancy in the Research and Development Department.

DEVELOPMENT ANALYST

c. £8,100

Working in a small team, this position involves analytical instrument evaluation and method development. You will be reporting to the Technical Advisor — Rapid Analytical Method Development and should be a Graduate in Analytical Chemistry with at least one year's experience in modern techniques of analysis associated with the food industry

A current driving licence is essential.

In addition to the salary detailed, 22 days holiday and membership of the Company's pension scheme are available on completion of the necessary qualifying period.

Application forms obtainable from:

Mr. G. D. Evans,
Personnel Officer — Central Staff,
Express Dairy UK Ltd.,
Block I, 430 Victoria Road,
South Ruislip,
Middlesex, HA4 0HF.
Tel: 01-845 2345 Ext. 3551/3370.

(6680)A

Immunodiagnostics

Cambridge BioScience Corporation, a biotechnology company specializing in infectious disease diagnostics offers exciting positions in its Hopkinton, Massachusetts laboratories. We are seeking advanced level scientists as well as research associates

Molecular Biology

- All levels with extensive background in recombinant-DNA, cloning and expression, minimum of 2 years academic or industrial experience

Virology/ Immunology

- Ph D level with experience in clinical virology
- Experience in hybridoma development
- Working knowledge of molecular virology

Cambridge BioScience offers a unique and stimulating environment and close working associations with academic institutions. **Qualified applicants should send resume and salary requirement to: Cambridge BioScience Corporation, Personnel Department, 35 South Street, Hopkinton, MA 01748.**

(NW1806)A

Cambridge BioScience

Experimental Nuclear Physicists

There are vacancies for experimental physicists to join the Nuclear Structure Division at Daresbury Laboratory, an establishment of the Science and Engineering Research Council which operates major national facilities for scientific research

The group is involved in carrying out and supporting a research programme on the Nuclear Structure Facility (NSF), a large tandem accelerator which is operating at up to 20 MV on terminal.

Senior Scientific Officer

Ref. DL/911

The successful applicant will be required to organise and direct work necessary for the operation, maintenance and development of major equipment for charged particle identification and detection used in programmes aimed at studies of nuclear collision dynamics, spectroscopy, and nuclei far from stability. Candidates will be expected to carry out research in conjunction with university users on the NSF and to play an active role in initiating and developing new programmes. Other duties will involve the overall coordination and scheduling of the scientific programme and the assessment of future needs for instrumentation and other facilities on the NSF.

Applicants (male or female) should have a good honours degree (or equivalent qualification) in physics and several years postgraduate experience in experimental nuclear research. A Ph.D qualification in nuclear physics and a period of relevant post-doctoral experience would be an advantage.

Higher Scientific Officer

Ref. DL/913

The successful applicant will be required to liaise with university research teams using the NSF. Candidates will also be expected to collaborate in nuclear research programmes and to play an active role in initiating and developing new programmes. Other duties will involve work on the design and development of major equipment through to commissioning, operation and maintenance. As a member of the in-house team of scientists he/she will be expected to provide expertise in the methods of experimental nuclear physics to aid and direct the technical and scientific support staff in operating the NSF and for diagnosing problems as they arise.

Applicants (male or female) should have a good honours degree (or equivalent qualification) in an appropriate discipline with a period of relevant post-graduate experience. A Ph.D degree in nuclear physics and a period of post-doctoral experience in experimental nuclear research would be an advantage.

The salary scales according to qualifications and experience for the Higher Scientific Officer and Senior Scientific Officer posts are £7,435 to £10,039 per annum and £9,329 to £12,050 per annum respectively. These salary scales are currently under review.

There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

CLOSING DATE: 30th June 1985.

For further information please write or telephone Dr. J. S. Lilley on Warrington (0925) 65000, Ext 558.

Application forms may be obtained from and should be returned quoting the appropriate reference number to: The Personnel Officer, Science & Engineering Research Council, Daresbury, Warrington, WA4 4AD. (6624)A

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. **Send vita, a brief description of past and present research experience, and names of three or more references to**

Dr. A. M. Brown, Incoming Chairman,
Physiology and Molecular Biophysics Department

Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.

An Equal Opportunity A/A Employer

(NW1814)A

SENIOR SCIENTIFIC PROGRAMMER

National Optical Astronomy Observatories

The National Optical Astronomy Obervatories

(NOAO) needs a senior level programmer for its Detector R&D Program in Tucson. Working with scientists and engineers, the position involves development of software to operate state-of-the-art infrared arrays and signal processing equipment for astronomical instrumentation. We require skills in the development of real-time software for data acquisition and process control applications and date reduction and analysis programs. Applicants should have experience in FORTRAN and one of the structured languages such as "C". Experience in a real-time programming environment and software signal procesing is required. Knowledge of FORTH, UNIX , and DEC computers is desirable. An Electrical Engineering or Computer Science degree and at least five years experience as a programmer are required. Other degree background will be considered with more extensive programming experience.

The NOAO, headquartered in Tucson, Arizona, operates Kitt Peak National Observatory near Tucson; National Solar Observatory sites at Sacramento Peak, New Mexico, and on Kitt Peak; Cerro Tololo Inter-American Observatory in Chile, and the Advanced Development Program in Tucson.

Offering forefront technology challenges, and unclassified workplace, and a Sunbelt location, we are an EEO/AA employer with an excellent compensation package, including 24 days annual vacation and, after one year, a fuly vested retirement plan.

Send résumé in confidence to: Judy Ruffino, Personnel Manager, P.O. Box 26732, Tucson, Arizona 85726.

Equal opportunity employer. (NW1804)A

MACROMOLECULAR CRYSTALLOGRAPHER

Three-year Postdoctoral Research Associate position. Seeking motivated, talented person with experience in experimental aspects of structure determination to conduct x-ray diffraction studies. Several ongoing projects at different stages under the overall theme of intermolecular interactions. Excellent facilities for data collection, reduction, and analysis: rotating anode generators, area detector, oscillation/precession cameras, 2 VAX 11-750s, node to CRAY, color vector and raster graphics. Exciting work environment within a strong departmental structure group including six faculty crystallographers. Salary negotiable. Superb working setting in new building located on cliffs overlooking ocean. Send C V and three references to:-

Elizabeth Getzoff or John Tainer, Department of Molecular Biology — MB3, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037 (NW1799)A

THE ROYAL VETERINARY COLLEGE
University of London

Division of Preclinical Studies
Department of Physiology, Biochemistry and Pharmacology

Lecturer in Biochemistry

Applications are invited from veterinary, science, or medical graduates for a post involving both teaching and research which it is hoped to fill by 1st October 1985. An interest in metabolism and previous experience in lipid and lipoprotein biochemistry would be of advantage.

Salary Scale (under review): £8,753 to £16,158 including London Allowance. Universities Superannuation Scheme. Removal expenses up to £1,400 reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. (Tel: 01-387-2898).

Closing date for applications: 17 June 1985 (6630)A

UNIVERSITY OF NATAL
DEPARTMENT OF ZOOLOGY
ZOOLOGY

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin for appointment to the post of.

LECTURER IN BIOLOGY

Minimum qualifications would be a PhD in Zoology, Botany, Biology or a closely allied field and/or suitable academic or research experience.

The successful applicant will be responsible for teaching a first year introductory course, which is essentially a service for students in the Faculty of Agriculture. The four major areas covered by the course are Cell Biology, Plant Anatomy and Development, Animal Physiology, and the Ecology and Evolution of Animals and plants. No particular area of expertise is required beyond a sound and comprehensive knowledge of biology, but the appointee will be encouraged to actively participate in research and to supervise post-graduate students.

The appointment carries an attractive salary package, details of which are obtainable from the Staffing Section on request (telephone 0331-63320). The salary offered will be determined according to the qualifications and/or experience of the successful applicant.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, service bonus, staff bursary, housing loan, and subsidy schemes, long leave conditions and travelling expenses on first appointment, are available from the Registrar, University of Natal, PO Box 375, Pietermaritzburg, South Africa, 3200, with whom applications on the prescribed form must be lodged not later than 28 June 1985 quoting reference PMB 29/85.

Application forms for overseas applicants are available from the Secretary, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. (W1895)A

COMMONWEALTH AGRICULTURAL BUREAUX

THE LEADING WORLD AGRICULTURAL INFORMATION SERVICE FOR SCIENTISTS AND TECHNOLOGISTS

SCIENTIFIC INFORMATION OFFICER

REQUIRED AT THE
COMMONWEALTH BUREAU OF PLANT BREEDING AND GENETICS, CAMBRIDGE

Duties:
Preparing abstracts for the Journal *Plant Breeding Abstracts*, which covers world scientific literature in this field: indexing, and answering scientific enquiries.

Qualifications:
A degree in botany, genetics, or a related subject is essential, plus a reading knowledge of at least one European language (Slavonic languages preferred) and the ability to write good English. Applicants must be able to render the contents of scientific publications into concise, accurate and readable abstracts.

Salary:
In the scales £5,909—£8,153 p.a., £7,435—£10,039 p.a. Starting salary according to qualifications and experience.

Application forms and further particulars from
Commonwealth Bureau of Plant Breeding and Genetics
Department of Applied Biology
Pembroke Street, Cambridge CB2 3DX
Tel. No: Cambridge (0223) 358381, etxn.216.
Closing date for applications: 20th June, 1985.

(6689)A

Bureau of Mineral Resources
Geophysics Division
Canberra
GEOMAGNETISM SECTION MANAGER
A$34757 - A$37028

The Geomagnetism Section of the Division of Geophysics, Bureau of Mineral Resources, comprises a staff of 15. The Section is responsible for operating the magnetic observatories and conducting magnetic surveys in the Australian and Antarctic regions, and also runs a modern palaeomagnetic facility. Mission oriented research is carried out in the fields of solid-Earth geomagnetism and palaeomagnetism.

Duties:
The Manager, acting under the Section Head, is responsible for administration of the section, and the co-ordination of magnetic (including palaeomagnetic) observatory and survey operations. The person appointed will be expected to initiate and participate in aspects of the research program

Qualifications:
An appropriate degree or equivalent, ability to manage a scientific team, experience in magnetic (including palaeomagnetic) observatory and survey operations, the ability to carry out original research in geomagnetism.

Conditions:
The BMR is part of the Australian Public Service. To qualify for permanent appointment, applicants must be Australian citizens. Non-citizens may be appointed under a 3-year contract, with the expectation that they seek permanent appointment. Conditions of service (pension scheme, leave entitlement, etc.) are those pertaining to the Australian Public Service. Moving and settling-in allowances are paid to persons, and their dependent families, appointed from overseas.

Canberra, the national capital, is located approximately 280km southwest of Sydney and has excellent educational, recreational and sporting facilities.

Applications, including a curriculum vitae, statement of research interests, and names of professional referees should be addressed to: Staff Section, Bureau of Mineral Resources, P.O. Box 378 Canberra, A C.T. 2601, Australia.

Please quote reference: Position No. 2409, Geomagnetism. Applications should be submitted before 30th June 1985 (W1899)A

AUSTRALIA

Consejo Superior de Investigaciones Cientificas
INSTITUTO CAJAL
(Madrid, Spain)

Three permanent research positions will be available in 1985 at The Cajal Institute for Neurobiology. One of the positions is open for neurophysiologist and the other two for specialists in any other branch of Neuroscience. The appointments will be at the Research Associate level. Applicants must be Spanish citizens and have a M.D. or Ph.D. degree.

Further information may be obtained from:
The Director, Instituto Cajal, Veláquez 144, 28006-Madrid, Spain. (w1889)A

Postdoctoral or Research Associate Position

Available July 1, 1985. Project involves function of actin isoforms, post-translationally modified tubulin species, and microtubule-associated proteins. Biochemisty or molecular biology background is desirable; the lab uses these, as well as peptide antibody techniques, HPLC, cell culture, and immunostaining. Salary depends on experience. Send curriculum vitae and the names of three references to.

Dr. J.C. Bulinski, Department of Biology, University of California, Los Angeles, CA 90024. (NW1805)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions

Research School of Earth Sciences

POSTDOCTORAL FELLOW/RESEARCH FELLOW/ SENIOR RESEARCH FELLOW NOBLE GAS GEOCHEMISTRY

Applications are invited for an appointment in the School's Geochronology and Isotope Geochemistry Group, the field of interest being noble-gas geochemistry The appointee will work with Dr Ian McDougall, Senior Fellow, in developing research in this field, which is new to the School. The School wishes to make the appointment at the Research Fellow level, but will be prepared to consider applications at the level PDF or SRF

Noble gas investigations are being initiated to assist in elucidation of the geochemical evolution of the mantle, the mantle-crust system, and the atmosphere Research initially will concentrate on mantle-derived rocks, with subsequent development of studies on fluids in the crustal environment Provision of a suitable high-resolution gas-source mass spectrometer is in hand, and construction of appropriate extraction lines is planned.

Persons with suitable backgrounds in the physical sciences, and with particular interests in geochemistry, are encouraged to apply Experience in noble gas studies on geological samples would be a great advantage.

The appointee will assist in developing the laboratory facilities, and will be expected to undertake discrete projects within this broad field of study Active collaboration with other workers in isotope geochemistry is envisaged. Enquiries may be directed to Dr I McDougall (062 49 4136)

Closing date. **19 July 1985.** Rf: ES/1.5.1.

Faculty of Science
DEPARTMENT OF ZOOLOGY
POSTDOCTORAL FELLOW

The successful applicant will work on a NH & MRC funded project involving gene cloning of sheep parasites particularly larval tapeworms and liver fluke Particular interest will be centred on those genes which code for antigens that are involved either in resistance to reinfection or immunopathological phenomina. A principal objective will be to obtain expression of these antigens in prokaryotic systems Experience in gene cloning techniques is essential and some knowledge of immunological procedures would be highly desirable The successful applicant will work in the Centre for Recombinant DNA Research, Research School of Biological Sciences (Executive Director. Professor J A Pateman FRS). The position is funded for 3 years Previous applicants need not reapply as all applications will be considered

Further information is available from Dr M J Howell, (062) 49 3060 or (062) 49 4225

Closing date **15 July 1985.** Ref: FS/1 5.1

CONDITIONS OF APPOINTMENT: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow $A21,411-$24,528 pa; Research Fellow $A24,840-$32,634 pa; Senior Research Fellow $A34,699-$41,390 pa Current exchange rate is $A1 00 – Stg 0 48.

Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities Maternity leave is available The University reserves the right not to make an appointment or to make an appointment by invitation at any time **Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be sent to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. General Information also available from the Secretary General, Association of Commonwealth Universities (Appts) 36 Gordon Square, London WC1H 0PF**

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W1880)A

UNIVERSITY OF TORONTO
DEPARTMENT OF BOTANY
POSTDOCTORAL FELLOW

required to work in group studying molecular basis of metal-tolerance in micro-organisms and higher plants Applicants should have experience in recombinant DNA techniques; knowledge of microbial physiology and plant ecology an advantage. Salary is in range of $21,500 Canadian Position is available immediately. **Please send detailed letter and C.V., plus three letters of reference to Prof. T.C. Hutchinson, Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1.** (NW1809)A

VICE PRESIDENT FOR RESEARCH
THE UNIVERSITY OF GEORGIA

The University of Georgia invites nominations and applications for the position of Vice President for Research.

Located in Athens, Georgia, The University of Georgia is the nation's oldest chartered state university. The University ranks among the top 50 research institutions in the country and operates with annual research expenditures in excess of $80 million across its 13 schools and colleges.

The Vice President for Research will administer the University's research resources, serve as an executive officer of the University and report to the President. He/she will facilitate and coordinate a vigorous expansion of competitively-funded research programs, and seek new avenues for creative partnerships with the private sector and government agencies She/he will exert strong leadership in the organisation and funding of new, interdisciplinary research programs, and will formulate reward and incentive systems for faculty participation in sponsored research

Nominees/applicants should have a record of executive-level accomplishments, knowledge of current R&D environments encompassing national and international perspectives in private, public, and governmental sectors, and should possess a working knowledge of higher education, including the nature and scope of university research activities. Candidates should have the terminal degree or equivalent experience in their discipline, and credentials suitable for appointment at the level of professor in the University.

Nominations should include the nominee's address and telephone number. Applications should include a letter of interest, a curriculum vitae, and the names and addresses of 5 persons who can serve as references. The closing date for receipt of completed applications is **July 15, 1985.** The position may be occupied as early as January 1, 1986

Address all nominations and applications to:

Vice President for Research Search Committee
c/o Dr Luis M. Proenza
Office of the President
The University of Georgia
Athens, Georgia 30602

The University of Georgia is an Equal Opportunity/Affirmative Action Employer

(NW1795)A

MAMMALIAN CELL BIOLOGIST

A Ph.D. with academic or industrial post-doctoral experience is sought for a position in a multidisciplinary group studying high density mammalian cell culture in the Biotechnology Laboratory of Central Research at The Dow Chemical Company. The successful candidate will have experience in handling multiple cell lines and associated analytical techniques and will have responsibility for the planning, organization and execution of a high priority program in the development of optimal culture conditions

For further information, please contact:

Dr. Kerr Anderson
Central Research
Biotechnology Laboratory
1701 Building
The Dow Chemical Company
Midland, MI 48674

(NW1808)A

SCOTTISH CROP RESEARCH INSTITUTE
DIRECTOR

The Governing Body of the Scottish Research Institute invites applications for the post of Director which will become vacant on the retirement of Professor C E Taylor CBE in March 1986.

SCRI, which is funded by an annual grant from the Department of Agriculture and Fisheries for Scotland, is a major crop research institute within the Agricultural and Food Research Service. It's research programme requires a wide range of scientific disciplines associated with the production and breeding of arable and horticultural crops.

Candidates should have an established record of scientific leadership and the capability to manage an organisation with over 300 staff.

Salary; £26,909 per annum non-contributary superannuation scheme

The Institute is an equal opportunities employer

Further particulars can be obtained from Mr N D Anderson, The Secretary to the Governing Body

Applications accompanied by a full curriculum vitae and the names and addresses of three referees should be lodged with the Secretary to the Governing Body, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA.

Closing date 12 July 1985. (6679)A

DROSOPHILA DEVELOPMENT

University of Arizona

Two postdoctoral positions available to work on molecular aspects of pattern formation and morphogenesis in Drosophila. One position state-funded at the level of Visiting Lecturer, with opportunities for teaching. Salary commensurate with experience Send CV, statement, and names, addresses, and telephone numbers of at least three referees to. Dr. Danny Brower, Department of Molecular & Cellular Biology, Biosciences West Building, University of Arizona, Tucson, AZ 85721, USA Position to begin Fall 1985 An equal opportunity/affirmative action employer (NW1801)A

TWO RESEARCH ASSOCIATE POSITIONS

Available immediately. Experience in proteins and recombinant DNA. Salary $18,000 to $22,000
Send CV and three references to Dr O P. Bahl, Dept. of Biological Sciences, SUNY at Buffalo, Cooke Hall 347, Buffalo, NY 14260. Equal Opportunity/Affirmative Action Employer (NW1811)A

Post Doctoral Fellow Experimental Pathology

At Smith Kline & French we wish to appoint a Post Doctoral Fellow within a new laboratory of our expanding Pathology Department.

The work will be to apply cell and organ culture techniques to the investigation of cell pathology related to new and model compounds. Integrated projects with histopathologists, EM and clinical pathologists will also be involved.

A PhD and post doctoral experience in the use of *in vitro* techniques applied to experimental pathology is required. Experience in bone marrow CFU and lymphocyte culture would be an advantage.

We offer a competitive salary and benefits for this two year fixed term appointment.

Please write for an application form or send a detailed CV outlining your qualifications and experience quoting reference number N/123/JW to John Ward, Personnel Officer, Smith Kline & French Research Limited, The Frythe, Welwyn, Herts AL6 9AR.

SK&F

UNIVERSITY OF MANITOBA
Department of Plant Science

POSTDOCTORAL POSITION PLANT CYTOGENETICS

Applications are invited for a position available September 1 to carry out research on wheat-rye chromosome substitutions in triticale These studies concern the use of chromosome banding techniques for the identification and establishment within an isogenic background, disomic substitution lines involving individual chromosomes of rye and their homoeologues from wheat. Experience with chromosome banding techniques and electrophoretic analysis of plant proteins in desirable The appointment is initially for one year with a maximum of two years. Starting salary from $18,500 to $20,500 per annum according to experience.
Send curriculum vitae with names and addresses of two referees to:- Dr. E.N. Larter, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.(NW1807)A

DEVELOPMENTAL BIOLOGIST

CORNELL UNIVERSITY MEDICAL COLLEGE

Applications are invited for a senior or junior tenure-track faculty position. Applicants should have at least two years of postdoctoral experience and maintain a research program in early development or morphogenesis, using genetic, recombinant DNA, biochemical, immunological, and/or cell biological tools to explore mechanisms of pattern specification and determination in a vertebrate or invertebrate system The position includes supervision of graduate students and teaching in a related area

Send curriculum vitae, research summary, and three letters of reference to: Dr. Rosemary Bachvarova, Developmental Search Committee, Department of Cell Biology and Anatomy, Cornell University Medical College, 1300 York Avenue, New York, NY10021. An Equal Opportunity/Affirmative Action Employer. (NW1767)A

POSITION OPEN

RESEARCH ASSOCIATE POSITION

available immediately to study the dynamics of membrane phase transitions using a novel volume-perturbation kinetic calorimeter which we have developed We are seeking a person with a Ph.D in Biochemistry, Biophysics or related field for a Research Associate position to work on this project.
Our calorimeter measures the thermodynamic phenomenon associated with phase transitions in membrane systems via temperatures and pressure changes and optical properties. The person we seek must be able to synthesize artificial lipid membranes in pure form and mixed with other constituents, and be able to reconstitute protein recombinant systems so the proteins retain their native functions. This person will conduct experiments, do some hardware modification, and modify and use computer software for data acquisition and experimental analysis. The individual must have a working knowledge of membrane systems. He/she must also be experienced with computer hardware and software methods for data acquisition, instrument control, data analysis and modelling studies.
Send Curriculum Vitae and names of referees to Dr. Michael L. Johnson, Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908. An Equal Opportunity/Affirmative Action Employer. (NW1813)A

CELL BIOLOGIST

UNIVERSITY OF ALABAMA AT BIRMINGHAM

Applications are invited for two senior, tenured positions in the Department of Cell Biology at the level of Associate or Full Professor. Candidates must have achieved international recognition in cell or molecular biology, have a productive and well funded research program and be willing to move the program to Birmingham. Preference will be given to individuals employing molecular techniques to study the structure and function of cell components, growth control, regulation of gene expression, cell development or motility. Successful candidates will be offered nationally competitive salaries, excellent laboratory space and facilities, and an outstanding research environment in a rapidly expanding medical center Ample opportunities exist for teaching in the Schools of Medicine and Dentistry including gross anatomy, neuroanatomy, histology or in a new graduate program in cell biology.

Applicants should send a curriculum vitae and a brief description of past and present research experience and the names of three or more references by July 1, 1985 to: B. R. Brinkley, PhD, Professor and Chairman, Department of Cell Biology and Anatomy, Schools of Medicine and Dentistry, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294. An Equal Opportunity/Affirmative Action employer. (NW1785)A

Southampton THE UNIVERSITY

Department of Chemistry

Production and properties of Silicon-on-Insulator materials

A Postdoctoral position will be available from October 1985 for a Physical Chemist or Physicist to participate in a fundamental study of the production of new semiconductor materials. The project will employ a novel combination of techniques, including electrochemistry and in-situ ellipsometry applied to porous silicon substrates, which are being evaluated as the basis of the next generation of fast devices.

Previous experience in electrochemistry or spectroscopy would be an advantage, and some knowledge of computer programming or computerised instrumentation is necessary. The salary will be at a point on the SERC 1A scale commensurate with the age and experience of the appointee

Applications together with the names of two referees should be sent as soon as possible to: Dr. R. Greef, Chemistry Department, The University, Southampton SO9 5NH, UK, from whom further information can be obtained. (6670)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE (UNIVERSITY OF LONDON)

DEPARTMENT OF MEDICAL PROTOZOOLOGY

MOLECULAR BIOLOGIST

A post-doctoral position will shortly become available in this Department for a 3-year period for a suitable individual who wishes to join a team investigating the mechanisms of drug-resistance in malaria, under the direction of the Head of the Department, Professor W Peters. The project will be carried out in close association with the members of the Wolfson Unit of Molecular Medical Microbiology and Parasitology who are based in this Department. Applicants must have training and experience in recombinant DNA technology and will work closely with other members of the team who are investigating the very practical problem of drug-resistant malaria from other points of view, including the evaluation in depth of promising new antimalarial compounds. The appointment, which will be for up to three years in the first instance, carries a starting salary in the range £8,543 to £13,173 per annum.

Candidates should send, as soon as possible, a full curriculum vitae describing their previous training, research experience and research interests, together with the names of three referees, to the Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT. Please quote reference MP2.

(6632)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

Positions are available for a temporary lecturer, postdoctoral worker and SERC-CASE student. All posts are tenable from the 1st of October, 1985.

The temporary lectureship is available for 3 years and results from an MRC-replacement scheme award to Dr H C Nimmo. The appointee would be encouraged to undertake research and to collaborate with existing groups within the Department.

The postdoctoral position is available for 2 years to work with Professor Houslay's group on guanine nucleotide regulatory proteins and their interaction with adenylate cyclase. This appointment is funded by the MRC and will be made within the first three points of the 1A salary scale for research workers.

An SERC-CASE studentship award for the study of a PhD is available to work jointly with Professor Houslay at Glasgow and Dr Baxter at Glaxo. This project is aimed at understanding the physiological importance of the tyrosine kinase activity associated with the insulin receptor. The conditions of this award are as generally applicable to research council studentships.

Further information may be obtained from, and informal enquiries may be made to, Professor M D Houslay, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, to whom application (3 copies), should be made by 30th June, 1985. (6691)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ZOOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE IN NEUROBIOLOGY

Applications are invited for a postdoctoral Research Associate to work with a small group, supported by the SERC investigating the physiology and structure of insect brain neurones. Interests are both in the operation of synapses which identified visual neurones make amongst each other, and in the function of these neurones in the control of behaviour. In particular, we plan to compare the membrane properties and synaptic structure of different neurones, with the aim of understanding more about the roles of spikes in regulating transmitter release from neurones. Applicants should have experience in ultrastructural or in electrophysiological techniques, and are required either to have or to be close to completing a PhD. The appointment is for 3 years tenable from 1st July 1985 (deferable for up to 6 months).

Salary will be up to £8,450 per annum on the Range 1A scale (£7,520 - £12,150) according to age, qualifications and experience.

Applications (2 copies of curriculum vitae and research experience), plus names of 2 referees, or enquiries to: Dr P J Simmons, Department of Zoology, University of Newcastle upon Tyne NE1 7RU not later than 13th June 1985.

(6627)A

NATURE CONSERVANCY COUNCIL

(Commissioned Research —)

(Contract Post)

DEPARTMENT OF ENTOMOLOGY

HIGHER SCIENTIFIC OFFICER DATA HANDLING/ ENTOMOLOGIST

Applications are invited for a one year contract post funded by the Nature Conservancy Council, to be based in the Entomology Department, Rothamsted Experimental Station, Harpenden, Herts. The successful applicant will be responsible for analysing light trap data for moths gathered by the Rothamsted Insect Survey. The project will investigate whether any long term trends are apparent for the polulations of particular moth species, or for the fauna of selected sites. The RIS moth data is stored on computer file and so previous computing experience is essential, and some familiarity with the British moth fauna would be an advantage.

Qualifications. Candidates should have a first or second class honours degree, or equivalent, in computing studies or in zoology and with at least two years relevant postgraduate experience.

Salary depending on age and qualifications Higher Scientific Officer £7,435 rising to £10,039 per annum.

Application forms and further details are available from Miss B A Meredith, Personnel 1, Nature Conservancy Council, Northminster House, Peterborough PE1 1AU.

The closing date for receipt of completed application forms is 21 June 1985 The Nature Conservancy Council is an equal opportunity employer. (6653)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY PATHOLOGY

MOLECULAR VIROLOGY UNIT

A molecular virology unit has been established to develop novel vectors for the expression of viral antigens in mammalian systems. This unit works in close collaboration with virilogists and molecular biologists in this department and at the Beatson Institute.

Three positions, supported by the National Institutes of Health, are immediately available. Applications are invited for 2 post-doctoral fellows (salary range £8,450 to £10,720 per annum) and a research assistant (£6,600 per annum). The research assistant may apply to register for a higher degree.

The applicants should have experience in recombinant DNA technology and a background in virology would be an advantage. Applications should be sent to, and further particulars may be obtained from, Professor W F H Jarrett, FRS, University of Glasgow Veterinary School, Bearsden, Glasgow G61 1QH (telephone number 041-942 2301). (6692)A

UNIVERSITY OF LONDON THE CARDIOTHORACIC INSTITUTE

DEPARTMENT OF MEDICINE

NEW BLOOD APPOINTMENT

Applications are invited for a "New Blood" appointment in the Department of Medicine. The successful candidate is likely to be a clinical or non-clinical scientist with experience in the application of immunological and cell biological methods to study of the pulmonary macrophage. He or she will be expected to be sufficiently experienced to contribute new basic scientific ideas in this area and to carry these through experimentally in the current programme of research being conducted in the Department of Medicine into the cellular, biochemical and host defence aspects of pulmonary disease. A clinical appointee would be expected to co-ordinate the programme of laboratory research as it is applied to in-patients and out-patients, and to participate in the teaching activities of the Department.

Candidates should not hold a permanent University appointment in the UK. Appointment will be on the Clinical or non-Clinical Lecturer scale. A London Allowance is payable.

Applicants should apply by letter, enclosing 8 copies of their curriculum vitae, a statement of not more than 500 words on why they are applying for this post, and naming 3 referees to The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP from who further particulars can be obtained. Closing date for application 21 June 1985.(6645)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING

POSTDOCTORAL AND POSTGRADUATE RESEARCH ASSISTANTS RESEARCH TECHNICIAN

Applications are invited from biochemical and chemical engineers, biochemists and microbiologists for four posts in the Department of Chemical and Biochemical Engineering to work on two research programmes.

(1) Biocatalytic reaction involving water-insoluable organic compounds (supported by SERC).

(2) Extended use of biocatalysts (supported by DTI and seven companies through The Institute for Biotechnological Studies).

Appointments for three years starting as soon as possible will be made on the 1A and 1B scale for research assistants, and grade 3 scale for the technician.

Applications including CV and names and addresses of two referees should be sent to Professor M D Lilly, Department of Chemical & Biochemical Engineering, University College London, Torrington Place, London, WC1E 7JE. (6652)A

THE NATURE CONSERVANCY COUNCIL

SENIOR SCIENTIFIC OFFICER

Co-ordinator for remote sensing of the environment

The successful candidate will be required to assist with the management and co-ordination of the National Countryside Monitoring Scheme. The duties will include advising NCC staff on digital cartography and the development of NCC's use of satellite imagery for monitoring purposes. Other responsibilities will include liaising with the Department of Environment, Countryside Commission, Countryside Commission (Scotland), Institute of Terrestrial Ecology and other organisations concerned with the monitoring of countryside feature through remote sensing.

Candidates should have a degree in an appropriate subject and at least four years post graduate experience. Essential experience will include digital cartography, photogrammetry, statistical analysis and sampling strategies involving remote sensing, the ability to write and edit the necessary computer software and management experience of remote sensing programmes.

Salary range: £9,329 to £12,050.

For further details and an application form, please contact Mr S Woodfield, Personnel 1, Nature Conservancy Council, Northminster House, Peterborough PE1 1AU to whom completed forms should be returned by the 19 June 1985.

The Nature Conservancy Council is an equal opportunity employer (6654)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GEOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT/ SENIOR RESEARCH ASSISTANT/ RESEARCH ASSOCIATE

to work on a study of fault geometry and mechanics using data from colliery plans and records. Applicants should be graduates in a branch of Earth Sciences and have a good grounding in either structural geology or geophysics. An interest in computing and/or quantitative methods in geology would be an advantage. There will be no opportunity to register for a higher degree. A candidate currently registered for a higher degree will only be considered if thesis submission is imminent.

Appointment will be from 1st July, 1985, initially for one year. Initial salary will be within the range £6,600 — £9,390 per annum.

Applications, together with the names of three referees, should be received not later than 14th June, 1985, by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained.

Quote ref RV/294/N. (6662)A

VICTORIA UNIVERSITY OF WELLINGTON

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURER IN BIOCHEMISTRY

Applications are invited for the above position, which is available from 1 February 1986. Applicants should have recently obtained a PhD in contemporary biochemistry. The most desirable areas of expertise are those of molecular biology (in particular recombinant DNA technology), molecular enzymology or protein chemistry. The research interest of the Department lie in mechanistic studies in biochemical pharmacology and toxicology with a bias towards projects of agricultural significance The appointeee will be expected either to redirect his or her expertise into existing projects or to initiate new research compatible with departmental interests. Experience in relevant fields such as biochemical pharmacology or insect biochemistry or toxicology would therefore be advantageous

Salary will be the range NZ$23,622 — NZ$27,928 per annum. The appointment will initially be for a period of two years. Extension for a third year may be possible depending on the outcome of a review of the Life Sciences at Victoria University.

Conditions of Appointment and method of application are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or the Administrative Assistant (Appointments), Victoria University of Wellington, Private Bag, Wellington, New Zealand, with whom applications close on 26 July 1985. (W1901)A

THE SCHOOL OF PHARMACY

University of London

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

POSTGRADUATE Research Opportunities in Pharmaceutical Chemistry

Applications are invited for a number of positions for postgraduate research leading to higher degrees Candidates should have or expect to have a good honours degree in pharmacy, chemistry, biochemistry or biophysics.

Research Council and industrially sponsored projects on offer include.

(a) Diffusion and light scattering of biopolymer systems
(b) Applications of fast atom bombardment mass spectrometry in pharmaceutical and biological chemistry
(c) Chemical and biological NMR spectroscopy; and
(d) Synthesis and structure of natural products and peptides.

Applications should indicate area(s) of interest, giving names of two academic referees and two copies of CV and, to be sent to: Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX (6674)A

MICROBIOLOGIST

Molecular Immunology Division

The Molecular Immunology Division of Allelix Inc. is seeking a microbiologist with experience in the culture, maintenance and isolation of clinically important infectious agents for the Product Development Group. The successful candidate will work in a team developing rapid diagnostic immunoassays for the diagnosis of infectious diseases. This individual will possess a Ph.D. in microbiology and at least two years' post-doctoral experience in clinical microbiology. Experience in immunoassay and protein chemistry techniques will be advantageous.

Allelix provides the opportunity to learn from and interact with experienced staff scientists, in addition to the immediate project leader, in a well-equipped laboratory. Salaries and benefits are at fully competitive levels.

Interested candidates are asked to send complete resumés, including three references and quoting file number MI001, to:

Dr. J.G.R. Hurrell

Allelix INC

6850 Goreway Drive
Mississauga, Canada
L4V 1P1

(NW1810)A

UNIVERSITY OF LEICESTER

DEPARTMENTS OF ZOOLOGY AND OBSTETRICS AND GYNAECOLOGY

SENIOR DEMONSTRATOR IN REPRODUCTIVE PHYSIOLOGY

Applicants are invited for a post of Senior Demonstrator in Reproductive Physiology. This will be a joint appointment in the Department of Zoology and Obstetrics and Gynaecology Applicants should have experience in tissue culture and/or the culturing of mammalian embryos, and should preferably be post-doctoral. The appointee will be required to assist in collaboration with the two departments in developing a programme of extracorporeal fertilisation There will also be a teaching commitment in the area of cell and developmental biology in the Department of Zoology. The appointment will be for two years.

Initial salary will depend on age and experience on the scale £7,520 to £12,150 (under review).

Further particulars from the Registrar (Appointments), University of Leicester, University Road, Leicester LE1 7RH (telephone 0533 554455 ext 233), to whom applications on the form provided should be sent by 17 June 1985. Informal enquiries can be made to Professor H C Macgregor, Department of Zoology Telephone 0533 554455 ext 115 (6642)A

UNIVERSITY OF THE WEST INDIES

St Augustine, Trinidad

DEPARTMENT OF MECHANICAL ENGINEERING, INDUSTRIAL ENGINEERING PROGRAMME

LECTURER/ ASSISTANT LECTURER

Applications are invited for the post of Supernumerary Lecturer/Assistant Lecturer in the Department of Mechanical Engineering, Industrial Engineering Programme, specialising in Operations Research and Production/Investment Planning. The appointment will be two years.

Salary scales: Lecturer: TT$54,708 × 1,908 – $66,156 (Bar) × 1,908 – $77,604; Assistant Lecturer: TT$45,480 × 1,908 – $49,296. FSSU Unfurnished accommodation if available at 10% or furnished at 12% or housing allowance of 20% of pensionable salary. Up to five full economy passages on appointment and on normal termination. Study and Travel Grant

Detailed applications giving qualifications and experience and naming three referees to the Campus Registrar, University of West Indies, St Augustine, Trinidad, West Indies. Details of post sent to all applicants, but also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1902)A

CANCER RESEARCH

The AMC Cancer Research Center seeks to expand its laboratory and clinical research programs. Individuals at all levels, from Post-doctoral Fellows to Department Chairmen, will be considered. Current interests include tumor cell biology, tumor immunology, experimental hematology, monoclonal antibodies, cell kinetics, nucleic acid metabolism, nuclear magnetic resonance imaging, as well as clinical and national community research and control programs. Scientists (Assistant Professor equivalent) will be expected to vigorously develop independent research. Senior Faculty must be of national prominence and hold significant external funding. Opportunities exist for collaboration and growth Facilities, equipment and initial research support are available. Clinical resources on campus include a 32 bed inpatient unit, outpatient clinics and services. Individuals with interests bridging laboratory and clinical cancer research are particularly sought. Send Curriculum vitae and other pertinent information to:

Dr. Philip Furmanski
Associate Director
AMC Cancer Research Center
1600 Pierce Street
Denver, Colorado 80214

(NW1798)A

UNIVERSITY OF THE WEST INDIES MONA, JAMAICA

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the following posts in the University of the West Indies

LECTURER/ASSISTANT LECTURER

It is desirable for teaching purposes that the successful applicant should have a broad training in Biochemistry. Certain areas of research interest are particularly welcome, eg plant biochemistry, toxicology, nutrition, immunology, biotechnology. Duties to be assumed as soon as possible.

Salary scales (under review): Lecturer (non-medical) J$23,688×768 - 28,296 (B)×825-33,246. Assistant Lecturer (non-medical) J$20,379×717-21,813.

Up to five full economy class passages plus baggage allowance up to US$900 on appointment and normal termination. Special allowance up to US$900 on appointment and normal termination. Special allowance up to US$300 for shipment of academic books and teaching/research equipment on appointment. Unfurnished accomodation at 10% of pensionable salary or housing allowance of 20% of pensionable salary. UWI contribution of equivalent of 10% of salary to Superannuation Scheme. Subsidized Health Service. Annual Book Allowance. Annual Study and Travel Grant for self, spouse and three children. Transportation allowance to persons who own and operate a motor vehicle.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status, and the names and addresses of three referees should be sent as soon as possible to the Campus Registrar, UWI, Mona, Kingston 7, Jamaica. Applicants in the UK should also send one copy to the Assistant Secretary General (Appointments), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. Further details obtainable from either source. (W1905)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
London W1P 6DB
(University of London)

DEPARTMENT OF ANATOMY AND BIOLOGY AS APPLIED TO MEDICINE

POST-DOCTORAL RESEARCH ASSISTANT 1A

Applications are invited for this SERC supported post, tenable from October 1, 1985 (or earlier), to work for 3 years on cell contact and movement using advanced fluorescence methods.

Written applications, including the names of two referees, should be sent to Professor D Gingell at the above address. (6650)A

TECHNICAL UNIVERSITY OF GRAZ, AUSTRIA

The Chemistry Department of the Technical University of Graz invites applications for the position of a

FULL PROFESSOR OF PHYSICAL CHEMISTRY

at the Institute of Physical and Theoretical Chemistry. Applicants are expected to be internationally recognized researchers having appropriate teaching experience to teach the full course program of Physical Chemistry. Willingness to cooperate with existing research groups at the institute (crystal structure, theoretical chemistry, spectroscopy and magnetochemistry, solid-state chemistry, quantum chemistry) and interest in interdisciplinary team work is expected. Candidates are asked to send their resume (curriculum vitae, teaching experience, list of publications etc) and reprints of five of their most important publications.

Application deadline is September 16, 1985. Submit your application and resume to: Dekan der Technisch-Naturwissenschaftlichen Fakultät der Technischen Universität Graz, Petersgasse 16, A 8010 Graz, Austria, where also additional information may be requested. (W1891)A

UNIVERSITY OF CALIFORNIA

DEPARTMENT OF PHYSICS

ENDOWED CHAIR PROFESSOR OF PHYSICS

The UCLA Physics Department has an open Endowed Chair position. An appointment will be considered in any field of experimental or theoretical physics. The successful candidate will have a regular University of California tenured faculty position; the endowment income will support the candidates' program.

The successful candidate will be expected to lead an internationally recognized research program, and to teach at the graduate and undergraduate level.

Applicants should send a curriculum vitae, a brief statement of past, present, and future research interests, and the names of 5-10 referees to C Kennel, Chair, Department of Physics, University of California, Los Angeles, CA 90024.

The department is especially interested in identifying women and minority candidates.

The University of California is an affirmative action/equal opportunity employer. (NW1435)A

UNIVERSITY OF SOUTHAMPTON

CHAIR OF OCEANOGRAPHY

Applications are invited for the Chair of Oceanography which will become vacant on the retirement of Professor H Charnock, FRS, on 30 September 1986. Applicants should have research interests in the physically-related marine sciences and the ability to foster work in a multi-disciplinary field.

Further details may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH, to whom applications (10 copies from persons in the UK) should be sent before 12 July 1985. (6626)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF PHYSIOLOGY

RESEARCH ASSISTANT

Applications are invited for the above post, tenable for up to three years, to work on a project concerned with thermal changes in the immersed human body. This phase of the work is a theoretical study and will involve mathematical modelling. Applicants should have, or expect to get, a good honours degree, ideally with some experience of modelling biological systems but ability and enthusiasm could offset lack of experience.

Salary within range £6,600 — £7,520 on the 1B Scale for Research and Analogous Staff, with appropriate placing (scale under review).

Further particulars and application forms from The Secretary, The University Aberdeen, with whom applications (2 copies) should be lodged by 28 June 1985. (6622)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following position:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF DEVELOPMENTAL BIOLOGY

POSTDOCTORAL FELLOW - MOLECULAR ANALYSIS OF DEVELOPMENT PROJECT

A Postdoctoral Fellow is required in the Department of Developmental Biology for a project on genetic analysis of events in the cell cycle of Chlamydomonas. A background in genetics, biochemistry and ultrastructural analysis would be advantageous. The position will commence on 1 January 1986.

Further particulars can be obtained from Professor B Gunning, Head of Department (062) 49 2372, or from Dr P C L John (address until 31 July 1985, Department of Botany, Queen's University of Belfast, Belfast BT7 1NN, UK).

Closing date: 31 July 1985. Ref: BS 15/5/1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow $A22,614 - $25,905 pa.

Appointment, unless otherwise stated, will be: Postdocotral Fellow normally two years, with the possibility of extension to maximum of three years.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when requesting further particulars, and when submitting applications, which should be sent to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. Further information also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an equal opportunity employer. (W1904)A

POSTDOCTORAL POSITION

Is available immediately to study functional role of insulin in the brain at the cellular level with Drs. D.W. Clarke and M.K. Raizada. Experience in cell culture and biochemical techniques is a disirable. Experience with immunocyto-chemistry would be helpful. **Contact Dr. Mohan K Raizada, Dept of Physiology, J274, Univ of Fla Gvl, FL 32610 or telephone 904 392-3791.**

(NW1802)A

AFRC FOOD RESEARCH INSTITUTE — BRISTOL
LANGFORD,
BRISTOL BS18 7DY

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER (85/17)

ASSISTANT SCIENTIFIC OFFICER (85/19)

SHORT TERM CONTRACTS (3 YEARS)

Applications are invited for 2 short term contracts (3 years) to examine gaseous forms of preslaughter anaesthesia in pigs and poultry. The project is aimed at resolving some of the humanitarian problems associated with the slaughtering of livestock

Salary SO/HSO £5,909 — £10,039. ASO £4,456 — £6,315.

Qualifications SO/HSO (Post 85/17) Degree in a Biological Science with relevant post-graduate research experience

ASO (Post 85/19) HNC in Biology.

Further particulars and an application form from the Personnel Officer at the above address.

The Agricultural and Food Research Council is an equal opportunities employer (6649)A

UNIVERSITY OF GLASGOW

DEPARTMENTS OF BIOCHEMISTRY AND PHARMACOLOGY

WELLCOME LECTURESHIP IN MOLECULAR PHARMACOLOGY

Applications are invited for a Wellcome Lectureship in Molecular Pharmacology to be held jointly in the Departments of Biochemistry and Pharmacology. The appointment will be for three years: it may be renewed for a further two years subject to satisfactory progress. Applicants should not be more than 35 years of age and persons having a permanent University post in the UK are ineligible.

The successful applicant will contribute to the teaching in both departments and in research will become part of an active interdepartmental group. The departments are in the process of appointing a 'New Blood' lecturer to the same group.

The salary will be within the range of £7,520 — £14,925 per annum of the Lecturer's scale, with placement according to age, qualifications and experience.

Those wishing to be considered should send eight copies of a statement of their qualifications and experience, including a list of publications and the names and addresses of not more than three persons to whom reference may be made, to Professor R M S Smellie, Department of Biochemistry, University of Glasgow, Glasgow, G12 8QQ, not later than 21st June, 1985, from whom further particulars may be obtained. The applications must be accompanied by a summary of not more than 500 words outlining the proposed research. (6666)A

HEINRICH-PETTE-INSTITUT FÜR EXPERIMENTELLE VIROLOGIE UND IMMUNOLOGIE
AN DER UNIVERSITÄT HAMBURG

Applications are invited for the Tenure Position of

HEAD OF DEPARTMENT OF MOLECULAR BIOLOGY

Candidates with extensive scientific experience are expected to lead a research group in the general field of molecular biology. Budget and tenure positions are available. The Heinrich-Pette-Institut is an independent research establishment, active in the fields virology, immunology, and cell biology. Administrative experience and willingness to participate in running affairs of the institute are required. Teaching at the University of Hamburg is on voluntary basis. The salary is according to BAT I and depends on age and family status.

Applications including curriculum vitae and list of publications should be sent: An das Direktorium, Heinrich-Pette-Institut, Martinistraße 52, D-2000 Hamburg 20, Federal Republic of Germany. Closing date 31 August 1985. (W1892)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
DEPARTMENT OF ENTOMOLOGY

LECTURER OR SENIOR LECTURER

Applications are invited from medical entomologists for a tenured post in the Entomology Department. Preference will be given to applicants combining tropical experience with an outstanding record in research, preferably in biochemistry, ecology, population dynamics, or insect behaviour, but experience in any field within entomology applied to human health will be considered.

The person appointed will be expected to develop active research programmes, with overseas connections, and the extensive facilities of the London School will be available for this.

Appointment will carry a salary in the range £8,753 to £18,938 (inclusive of London Weighting) on the Universities' Lecturer or Senior Lecturer scale according to age, qualifications and experience, with membership of USS.

Further information may be obtained from Professor A N Clements (01-636 8636 ext 348). Applications with full curriculum vitae and the names of three referees should be sent to the Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

Closing date: 5 July 1985 (6635)A

We are a major Italian pharmaceutical company with a growing interest in biotechnology.
We are looking for an experienced

BIOCHEMIST/ IMMUNOCHEMIST

For a newly created laboratory of biochemistry and immunology, located in the urban area of Milan, Italy.

Potential candidates should have a doctorate degree and post-doctoral training Previous experience in hybridoma technology for diagnostic purposes would be considered an advantage

We Offer:
- career opportunities based on performance;
- a competitive salary in accordance with experience and qualifications;
- a stimulating, dynamic work environment;
- assistance in relocation expenses

Candidates from EEC Countries are encouraged to apply.

Please contact in confidence:
P.O. Box 10119
Personnel Dept.
20110 Milan, Italy (W1898)A

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY
(University of London)
HAMMERSMITH HOSPITAL

RESEARCH ASSISTANT

Applications are invited for the post of research assistant for up to 3 years to work on arachidonic acid metabolism in fetal growth retardation. Candidates should have or expect to obtain a 1st class or upper 2nd class honours degree in Biochemistry. The successful applicant will be expected to register for a PhD in London University.

Further information from and applications to Professor M G Elder Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London W12 0HS. (6633)A

THE UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

BIOCHEMISTRY LABORATORY

WELLCOME LECTURESHIP IN BIOCHEMISTRY

Applications are invited for the Wellcome Lectureship in Biochemistry Applicants should have research experience, preferably in the area of the biochemistry of secretion of polypeptide hormones, growth factors or neuropeptides. Those holding tenured positions are not eligible for consideration, and applicants should not normally be over 35 years in age.

Appointments will be for 3 years in the first instance, renewable for a further 2 years, subject to a satisfactory review. The salary will be on the University Lecturer scale (£7,520 to £14,925 pa).

Send self addressed envelope (6'' x 9'') for further particulars and an application form to Mrs L Vivian, Personnel Office, Sussex House, The University of Sussex, Falmer, Brighton BN1 9RH, Sussex. Closing date for applications 10th June 1985 (6629)A

UNIVERSITY OF BATH

SCHOOL OF CHEMISTRY

POST DOCTORAL RESEARCH OFFICER

Applications are invited for a Research Officer to work with Dr S Mann for three years beginning October 1985 to investigate the structural and mechanistic properties of magnetic solids formed in bacteria. Applicants should have a strong interest in structural inorganic chemistry and have some experience in electron microscopy and electron diffraction.

Starting salary up to £7,980 pa (under review).

Application forms from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting ref no 85/79. Closing date. 13/6/85. (6619)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY PATHOLOGY

MOLECULAR VIROLOGY UNIT

A molecular virology unit has been established to develop novel vectors for the expression of viral antigens in mammalian systems. This unit works in close collaboration with virologists and molecular biologists in this department and at the Beatson Institute.

Three positions, supported by the National Institutes of Health, are immediately available. Applications are invited for 2 post-doctoral fellows (salary range £8,450 to £10,720 per annum) and a research assistant (£6,600 per annum). The research assistant may apply to register for a higher degree.

The applicants should have experience in recombinant DNA technology and a background in virology would be an advantage. Applications should be sent to, and further particulars may be obtained from, Professor W F H Jarrett, FRS, University of Glasgow, Veterinary School, Bearsden, Glasgow, G61 1QH (telephone number 041-942-2301). (6658)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals, Houndmills, Basingstoke, Hants. RG21 2XS, UK

or

Paul Siman, Bio/Technology, 65 Bleecker St., New York, NY 10012.

MOLECULAR BIOLOGIST

Applications for an assistant professorship are invited from individuals with major commitment toward research and whose interests are in the structure, regulation or expression of viral, or mammalian, genes. Expertise in genetic engineering of enzymology-protein chemistry, an asset. Knowledge of or readiness to learn French essential. Send applications to: Dr Pierre Bourgaux, Directeur, Département de Microbiologie, Faculté de Médecine, Centre Hospitalier Universitaire, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.

(NW1790)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL

(University of London)

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

required to investigate the organisation of Campylobacter DNA, particularly the structure and expression of toxin genes. Previous experience, preferably postdoctoral, in prokaryotic molecular genetics and recombinant DNA techniques is essential. Experience with expression vectors is desirable The successful applicant will join a group working on various applications of molecular biology to clinical problems.

The post is available immediately for a fixed term period of up to three years. Salary will be on Scale 1A for Research and Analogous staff, (£7,520 — £12,150 pa) at a point appropriate to age and experience plus £1,233 pa London Allowance.

For further information concerning the project or the department, telephone Dr L Archard, 01-741 1858.

Applications on forms obtainable from The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London, W6 8RP, to be submitted within three weeks of the appearance of this advertisement.

(6625)A

UNIVERSITY OF LONDON

RANK CHAIR OF PHYSIOLOGICAL BIOCHEMISTRY TENABLE AT IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

The Senate invite applications for the above Chair, tenable in the Department of Biochemistry at Imperial College of Science and Technology.

Candidates should have demonstrated an interest in cellular biochemistry and/or control of integrated physiological processes. They should have significant research accomplishments and an interest in the teaching of these aspects of biochemistry in advanced undergraduate courses.

Applications (10 copies) must be received not later than 15 July 1985 by the Academic Registrar (N.), University of London, Malet Street, London WC1E 8HU, from whom further particulars should first be obtained

(6631)A

NEUROENDOCRINE RESEARCH GROUP

DEPARTMENT OF ANATOMY

UNIVERSITY OF CAMBRIDGE

We are seeking a postdoctoral physiological psychologist, or relevantly qualified zoologist or clinical graduate to join a multidisciplinary research tea investigating the neuroendocrine basis of social behaviour and anxiety in an experimental setting. The position is funded by the MRC and available immediately; the initial appointment is for 1 year, renewable for up to 5 years in total

Please send applications (including CV) to Mrs J Penton, Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY by July 1st, and arrange for references to be sent direct from 2 referees.

(6686)A

THE ANIMAL VIRUS RESEARCH INSTITUTE

POST-DOCTORAL MOLECULAR BIOLOGIST

Applications are invited for a 3 year appointment to work on analysis of the genome structure of classical swine fever (hog cholera) virus and its relationship to bovine virus diarrhoea. The work will involve molecular cloning of the genome RNA and nucleotide sequence determination. The successful applicant will work with Dr Charles Penn in the Division of Molecular Biology He/she should have a degree in biochemistry, microbiology or a related subject and a PhD. Experience in gene cloning would be an advantage.

The salary will be in the scale of £7,435 - £10,039 (Higher Scientific Officer grade) pay award pending. Sick leave and non-contributory superannuation schemes, 22 days annual leave. Accomodation may be available. The AVRI is an equal opportunity employer.

Application to include a curriculum vitae and the names of two referees should be sent as soon as possible to the Secretary, Animal Virus Research Institute, Ash Road, Pirbright, Woking Surrey GU24 0NF. Tel: 0483 232441. (6640)A

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURESHIP

A vacancy exists for a Temporary Lecturer as a result of Dr J R Burton being awarded a five-year Research Fellowship by the Lister Institute. Candidates with research interests in any area of Biochemistry are eligible to apply for preference may be given to those with experience of recombinant-DNA technology or immunology. It is expected that the successful candidates will be appointed for a period of three years with the possibility of extension for a further two years.

Salary within the range £7,520 — £11,675 on the non-clinical lecturers salary scale.

Applications including the names of three referees should be sent to the Personnel Department (Academic Staffing), the University, Sheffield S10 2TN by 14 June 85 from whom further particulars are available. Quote ref: R275/G (6617)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

RESEARCH ASSISTANT

Applications are invited for the above post, tenable immediately for up to two years, to work on the isolation of cNDA clones for the structural glycoprotein present in human aorta. A range of microbiological, immunological and gene cloning techniques will be involved. Applicants should have an honours degree in an appropriate discipline.

The appointment is funded by the Maitland Ramsay Trust. Starting salary within the range £6,600 to £7,980 per annum (under review) according to age and experience, on the 1B scale, plus USS.

Further information can be obtained by writing or by telephone from professor Serafini-Fracassini (tel 0334-76161, ext 275).

Applications (2 copies preferably in typescript) enclosing a full curriculum vitae and the names of 2 referees should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ to arrive not later than 20 June 1985.

(6661)A

UNIVERSITY OF OXFORD

DEPARTMENT OF NUCLEAR PHYSICS

APPOINTMENT OF RESEARCH OFFICER

The department will shortly appoint a Research Officer in Particle Physics. Whilst mainly a research appointment, the appointee will be required to undertake seventy-two hours a year of laboratory demonstrating and he/she will be encouraged to give graduate lectures. The research programme of the experimental particle physics group includes the Soudan II search for proton decay, a major contribution to the DELPHI detector for LEP (CERN), preparation for an experiment at HERA (DESY), and development of an indium detector for solar neutrinos.

The appointment will be for three years with possible extension to five years. Salary will be on the Lecture scale £7,520 - £15,930 (under review).

Applications with the names and addresses of two referees should be sent to Mr M S Gautrey, Department of Nuclear Physics, Keble Road, Oxford from whom further particulars may be obtained. The closing date is September 5th 1985.

(6676)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY DIVISION OF IMMUNOLOGY

SCIENTIST

A Scientist with a post-graduate qualification in Biochemistry or Organic Chemistry is required to join in a project investigating the development of immunoassay systems using red cell-labelled antibodies. Experience in protein chemistry or immuno-chemistry desirable The appointment is for two years in the first instance, possibilities of an extension.

Enquiries to Professor R R A Coombs, Division of Immunology, New Adenbrooke's Hospital, Hills Road, Cambridge. Tel Cambridge 0223 245171 ext 336. Age up to 30

Salary range: University scales age related.

Formal applications together with curriculm vitae and the names and addresses of two referees to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. (6667)A

UNIVERSITY OF LEICESTER/DARESBURY LABORATORY

POST-DOCTORAL RESEARCH ASSOCIATE IN EXPERIMENTAL X-RAY DIFFRACTION AT HIGH PRESSURES

Applications are invited for the above post, funded by SERC, tenable from 1st October 1985, or as soon as possible thereafter, for up to three years.

The successful candidate will be expected to pursue research at the Synchrotron Radiation Source at Daresbury into structural changes in solids at very high pressures, on the newly commissioned Power Diffraction Station on the Wiggler beam line. The project involves application of novel high-resolution powder diffraction techniques to materials at very high pressures, and is backed by a major theoretical programme of molecular dynamics simulations.

Applicants should hold a PhD in experimental science. Experience with X-ray or neutron crystallography, and computing would be an advantage. The starting salary will be in the range £7,520 to £8,450 according to age and experience.

Applications, together with a full curriculum vitae and the names of two referees to Dr D M Adams, Department of Chemistry, University of Leicester, Leicester LE1 7RH by 15th July 1985. (6682)A

SANDWELL HEALTH AUTHORITY

The Midland Centre for Neurosurgery and Neurology

DEPARTMENT OF PATHOLOGY

ELECTRON MICROSCOPIST in charge of Section

(Basic/Senior Science Graduate)

required on a Locum basis (available grading up to Senior as defined by Whitley Council PTA regulations). Well housed department with two other staff, two Siemens microscopes, several ultramicrotomes. Successful applicant will be required to continue and develop service in Muscle, Central and Periperal Nervous System Pathology, to Hospital and Region. Collaboration with current research projects will be expected and there is an excellent opportunity to develop a personal research programme on the readily available material.

Intending applicants are welcome to visit by appointment with Dr M V Salmon (Neuropathologist) - 021-558 3232 ext 314.

Job description and application form from the Administrator, Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick, Warley, West Midlands B67 7JX. Tel: 021-558 3232 ext 251. (6677)A

Cell Biologist/Biochemist/ Neurobiologist

Postdoctoral research position is immediately available for PhD with experience in cell biology, biochemistry, or neurobiology to investigate hereditary neuromuscular disorders. Initial research will focus on mechanisms of regulation of cell growth and the developmental expression of surface proteins in nerve, muscle, and related tissues. Applicants should have a PhD and experience in: cell biology and biochemistry, culture of nerve, muscle and fibroblasts; immunological methods for the study of cell proteins. Extensive possibilities exist for collaboration with expert neuromuscular morphologists and with active molecular genetics/recombinant DNA laboratory. Excellent support; salary commensurate with experience. Direct inquiries to Dr. Robert H Brown, Jr , Day Neuromuscular Research Center, Massachusetts General Hospital Warren 370/374, Boston, MA 02114. (NW1792)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

ENDOCYTOSIS

A post-doctoral research assistant is required to work on a cell-free assay for studying the pathway of adsorptive endocytosis. The aim is to understand the molecular basis of this process. Preference will be given to candidates with training in membrane biochemistry and/or enzymology.

This project is funded by the MRC and the position is for three years commencing 1st January 1986. The salary will be in the range £7,520-£12,150 per annum.

Applications, including a curriculum vitae, research summary and the names and addresses of three referees, should be sent to the Personnel Officer, The University, Dundee, DD1 4HN, Scotland as soon as possible. Informal enquiries concerning this project should be addressed to Professor Graham Warren, who is presently at the European Molecular Biology Laboratory, Postfach 10 2209, 6900 Heidelberg, West Germany. Tele phone number 010 49 6221 387324. Please quote Ref EST/66/85J. (6466)A

RESEARCH ASSOCIATE POSITION

Opportunity exists to work on a project concerned with the biochemical mechanisms of hormone (insulin) action to control phosphorylation and redox state Applications are invited from qualified candidates with a Ph D or M.D. degree. Submit a resume of research interests, curriculum vitae and three letters of recommendation to Dr Joseph Larner, Department of Pharmacology, Box 448, University of Virginia School of Medicine, Charlottesville, Virginia 22908. An affirmative Action/Equal Opportunity Employer (NW1812)A

AGROGEN BIOTECHNOLOGIES SENIOR SCIENTISTS — PLANT TISSUE CULTURE

Agrogen Biotechnologies Inc , a Vancouver, BC based Canadian company interested in plant tissue culture, micropropagation of woody ornamentals and forest tree species and related biotechnologies is seeking Senior Scientists in plant tissue culture to provide project leadership and research support to its micropropagation program.

The positions require a PhD with a strong background in plant physiology, cell biology, genetics or biochemistry with several years research experience in plant tissue culture. Agrogen offers the opportunity to join an energetic interdisciplinary research team, excellent research facilities, competitive salary and fringe benefits.

Please forward your curriculum vitae and other pertinent information in confidence to: Dr. D. Paul Webb, Director of Research, Agrogen Biotechnologies, Inc. 1710-1066 West Hastings Street, Vancouver, BC V6E 3X2. (NW1793)A

THE UNITED MEDICAL AND DENTAL SCHOOLS

ST THOMAS'S CAMPUS

RESEARCH ASSISTANT

(post-doctoral)

required to work on project on the role of Coxsackie-B Viruses in the pathogenesis of insulin-dependent diabetes mellitus (type 1).

Sound virological experience essential; experience with such techniques as ELISA, monoclonal antibody production and PAGE an advantage. Funding is being sought for one year appointment from 1 September 1985 in the first instance Salary not less than £7,520 plus £1,233 LWA pa (grant application pending).

For further information, contact Professor J E Banatvala, Dept of Virology, tel: 01 928 9292 ext 2405.

Applications giving full cv and names and addresses of 2 referees to Staffing Officer, Dean's Office, St Thomas's Hospital Medical School, London SE1 7EH by 13 June 1985. (6664)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF GEOLOGY

POST-DOCTORAL/ POST-GRADUATE RESEARCH ASSISTANT IN SEISMOLOGICAL COMPUTING

To work on the acquisition, digitisation processing and interpretation of the data from an international seismic refraction experiment to be carried out in Kenya in August 1985 The data processing will require the development, maintenance and operation of Fortran 77 programmes on VAX 11/70-8600 system. The anpointment will be for two years from July 1985 with a salary of £6,600 — £7,055

Applications, including a CV and the names of two referees, should be sent by June 7 to Dr M A Khan, Department of Geology, The University, Leicester LE1 7RH from whom further particulars may be obtained. (6641)A

UNIVERSITY COLLEGE LONDON

RESEARCH TECHNICIAN

Grade 4

Cell Biology

Applications are invited for the above post to assist in the development of a new project on the intracellular transport of membrane proteins, funded for 3 years by the MRC.

The person selected to join our small friendly group will have an interest in research and be qualified to at least HNC standard. Experience with tissue culture, cell fractionation or protein purification would be an advantage.

Salary £6,106 — £6,837 + £1,250 London Weighting.

Applications, including CV and the names of 2 referees should be sent to Personnel Officer (Technical Staff GE6), University College London, Gower Street, London WC1E 6BT. (6651)A

ROYAL HOLLOWAY AND BEDFORD COLLEGES

RESEARCH ASSISTANT

Applications are invited from Physicists, Engineers or Applied Mathematicians, to join a team engaged in a range of projects on fundamental aspects of Electromagnetic Antennas for Millimetric Wavelengths This project involves both theoretical work (using analytical and computational techniques) and supporting experimental studies in one or more of the following areas:-

(1) Electromagnetic Theory
(2) Numerical Control Engineering
(3) Applied Optics

Candidates should have an aptitude for research and a good degree or a postgraduate qualification in a relevant subject. Salary scale £9,863 — £11,093 (under review).

Applications (2 copies) with curriculum vitae and names and addresses of 2 referees should be sent immediately to the Personnel Officer, Royal Holloway and Bedford Colleges, Egham Hill, Egham, Surrey TW20 0EX. (6648)A

FACULTY POSITION

The Psychology Department of the Massachusetts Institute of Technology announces the opening of a faculty tenure-track position in neuroscience. Applicants with a PhD or equivalent degree are sought with training and research background in mammalian brain-morphology and function

Interested applicants should send a letter of inquiry with curriculum vitae and three references by July 31, 1985, to:

Dr. Peter H. Schiller
Psychology Department
Search Committee
Massachusetts Institute of Technology, E25-634
77 Massachusetts Ave.
Cambridge, MA 02139.

Qualified minorities and women are encouraged to apply. MIT is an affirmative action/equal opportunity employer. (NW1791)A

MIT

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

WELLCOME LECTURESHIP

Applications for the above post are invited from candidates in the field of neuroscience with expertise and interest in pursuing research involving the use of an in-vitro brain slice. Candidates wishing to visit the department or make provisional enquiries should contact Professor A M Sillito (0222)-44211 ext 2062.

Candidates should be less than 35 years old and must not currently hold a permanent university appointment. The post is tenable for five years (subject to a review after three years) and should commence on the 1st October 1985, although this date could be negotiated for a suitably qualified date. Appointment will be made at the appropriate point on the lecturer scale (scale is £7,520-£14,925).

Applications (three copies) with curriculum vitae, 500 word synopsis of the proposed research and the names and addresses of three referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P O Box 78, Cardiff CF1 1XL from whom further particulars may be obtained. Closing date 24th June 1985 Reference 2967 (6678)A

UNIVERSITY OF DUBLIN
Trinity College

TEMPORARY LECTURER IN ZOOLOGY

Applications are invited for the above post in the Department of Zoology at Trinity College, Dublin. The appointment will be tenable from 1st October, 1985 for one year, and may be renewed for a further year. Preference will be given to candidates having interests in Invertebrate Reproduction

Salary IR £9,302 per annum

Application forms and further particulars relating to this appointment may be obtained from.

Establishment Officer
Staff Office
Trinity College, Dublin 2
Tel 772941 Ext 1775

to whom completed application forms should be returned by **not later than Tuesday, 11th June, 1985** (6638)A

UNIVERSITY OF ZUERICH
DEPARTMENT OF PHYSIOLOGY
A MOLECULAR BIOLOGIST

is required for a three year postdoctoral position, to work on the regulation of mRNA by oxygen availability in cultured kidney cells Applicants should have PhD or equivalent in Biochemistry/molecular Biology and a minimum of two years of postdoctoral experience in these areas Knowledge in cell culture techniques would be an advantage Salalry in range sFr 47 000-60 000/yr, depending on experience and qualification. The position is available now, starting date to be agreed

Application with the names and addresses of two referees should be sent to Professor C. Bauer, Dept. of Physiology, University of Zuerich, Winterthurerstr 190, 8057 Zuerich, Switzerland. (W1894)A

PROFESSOR & HEAD
Biological Sciences
Carnegie-Mellon University

The Department of Biological Sciences invites applications from qualified individuals to serve as Professor and Chairperson We seek an individual with an established reputation in any field of biological research and an ability to provide leadership in the continuing growth of this department We expect to appoint the successful candidate by 30 June 1986 or before

Applications or inquiries should be sent to Professor W R McClure, Chairman, Search Committee, Carnegie-Mellon University, 4400 Fifth Ave , Pittsburgh, PA 15213
Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer.
(NW1765)A

CONFERENCES and COURSES

UNIVERSITY COLLEGE, LONDON:
SCHOOL OF MEDICINE

PART TIME COURSE IN TOXICOLOGY

A course in toxicology consisting of lectures and practical classes will be given weekly for 30 weeks, on Wednesdays at 15:45-19 00 hrs, starting on 2nd October 1985

A broad treatment of topics in toxicology will be presented, including basic histopathology, biochemical and genetic aspects and methodology. Regulatory aspects of toxicology will also be discussed The course is intended (1) for graduates with some experience in a relevant field; (2) for postdoctoral and other scientists wishing to broaden their knowledge of toxicology

The fee for the 30 week course is £600. Numbers will be restricted to a maximum of 25 participants Some assisted places where all or part of the course fees are waived are available to suitable applicants whose fees are not paid by their employer (e g persons working in Universities or similar institutions and holding non-tenured posts) Further details and application forms may be obtained from: —

Dr. I.C. Shaw
Toxicology Laboratory
Department of Clinical Pharmacology
University College London
5 University Street
London WC1E 6JJ, England
(01) 388-2411 Ext 212

Closing date for applications — 1st July 1985 (6656)C

Royal Postgraduate Medical School
(University of London)

A specialist scheme of study for graduates in medicine and biology leading to the award of:

DIPLOMA in ENDOCRINOLOGY

This is a one academic year, full-time, intensive laboratory-based training course open to graduates in medicine and biology who wish to develop skills in modern endocrinological investigative methods The selected applicants, who must be experienced in light or electron microscopical morphology, biochemistry or a related discipline, will undertake a series of research projects during their training period in recognised laboratories within this Medical School Award of the Diploma will depend on successful completion of an examination or by thesis

Further details and application forms may be obtained by writing to:

The School Office, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS

Completed application forms must be received before 31 July 1985 for registration on the 1985-86 Diploma course (6681)C

GRANTS and SCHOLARSHIPS

Research Award in Immunoregulation

Centocor, Inc., will award a $10,000 unrestricted research grant for the most outstanding human Interferon-Gamma scientific paper published (or accepted for publication) between 1/1/85 and 6/30/86.

For details, write to Centocor customer service.

CENTOCOR
244 Great Valley Parkway
Malvern, PA 19355
Fax (215) 644-7558
Telex 834823

(NW1726)H

WORKSHOP

ST JOHN'S COLLEGE CAMBRIDGE

α-1-ANTITRYPSIN MOLECULES AND MEDICINE

8-11 September 1985

Guest Chairman Professor C-B Laurell Sweden

An international workshop to sumarise progress and define goals in research and clinical management.

Enquiries: Professor R W Carrell St John's College, Cambridge CB2 1TP UK (6477)A

FELLOWSHIPS

THE UNIVERSITY OF SHEFFIELD

Faculty of Medicine

J G GRAVES MEDICAL RESEARCH FELLOWSHIP

Applications are invited for the above Fellowship tenable for a period of three years from 1 October 1985. The Fellow will be required to undertake full-time medical research in one of the medical or dental departments or hospital. Initial salary according to qualifications and experience within the range £7,520 — £8,920 a year, a higher salary may be offered to a candidate with appropriate experience and seniority. Additional funds may be available for the Provsision of laboratory assistance and materials.

Further particulars from the Personnel Department (Academic Staffing), the University, Sheffield S1Q 2TN to whom applications (10 copies), including the names and addresses of three referees, (indicating the line of research proposed), should be made by 17 June 1985. Quote refs R269/G. (6665)E

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

Supported by the British Gas Corporation. The work will be concerned with "Development of Membranes with Improved Selectivity" and will be directed mainly towards synthesis of novel polymers suitable for fabrication into membranes showing improved selectivity in gaseous diffusion processes combined with high stability at elevated temperatures.

The post will initially be for one year with a salary of up to £8,920 per annum depending on age, qualifications and experience. Superannuation is under USS conditions.

Applications are invited in the form of a curriculum vitae (3 copies) including the names and addresses of two referees, and should be sent to the Personnel Office (JLG), University of Surrey, Guildford, Surrey GU2 5XH as soon as possible, quoting reference 375. (6646)E

Please mention

nature

when replying to these advertisements

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Cell Culture of Marine Algae

Applications are invited for a Post-Doctoral Research Fellowship funded by the University of Aberdeen to work with Drs S B Wilson, F B Williamson and W F Long. The project will involve the development of continuous cultures of marine red algal cells primarily to produce materials with commerical value.

The post has initially been funded for one year but scope exists to obtain further financial support giving an appointee with flair and imagination a chance to share in the longer term future of this exciting new field. Applicants should preferably have a PhD and some experience in cell culture.

Salary on Range IA Scale for Research and Analogous Staff up to £8,920 per annum, with appropriate placing (scale under review).

Further particulars and application forms from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by 30 June 1985. (6668)E

POSTDOCTORAL FELLOWSHIP

In Molecular Biology/Biochemistry Position available Sept. 1, 1985 in Department of Microbiology at SUNY Stony Brook to study adeno-associated virus genetics, vector construction immunology or DNA replication. Send CV and references to Dr. Nicholas Muzyczka, University of Florida, College of Medicine, Box J-266 JHMHC, Gainesville, Florida 32610. (NW1797)E

POSTDOCTORAL FELLOWSHIP POSITIONS

Molecular Biology

Well-established cardiovascular research laboratory studying the molecular biology of lipoproteins and receptors is seeking molecular biologists with experience in cloning and DNA sequencing

Candidates with a Ph.D. in molecular biology or biochemistry are eligible

Send curriculum vitae and three letters of reference to: Personnel, c/o Dr. Brian McCarthy, The Gladstone Foundation Laboratories for Cardiovascular Disease, University of California, San Francisco, P.O. Box 40608, San Francisco, CA 94140-0608.

(NW1803)E

POSTDOCTORAL FELLOWSHIPS

California Biotechnology Inc. invites applications from recently qualified Ph.D.'s to join its postdoctoral research program. CBI is an established biotechnology company located in the Palo Alto area forty miles south of San Francisco. The company, which has about eighty scientific employees, is dedicated to applying the techniques of molecular biology, protein chemistry and cell biology to develop novel human and veterinary pharmaceuticals. It currently has major research programs in hypertension, atherosclerosis, inflammation, lung disease, reproductive biology, growth factors and polypeptide hormones. Recent graduates, who have experience in areas such as recombinant DNA technology, protein and peptide chemistry, cell biology and pharmacology, would join established research teams in one of the above areas. The postdoctoral fellowship would for a minimum of two years.

(NW1800)E

Please send applications, including a complete curriculum vitae to:

CBI

Dr. John Shine
Director of Research
California Biotechnology Inc.
2450 Bayshore Frontage Road
Mountain View, CA 94043

ESF PROGRAMME OF RESEARCH FELLOWSHIPS IN TOXICOLOGY

Short-term (up to 6 months) and Long-term (6-12 months) Research Fellowships (deadline: 30 September 1985) and Short-term (up to 1 month) Visiting Fellowships (no deadline) are offered to scientists working in European countries. No formal condition of age, level or field of training, provided the project contributes to furthering research in TOXICOLOGY in Europe. Information and application forms can be obtained from: Dr John Smith, European Science Foundation, 1, quai Lezay-Marnésia, F-67000 STRASBOURG, France; tel (88) 35 30 63; telex 890440. (W1890)E

EMBO
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
Autumn 1985 — Awards
Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany.

(W1893)E

STUDENTSHIPS

THE POLYTECHNIC OF CENTRAL LONDON

Faculty of Engineering & Science

RESEARCH POSTS IN THE SCHOOL OF BIOTECHNOLOGY

A. GENERAL AND MOLECULAR GENETICS OF FILAMENTOUS MICROORGANISMS

Post 1. SERC Research Studentship

Post 2 Research Assistantship

B. MICROBIAL PHYSIOLOGY/ BIOCHEMISTRY

Post. 3 SERC Biotechnology Research Studentship for a project entitled "Beta-lactum biosynthesis in free and immobilized Streptomycetes.

Post 4. Research Assistantship — candidates should have an interest in analytical biochemistry and particularly HPLC techniques.

Posts 1, 2 and 3 are tenable for three years from 1st October 1985. Applicants should hold or expect to gain a first or upper second class honours degree in a relevant discipline. The successful candidates will be expected to register for a PhD Degree.

Post 4 is available immediately or as soon as possible and applicants should hold or expect to hold a degree in an appropriate subject and is tenable for a minimum of three years. For suitably qualified and motivated candidates there may be an opportunity to register for an M Phil degree.

Salaries for posts 1 and 3 will be according to SERC current regulations. Salaries for posts 2 and 4 will be according to University Research Assistant Scales since the project "Extended Use of Biocatalysts" is undertaken by PCL jointly with University of Kent at Canterbury and University College London under the auspices of the Institute for Biotechnological Studies. The programme is financially supported by the Department of Trade and Industry and seven major industries.

Please send a curriculum vitae and the names of two referees to the Personnel Officer, The Polytechnic of Central London, 309 Regent Street, London W1R 8AL, as soon as possible.

PCL is an equal opportunities employer. (6615)F

OXFORD UNIVERSITY

Research Laboratory for Archaeology

RESEARCH STUDENTSHIP (SERC)

for thermoluminescence, laser-based optical dating, radiocarbon dating using a tandem accelerator or archaeomagnetism using a SQUID cryogenic magnetometer. Suitable for physicist, chemist or earth scientist anticipating good honours degree.

Details: Dr M J Aitken, FRS. 6 Keble Road, Oxford OX1 3QJ, telephone (0865) 55211. (6659)F

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF NEUROLOGY

POSTGRADUATE RESEARCH STUDENTSHIP

Applications are invited for a research studentship tenable in the Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital. The successful applicant will read for the degree of PhD Two topics are available. (i) intermediary metabolism in skeletal muscle and the origin of metabolic myopathies; (ii) the use of cytochemical techniques to study the molecular differentiation of the muscle cell surface during development and regeneration of the neuromuscular junction

Candidates should be qualified in an appropriate discipline (biochemistry, physiology, etc) and should possess or expect to obtain a First Class or Upper Second Class Honours degree.

Application should be made within two weeks of the appearance of this advertisement in the form of a curriculum vitae, and candidates should include the names of two academic referees The project for which application is being made should also be identified. All correspondence should be sent to Professor J B Harris, Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, from whom further information may be obtained. (6643)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GEOGRAPHY

POSTGRADUATE STUDENTSHIP

Applications are invited for a postgraduate studentship to work with Dr Ann Henderson-Sellers on Synthesis of cloud climatologies.

This project builds on previous work in the department and is a contributory part of the International Satellite Cloud Climatology Project (ISCCP). The student will be expected to have, or aquire, a sound knowledge of meteorology. Much of the data analysis is computer-based so that previous experience of computer programming would be useful. The project is funded by a grant from the US Air Force and will involve the student collaborative work with US and European scientists. It is anticipated that applicants will hold, or hope to gain, at least an upper second class honours degreem in physics, mathematics or physical geography or a similar science subject.

Applications, together with the names of two referees should be received not later than 20 June, 1985, by The Registrar, The University, PO Box 147, Liverpool L69 3BX Quote ref RV/291/N. (6620)F

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

Applications are invited from Chemistry graduates, or those about to graduate for the following research studentships which will become available as from 1st October, 1985.

(1) A CASE studentship in conjunction with ICI Pharmaceuticals to study A Thermodynamically-Based Scale of Hydrogen Bond Basicities. (Dr M H Abraham).

(2) A CASE studentship in conjunction with the Imperial Cancer Research Fund to study Mechanisms of Aromatic Hydroxylations. (Dr J R Jones).

(3) A Ministry of Defence sponsored studentship to study Aromatic Amine Cure of Epoxy Resins. (Drs G J Buist and J R Jones).

(4) A CASE studentship in conjunction with ICI to study The Synthesis of Polysulphones from Arylsulphonic Acids and PCl_5. (Professor J B Rose).

Interested persons should send their curriculum vitae and the names and addresses of two referees to Dr J R Jones, Chemistry Department, University of Surrey, Guildford GU2 5XH, as soon as possible (6647)F

GEORGE HENRY LEWES STUDENTSHIP

Applications are invited for this Studentship to pursue original investigations in Physiology. Appointment may be from 1 October 1985 for one year with a possibility of renewal for up to two further years. The award will provide an annual maintenance grant in line with the maintenance element of a Research Council research studentship; and the trustees will pay University and College fees for the holder at the rates charged to science research students from the UK.

Candidates should by 28 June 1985 send (a) a statement of their qualifications and their need for pecuniary help, (b) the subject of their proposed research, and (c) the names and addresses of two referees, to Professor R D Keynes, Physiological Laboratory, Downing Street, Cambridge CB2 3EG, from whom further particulars may be obtained. (6639)F

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF MICROBIOLOGY

RESEARCH STUDENTSHIP IN MOLECULAR BIOLOGY

Applications are invited from persons possessing or expecting to obtain at least an Upper Second Class Honours degree in a Biological Science.

The successful applicant will join a group involved in the study of microbial pathogenesis using molecular genetic techniques.

Further details may be obtained by contacting Dr I N Clarke on Southampton 0703 777222 ext 3853. Please quote reference number AJS/85/37/N. (6671)F

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

SERC RESEARCH STUDENTSHIP

Photosensitization of Tumours

Applications are invited for the above studentship, tenable from October 1985, to study fundamental biochemical aspects of photochemotherapy, a new approach to cancer treatment, involving photosensitization of tumours by porphyrin-containing drugs.

Candidates should have, or expect to obtain, a good Honours degree in Biochemistry or an appropriate related discipline.

Further information may be obtained from Dr S B Brown, Department of Biochemistry, University of Leeds, LS2 9JT (0532 431751 ext 7500), to whom applications (including the names of two referees) should be sent. (6663)F

UNIVERSITY OF SHEFFIELD

DEPARTMENTS OF BIOCHEMISTRY AND MICROBIOLOGY

SERC EARMARKED STUDENTSHIP FOR PROTEIN ENGINEERING

A studentship is available for three years from October 1985 to work for a PhD on in vitro mutagenesis of a bacterial glutamate dehydrogenase. The student would join an interdisciplinary team headed by Professor J R Guest (DNA manipulation), Dr P C Engel (enzymology) and Dr D W Rice (crystallography) but would primarily be involved in the DNA aspects of the project.

Applicants should have or expect to obtain a First or Upper, Second Class Honours Degree in Biochemistry or Microbiology.

Applicants naming two referees should be sent to Dr P C Engel, Department of Biochemistry, University of Sheffield, Western Bank, Sheffield S10 2TN by 14 June 1985. Quote ref: R247/G. (6616)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY

SERC CASE STUDENTSHIP

Applications are invited for a SERC CASE Studentship to work in collaboration with Dr F Y Liew (Wellcome Research Laboratories, Beckenham) on the protection potential of T cell responses in mice to genetically engineered specific HSV glycoprotein peptide fragments.

Applicants should have at least 2.1 Honours degree in Biochemistry, Biology, Immunology or Genetics.

Applications, together with the names of 2 referees should be made by 30 June 1985 to Dr W L Chan, Dept of Zoology and Comparative Anatomy, University College London, Gower Street, London WC1E 6BT Tel: (01) 387 7050 ext 428 (6675)F

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY

SERC STUDENTSHIP

Applications are invited for a 3 year studentship to work on the structure of squid rhodopsin and its role in activation of the photoreceptor enzyme cascade, using electron microscope image analysis and biochemical methods. Applicants should have, or expect to obtain, a good Honours degree in Biophysics, Biochemistry or related subjects.

Applications with a curriculum vitae and names of 2 referees should be sent as soon as possible to Dr H R Saibil, Department of Zoology, South Parks Road, Oxford OX1 3PS, to whom further enquiries may be made (Tel (0865) 56789 ext 317). (6657)F

UNIVERSITY OF READING

SUB DEPARTMENT OF HORTICULTURE

SERC CASE STUDENTSHIP IN PLANT PHYSIOLOGY

Applicants are invited for a CASE studentship tenable from 1 October 1985 to study genetic and environmental effects on the physiology and biochemistry of pod development in cocoa, in collaboration with Cadbury Schweppes plc.

Candidates should possess, or expect to obtain, a first or upper second class honours degree in Plant Sciences, Biochemistry or a closely related subject.

Further details may be obtained from Dr P Hadley, Sub Department of Horticulture, University of Reading, Earley Gate, Reading RG6 2AU. Applicants should send a curriculum vitae with the names of two referees not later than 10 June 1985 (6618)F

IMPERIAL COLLEGE (University of London)

CENTRE FOR BIOTECHNOLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited from candidates, preferably with a background in molecular biology, who hold, or expect to be awarded, at least two: one honours in biochemistry or biology for SERC research studentship. The award is for three years commencing October 1985. The project will involve the supression of gene expression in plants by anti-message RNA.

Informal inquiries may be made to Doctor Conrad Lichtenstein, Tel: (01) 589 5111 Ext 4112/7094.

Applications (cv and two academic referees) should be sent to me no later than June 30th 1985.

To: Centre for Biotechnology, Imperial College, London SW7 2AZ. (6690)F

UNIVERSITY OF ABERDEEN

DEPARTMENTS OF CHEMICAL PATHOLOGY AND THERAPEUTICS AND CLINICAL PHARMACOLOGY

POSTGRADUATE STUDENTSHIP

Applications are invited from graduates to work on a research project involving the use of isolated renal proximal tubular cells to investigate mechanisms of drug-induced nephrotoxicity. Candidates should have a good Honours degree in Biochemistry, Pharmacology or a related subject.

The studentship is initially for one year and the student will be registered for an MSc. Applicants, with a full curriculum vitae and the names of two academic referees, should be sent to Dr P Whiting, Department of Chemical Pathology, University Medical Buildings, Foresthill, Aberdeen AB9 2ZD as soon as possible. Further details are avalable from Dr Whiting (telephone 0224-681818, ext 2822) or Dr G Hawksworth (ext 2463). (6685)F

UNIVERSITY OF WARWICK

DEPARTMENT OF CHEMISTRY

SERC CASE AWARD

Solution Chemistry of Technetium : Redox and Extraction Properties

Applications are invited from graduates or those expecting to graduate this Summer with at least a class II-1 honours degree in chemistry, for this studentship. The project, carried out in collaboration with British Nuclear Fuels plc, will be concerned with kinetics of chain reaction of TcO_4^- with hydrazine and with the co-extraction of Tc with UO_2^{2+} ion. Normal SERC rates will be supplemented by £600 pa.

Further details may be obtained on application to the Personnel Office, University of Warwick, Coventry CV4 7AL. Please quote Ref No 42/A/85/O. Closing date for applications 21st June 1985. (6672)F

UNIVERSITY OF GLASGOW

BIOTECHNOLOGY UNIT

SERC CASE STUDENTSHIP — ICI

To clone and study expression of resistance and production genes from Streptomyces for an antibiotic of interest to ICI. The project is supervised jointly by Dr I S Hunter (Glasgow) and Dr P Groves (ICI, Corporate Lab, Runcorn).

Applicants should have, or expect to obtain, a first or upper second class honours degree in a related biological science. Interviews will be conducted towards the end of June.

Applications (CV and names of two referees) should be sent to Dr I S Hunter, Institute of Genetics, Church Street, Glasgow G11 5JS, before June 13th, 1985. (6596)P

AWARDS

ROSENSTIEL AWARD IN OCEANOGRAPHIC SCIENCE

Nominations are invited for the 1985 Rosenstiel Award in Oceanographic Science.

This award, administered on behalf of the Rosenstiel Foundation by the Rosenstiel School of Marine and Atmospheric (RSMAS) at the University of Miami recognizes outstanding contributions to marine science, including oceanographically relevant aspects of atmospheric science, and fundamental developments in ocean engineering. The award consists of a cash prize, currently $5,000, and a medal. The recipient of the award will be invited to spend a week at RSMAS for discussions with faculty and students. The award will be presented at that time.

To accumulate the multidisciplinary value of oceanographic science, the award recognizes, on a rotating basis, achievements in four broad disciplinary areas. In 1985 the emphasized discipline will be physical oceanography and air-sea interaction The achievements recognized may consist of contributions towards the development of ocean science in general, or of more focussed individual research of recognized impact on our understanding of the marine environment.

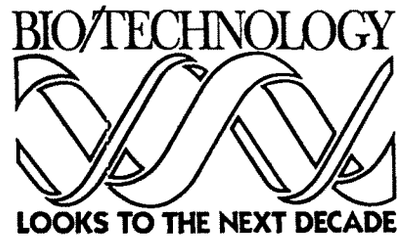
Nominations for the 1985 award for outstanding achievement in physical oceanography or air-sea interaction should be directed to Dean Alan Berman, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149-1098, before August 15, 1985 Nominations should include a brief justification together with relevant reference The selection panel would especially welcome nominations of outstanding younger scientists, whose early contributions suggest a continuing role of leadership in the field. (NW1796)N

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ASSOCIATESHIPS

Research Associateship in Experimental Nuclear Physics

There is a vacancy for an experimental physicist to join the nuclear physics group as a Research Associate at the Daresbury Laboratory, an establishment of the Science and Engineering Research Council situated in the north Cheshire countryside

The group is involved in a research programme on the Nuclear Structure Facility, a large tandem accelerator which currently is operating at up to 20 MV on terminal.

Currently, the principle areas of study are: direct reactions with light and heavy ions, production of exotic nuclei far from stability, nuclei with very high angular momentum, nuclear breakup and fragmentation and studies of isotope shifts and hyperfine structure using laser-induced resonance fluorescence Experimental equipment includes a Q3D magnetic spectrometer, advanced gamma-ray and neutron detection apparatus, and a 1m diameter scattering chamber An isotope separator with an associated dilution refrigerator and a beam line for laser studies has recently been brought into full operation, a recoil separator is in an advanced commissioning stage, and a heavy-ion polarised source is under construction

Applicants (male/female) should possess a PhD degree or expect to obtain one during 1985

An appointment will be made at a salary between £9,977 and £11,220 per annum depending on age, ability and experience The post is available for a fixed term of three years and is superannuable.

CLOSING DATE: Sunday 30th June, 1985

For further information please write or telephone.
Dr. J. Lilley on Warrington (0925) 65000 Ext 558

Application forms may be obtained from and should be returned quoting reference DL/912 to:
The Personnel Officer,
Science & Engineering Research Council,
Daresbury Laboratory, Daresbury,
Warrington, Cheshire,
WA4 4AD (6623)O

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

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ASSISTANTSHIPS

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COURTAULD INSTITUTE OF BIOCHEMSITRY
A POST DOCTORAL RESEARCH ASSISTANTSHIP IN MOLECULAR BIOLOGY

financed by The Cancer Research Campaign is available for work in a Molecular Toxicology group examining the role of glutathione and glutathione transferases in carcinogenesis, the cancer cell and cancer chemotherapy. The successful applicant would join several other molecular biologists from this group who work in association with Professor Roger Craig's Medical Molecular Biology Unit. The post is tenable for three years and the salary will be on the 1A scale according to age and experience.

Applications, in writing with curriculum vitae together with the names of two referees should be sent to: Professor Brian Ketterer, Cancer Research Campaign Molecular Toxicology Group, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London W1P 7PN. (6621)P

THE SCHOOL OF PHARMACY
University of London
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
POSTGRADUATE AND POSTDOCTORAL RESEARCH ASSISTANTSHIPS
in Chemical and Biological NMR Spectroscopy

Candidates should have shown scholarly achievement in chemistry, biochemistry, biophysics or pharmaceutical chemistry. Research in the department includes synthetic and medicinal chemistry, peptide and protein chemistry, recombinant DNA, receptor structure and function, enzyme structure and function, metabolism, surface and colloid chemistry, electroanalytical chemistry, mass spectroscopy, NMR spectroscopy, light scattering and diffusion

Emphasis is placed upon applications of chemistry and biochemistry to the pharmaceutical sciences.

Salaries offered (Research Assistants) range from £6,600 — £12,150 pa plus £1,233 pa London Allowance.

Applications, which should include the names of two referees and two copies of CV should be sent to Personnel Officer, The School of Pharmacy 29/39 Brunswick Square, London WC1N 1AX. (6673)P

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DEPARTMENT OF PHYSICS
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Initial salary £7,520 — £8,920 per annum on Range 1A plus superannuation

Applications (3 copies), naming three referees should be sent by 24 June 1985 to the Registrar, Science Laboratories, Durham DH1 3LE from whom further particulars may be obtained. (6644)P

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CHAIR OF BIOCHEMISTRY

Applications are invited from suitably qualified Biochemists for the post of Professor of Biochemistry which will carry with it in the first instance the Headship of the Department of Biochemistry & Soil Science. The appointment will be from as early a date as can be arranged. The salary will be in the professorial range (minimum £18,070).

Further particulars can be obtained from the Secretary and Registrar, UC NW, Bangor, Gwynedd, LL57 2DG.

Ten copies of the application (one from overseas applicants), together with the names and addresses of three referees, should be sent to reach the Secretary and Registrar by 1st July 1985. (6669)A

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Send CV to Dr G Currie, Marie Curie Memorial Foundation, Research Institute, The Chart, Oxted, Surrey RH8 0TL. Telephone 088388-2306/7. (6614)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
BIOPHYSICS GROUP

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Applications including curriculum vitae and names of two referees should be sent to Mrs A Varsey, Biophysics Group, Blackett Laboratory, Imperial College, London SW7 2AZ. (6634)A

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DEPARTMENT OF IMMUNOLOGY

POSTDOCTORAL RESEARCH FELLOW

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Scientific enquiries to Dr A Johnstone (01-672 1255 ext 4756) and further particulars and application forms from the Personnel Officer, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE (ext 4873). Closing date 27 June 1985. (6628)A

RESEARCH CENTER DIRECTOR

Applications and nominations are invited for a Director, Center for Atmospheric and Biospheric Effects of Combustion, located in the Applied Science Division. The scope of the Center is interdisciplinary and includes four major areas: Combustion and Abatement, Atmospheric Pathways and Chemistry, Radiative Balance, and Ecosystem Pathways and Effects. The Director will coordinate the efforts of a number of scientists, including chemists, physicists, engineers, biologists, geologists, meteorologists, and energy analysts to carry out the objectives of the Center; will be responsible for obtaining research support from governmental and non-governmental organizations; will represent the Laboratory at various related national and international meetings; and will also carry out research within the Center. This position requires a Ph.D., and a record of distinguished leadership, outstanding research accomplishments and an international reputation in one or more of the above areas. Experience and background in program planning, development, implementation, administration and evaluation is required. The incumbent should also have experience in obtaining research funds and have a familiarity with organizations that provide these research funds. Salary will be negotiable. The successful candidate will be appointed a Senior Staff Scientist at the Lawrence Berkeley Laboratory.

Applications and/or nominations should be forwarded to: Lawrence Berkeley Laboratory, One Cyclotron Road, Employment Office 90-1042, Berkeley, CA 94720. Please specify Job A/3330. An Equal Opportunity Employer M/F/H. (NW1777)A

LAWRENCE BERKELEY LABORATORY

Cardiovascular Group Leader

Roche is a major multinational pharmaceutical company with a firm commitment to the research and development of novel drugs.

A senior vacancy has arisen in the recently restructured department of Biology for an innovative and enthusiastic scientist to form and lead a new group within the Cardiovascular section.

The successful candidate will play a key role in the cardiovascular research programme, reporting to the Head of Cardiovascular Biology. He/she will have a high degree of scientific autonomy, but will also be expected to interact effectively with Chemists, Biochemists and other Pharmacologists as part of a multidisciplinary approach to drug research.

Ideal qualifications for the post would be a PhD in pharmacology plus a few years experience of industrial Cardiovascular research encompassing both in vivo and in intro techniques.

Research facilities at the Hertfordshire based UK Research Division Headquarters are outstanding as are the company benefits/salary we can offer you.

To take things further, call **Mrs Ann Judge** on **Welwyn Garden City 328128** or write to her, enclosing details, at

Roche Products Ltd,
PO Box 8,
Welwyn Garden City, Herts. AL7 3AY.

ROCHE

(6684)A

NORTH EAST BIOTECHNOLOGY CENTRE

Applications are invited for the following posts which will be based at:

TEESSIDE POLYTECHNIC

in the Department of Chemical Engineering.

READER IN BIOTECHNOLOGY

To lead research teams and supervise research students and develop research projects in areas of interest to the NEBC. The major strengths of the Centre are in Fermentation, Microbial Physiology and Downstream Processing of biological products.

Applicants should be well qualified academically in a discipline related to Biotechnology or Biochemical Engineering and will be expected to hold a PhD and have made a significant contribution to the literature relevant to the post.

Industrial experience would be preferable and membership of a professional institution an advantage.

Salary: £13,095 – £16,467 per annum.

PRINCIPAL LECTURER IN BIOCHEMICAL ENGINEERING

To take responsibility for the operation and further development of the Biochemical Engineering content of courses in process engineering run jointly by the department of Chemical Engineering and the North East Biotechnology Centre.

It is expected that the successful candidate will also undertake research projects, especially in aspects of downstream process design.

Applicants should be well qualified in Biochemical Engineering or a discipline of fundamental importance to this subject area. They will have demonstrated a proven research record in a relevant area, an effective approach to student teaching and appropriate industrial experience. Members of an appropriate professional institution would be an advantage.

Salary: £13,095 – £14,580 (work bar) – £16,467 per annum.

The salary on appointment will be no greater than £14,580 per annum.

LECTURER II/SENIOR LECTURER BIOCHEMICAL ENGINEERING

A Biochemical Engineer to join a team of Biotechnologists promoting research, teaching and consultancy in the North East Biotechnology Centre. The team will be expected to develop teaching and research programmes in one or more of the following areas: Downstream Processing, Control of Fermentation Processes, and the Application of Microbial Physiology to Fermenter Design.

Applicants should be well qualified academically in or a discipline related to Biotechnology or Biochemical Engineering; preferably with some industrial experience; membership of a professional institution would be an advantage.

Salary: £7,548 – £12,099 (efficiency bar) – £13,128 (work bar) – £14,061 per annum.

An appointment will be made at Lecturer II level and the salary on appointment will be no greater than £12,099 per annum.

Closing date for applications in respect of all posts: 24 June 1985.

An Equal Opportunity Employer.

Application forms and further particulars from: Personnel Section, Teesside Polytechnic, Borough Road, Middlesbrough, Cleveland TS1 3BA. Tel: 0642 218121 Extension 4114. (6636)A

THE UNIVERSITY OF ALBERTA

invites applications for the

CANADIAN PACIFIC – ALBERTA AGRICULTURE CHAIR IN PLANT BIOTECHNOLOGY

The University of Alberta is seeking an established scientist to occupy the Canadian Pacific – Alberta Agriculture Chair in Plant Biotechnology. The occupant of the chair will be expected to carry out research related to the improvement of agriculture in Western Canada, to provide leadership within the area of plant biotechnology in the Faculties of Agriculture and Forestry, and Science, and to communicate actively with the agricultural sectors of industry and government. Candidates presently located outside the scientific university community will be given serious consideration, although scientific credentials will be of paramount importance.

The successful candidate will be a member of the Department of Plant Science, in the Faculty of Agriculture and Forestry. The Faculty of Agriculture and Forestry has ninety academic members housed in a modern, well-equipped research complex. In addition, within the Facilty of Science, members of the Departments of Botany, Genetics, and Microbiology are currently involved in basic and applied plant research. The University of Alberta (approximately 25,000 students) is located in the capital city of Alberta. **Please send curriculum vitae, names and addresses of at least three referees, and an outline of research interests, including reprints of relevant publications, to:**

Dr. Mary Spencer
Department of Plant Science
Faculty of Agriculture and Forestry
University of Alberta
Edmonton, Alberta T6G 2P5
Canada

Closing date for receipt of applications is August 31, 1985. Salary will be by negotiation. Appointment will commence on January 1, 1986, or as soon as possible thereafter.

The University of Alberta is an equal opportunity employer but, in accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada. (NW1794)A

KAROLINSKA INSTITUTE, STOCKHOLM, SWEDEN

Invites applications for a position as

FULL PROFESSOR IN MOLECULAR BIOLOGY

The Karolinska Institute is seeking an outstanding Molecular Biologist to join the newly formed Biotechnology Center located at Huddinge University Hospital in Stockholm. The successful candidate is expected to develop a vigorous independent research program. He will be expected to interact with other research teams within the center and in other departments as Karolinska Institute working in related fields. Funding for 3 Post-Doctoral positions, 3 graduate students, 1 Secretary, 4 Technicians and Instruments as well as supplies will be available for the successful candidate.

Application including C.V., a short summary of previous scientific and educational experience, proposal for future research, list of publications as well as reprints or copies of publications or manuscripts (four copies of each) should be received at Karolinska Institutes Registratorskontor, Solnavagen 1,S-104 01 Stockholm, no later than Thursday June 13th, 1985. Applicants who wish to have a later deadline may submit material so that it reaches the above address no later than July 4th, 1985, provided that they, no later than June 13th, make clear to the Karolinska Institute (same address as above) that they wish to apply for the position, and that they state what material they will submit later.

Further information can be obtained from Professor Jan-Oke Gustafsson (Tel. No. 8/7463351) and Dr. Rune Toftgord (Tel. No. 8/7463361). (W1897)A

Tenure track Assistant Professor positions are available in the Department of Pharmacology, University of Iowa, College of medicine. Applications are invited from well-trained individuals who possess the Ph.D. and/or M.D. degree and who are active in an area suitable for development of a vigorous research program related to molecular and cellular pharmacology or more traditional pharmacological disciplines. The University of Iowa is an equal opportunity/affirmative action employer. Submit curriculum vitae and names of three references to:

Search Committee
Department of Pharmacology
Bowen Science Building
University of Iowa
Iowa City, IA 52242
(NW1762)A

ENDOCRINOLOGY BIOCHEMISTRY/ PHYSIOLOGY

Postdoctoral Opportunities

Broad range of Endocrine research projects: (i) isolation and identification of pituitary and lung peptides; (ii) physiologic and pharmacologic action; (iii) growth and control of function of fetal adrenals; (iv) role of growth factors in fetal development.

Send curriculum vitae, bibliography, three letters of recommendation, and short statement regarding career goals, interests and experience to: Dr Samuel Solomon, Endocrine Laboratory, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec H3A 1A1. (NW1772)A

SPECIAL RESEARCH INITIATIVES

1. **Research Fellow**
   Analytical Atomic Spectroscopy
2. **Research Assistant**
   Cretaceous Micropalaeontology
3. **Research Technician**
   Fish Disease/Cell Culture

For further details and an application form, please contact Personnel Officer at the address below or telephone (0752) 264639.

Closing date for applications: 21 June 1985.
(6660)A

Plymouth Polytechnic
Drake Circus, Plymouth
Devon PL4 8AA

Bureau of Mineral Resources, Geology & Geophysics

Canberra

MARINE RESEARCH GEOPHYSICISTS

The Division of Marine Geosciences and Petroleum Geology in the Australian Bureau of Mineral Resources, Canberra, has vacancies for two Marine geophysicists, one being at senior level.

The principal research aims of the Marine Division are to define the regional geological framework, petroleum prospectivity and evolution of the sedimentary basins of Australia's extensive continental shelf and margins; further to develop new scientific concepts of basin structures and evolution of passive margins and also the active margins of the South Pacific. The main objective is the stimulation of ongoing programs of offshore petroleum exploration. The Division has recently acquired a research vessel with full multichannel seismic capability and seismic processing centre.

SENIOR MARINE GEOPHYSICIST:

to initiate and lead research and resource assessment programs on the Australian Continental Margins and in Australian Territorial waters.

The successful candidate should have a strong background of research experience and an international reputation. He/she should be experienced in multichannel seismic operations, seismic structural and stratigraphic interpretation and in aspects of geohistory analysis as well as have associated experience in other fields of marine geophysics and petroleum. **(Salary at SPRS/PRS level depending on qualifications and experience.)**

MARINE GEOPHYSICIST:

to undertake research and resource assessment programs on the Australian Continental Margins and in Australian Territorial Waters.

The successful candidates should have a history of research, be experienced in multichannel seismic operations, in seismic structural and stratigraphic interpretation and in at least some aspects of geohistory analysis. Associated experience in other fields of marine geophysics and petroleum would be valuable. **(Salary at SRS/RS level depending on qualifications and experience.)**

Qualifications for Both Positions:

A Ph.D (or equivalent) in marine geophysics together with demonstrated research ability.

Salary:

Research Scientist (RS) A$26001-32083
Senior Research Scientist (SRS) A$33209-38243
Principal Research Scientist (PRS) A$39692-45083
Senior Principal Research Scientist (SPRS) A$47501-52212

Conditions:

Conditions of service include superannuation for appointees, long service leave, cumulative sick leave, four weeks annual leave and assistance with removal expenses to Canberra. Permanent appointment is available to Australian Citizens. A term engagement will be considered for persons not meeting this criterion.

Canberra, the national capital, is located approximately 280km southwest of Sydney and has excellent educational, recreational and sporting facilities.

Applications together with full personal and professional details and the names of at least three referees should be sent to:

The Director,
Bureau of Mineral Resources,
GPO Box 378, Canberra ACT
2601, AUSTRALIA.

Applications close 21 June, 1985.

AUSTRALIA

(W1903)A

Cell Biologist (PhD)

elf bio recherches

Elf Bio Recherches, a subsidiary of Elf Aquitaine and Sanofi, specialises in biotechnology. It is looking for a Cell Biologist for its laboratory of animal cell physiology and fermentation. This laboratory (2 scientists and 3 technicians) forms part of the recombinant animal cell technology unit. The position is to investigate the role of physiological factors involved in the expression of genes cloned into animal cells. Candidates should have a profound knowledge of animal cell physiology. Experience in medium or large-scale tissue culture will be an advantage. Knowledge of French would be appreciated. Elf Bio Recherches R&D facilities are located in the Toulouse area, in Southern France. Curriculum vitae and list of publications, together with a handwritten letter of application, should be sent to M. Fabre quoting reference **B/T 2018N.**

PA

(W1900)A

71 bis, allées Jean-Jaurès, 31000 Toulouse, France.
Tel: Toulouse 63.70.63.

NATIONAL OPTICAL ASTRONOMY OBSERVATORIES

Associate Director-NOAO and Director-Cerro Tololo Inter-American Observatory — Applications and nominations are invited for the position of Director, Cerro Tololo Inter-American Observatory. The position reports to the Director NOAO and carries the added designation of Associate Director, NOAO. The incumbent will be a key member of a management team who plans and guides the development of NOAO as a program characterized by authoritative in-house science and imaginative and effective support to the user community. The position will be located in La Serena, Chile, and will carry explicit responsibilities for the planning, implementation, and oversight of AURA's program in Chile. The post offers excitement, challenge, and the promise of deep personal satisfaction derived from direct participation in the growth of an institution dedicated to the highest standards of in-house research, service to the astronomical community, and innovative approaches to astonomy. Applicants must have an earned doctorate in astronomy, or a closely related physical science, and a distinguished record of achievement in astronomy; evidence of leadership ability of a high order will also be sought. Interested individuals who feel that their background and temperament match the opportunities and inherent challenge of this position are invited to forward applications, with names of at least three people from whom a confidential evaluation can be sought to The Chair, Search Committee for Director CTIO, National Optical Astronomy Observatories, 1002 North Warren Avenue, Tucson, AZ 85719. Closing date for applications is July 31, 1985.

Further information can be obtained from Dr. John T. Jefferies, Director NOAO, at 602/881-1960. An EEO/AA Employer.

(NW1786)A

LUDWIG INSTITUTE FOR CANCER RESEARCH
UNIVERSITY OF SYDNEY

Post Doctoral Research Fellow

Applications are invited from Post Doctoral Scientists for a Research Fellow to join a group studying in vivo growth regulation of solid tumours, microenvironment and the effects of cytotoxic drugs. Techniques currently in use employ tissue culture, syngeneic and xenogeneic animal tumours, and clinical material, with an excellent flow cytometry facility.

Applicants should possess a good background in cellular biology, and some experience in tumour growth kinetics would be an advantage.

An attractive salary within the range A$22,614-A$25,246 p.a. will be negotiated.

Further information from Dr. D. Hedley, (02) 692 3675/76.

Applilcations should be addressed to:

The Administrator,
LUDWIG INSTITUTE FOR CANCER RESEARCH,
Blackburn Building,
University of Sydney, NSW 2006. Australia.

(W1896)A

MRC
MEDICAL RESEARCH COUNCIL
CLINICAL PHARMACOLOGY UNIT
UNIVERSITY DEPARTMENT OF CLINICAL PHARMACOLOGY — OXFORD

NEUROCHEMIST

Applications are invited for a MRC SHORT-TERM NON-CLINICAL SCIENTIFIC post in this MRC Unit, tenable for 3 years in the first instance. Candidates with some post-doctoral experience will be preferred.

The successful candidate will be required to study the neuro-chemical mechanisms by which neurotransmitter systems respond to the short- and long-term effects of psychotropic drugs. Knowledge and experience of the biochemical mechanisms of neuronal membrane function, neurotransmitter synthesis and release, and techniques of brain slice and synaptosome incubation and stimulation would be advantageous. This work has strong links with an active clinical psychopharmacology section.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from Professor D.G. Grahame-Smith, University Department and MRC Unit of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE (Oxford (0865) 241091), with whom applications, including a full curriculum vitae and the names and addresses of two professional referees, should be lodged by 24th June 1985.

(6613)A

AN ACTIVE FERTILITY AND IN VITRO PROGRAMME IS DUE TO OPEN AT THIS HOSPITAL ON 1st AUGUST 1985

Applications are invited for the following position, for which date of commencement will be by mutual agreement.

TECHNICAL ASSISTANT/EMBRYOLOGIST

The successful applicant will be expected to undertake both routine laboratory and research work. Research is currently undertaken in collaboration with MRC (Carshalton) and the Institute of Animal Physiology (Cambridge).
Experience in mammalian embryology would be desirable and a PhD or MSc in reproductive physiology or embryology would be an advantage, but is not essential.
The Hospital offers a generous salary and excellent benefit package.
Applicants should forward a Curriculum vitae, including the names of three referees to: The Personnel Department, Humana Hospital Wellington, Wellington Place, London NW8
Closing Date 22nd June 1985. (6599)A

Humana Hospital Wellington
Wellington Place London NW8 9LE

UNIVERSITY OF WISCONSIN-MADISON AQUATIC BOTANIST

RESEARCH ASSOCIATE (postdoctoral) position available by 1 June, 1985, for laboratory and lake studies on integrated control of Eurasian watermilfoil and impact of control strategies on water quality. Additional experience in water chemistry, limnology, or mycology desirable. Funding for two years. Salary competitive and commensurate with qualifications.

Send résumé, including the names of three referees, to: Dr. John H. Andrews, Department of Plant Pathology, 1630 Linden Drive, Madison, Wisconsin 53706.

An affirmative action-equal opportunity employer.

(NW1781)A

POST DOCTORAL RESEARCH

position is available to study the molecular biology of the enzymes involved in connective tissue degradation. A research background in biochemistry or molecular biology is advantageous. Applicants should send curriculum vitae and three references to Gregory I. Goldberg, PhD, Washington University School of Medicine, Box 8123, St. Louis, Missouri 63110.

Equal Opportunity/Affirmative Action Employer.

(NW1788)A

MOLECULAR IMMUNOLOGY POST-DOCTORAL SCIENTIST

Salary from £9,000

Celltech, a leader in the field of biotechnology, has achieved its present success in the space of five years. Pioneers in several areas, our achievements are based on the foundations of scientific excellence, innovation and commercial expertise.

Celltech's Molecular Immunology group is expanding its activities in the molecular cloning and expression of immunological targets, principally antibodies. We are looking for a Molecular Biologist/Immunologist, either a post-doc or a graduate expecting soon to finish a PhD. Experience in molecular or cellular immunology would be desirable.

We are an ambitious and forward looking company, which places as much emphasis on its people as its products. You will work in a small friendly team, gaining invaluable broad-based experience, with plenty of opportunity for advancement.

Please send a detailed c.v. to Jane Walley, Celltech Ltd., 244–250 Bath Road, Slough, Berks SL1 4DY, quoting Ref. 268.

CELLTECH

Express Dairy UK Ltd., one of the country's largest Dairy Food organisations has a vacancy in the Research and Development Department.

DEVELOPMENT ANALYST

c.£8,100

Working in a small team, this position involves analytical instrument evaluation and method development. You will be reporting to the Technical Advisor — Rapid Analytical Method Development and should be a Graduate in Analytical Chemistry with at least one year's experience in modern techniques of analysis associated with the food industry.

A current driving licence is essential.

In addition to the salary detailed, 22 days holiday and membership of the Company's pension scheme are available on completion of the necessary qualifying period.

Application forms obtainable from:

Mr. G. D. Evans,
Personnel Officer — Central Staff,
Express Dairy UK Ltd.,
Block I, 430 Victoria Road,
South Ruislip,
Middlesex, HA4 0HF.
Tel: 01-845 2345 Ext. 3551/3370.

(6680)A

Immunodiagnostics

Cambridge BioScience Corporation, a biotechnology company specializing in infectious disease diagnostics offers exciting positions in its Hopkinton, Massachusetts laboratories. We are seeking advanced level scientists as well as research associates.

Molecular Biology

- All levels with extensive background in recombinant-DNA, cloning and expression, minimum of 2 years academic or industrial experience.

Virology/ Immunology

- Ph.D. level with experience in clinical virology
- Experience in hybridoma development
- Working knowledge of molecular virology

Cambridge BioScience offers a unique and stimulating environment and close working associations with academic institutions. **Qualified applicants should send resume and salary requirement to: Cambridge BioScience Corporation, Personnel Department, 35 South Street, Hopkinton, MA 01748.**

(NW1806)A

Cambridge BioScience

Experimental Nuclear Physicists

There are vacancies for experimental physicists to join the Nuclear Structure Division at Daresbury Laboratory, an establishment of the Science and Engineering Research Council which operates major national facilities for scientific research.

The group is involved in carrying out and supporting a research programme on the Nuclear Structure Facility (NSF), a large tandem accelerator which is operating at up to 20 MV on terminal.

Senior Scientific Officer

Ref. DL/911

The successful applicant will be required to organise and direct work necessary for the operation, maintenance and development of major equipment for charged particle identification and detection used in programmes aimed at studies of nuclear collision dynamics, spectroscopy, and nuclei far from stability. Candidates will be expected to carry out research in conjunction with university users on the NSF and to play an active role in initiating and developing new programmes. Other duties will involve the overall coordination and scheduling of the scientific programme and the assessment of future needs for instrumentation and other facilities on the NSF.

Applicants (male or female) should have a good honours degree (or equivalent qualification) in physics and several years postgraduate experience in experimental nuclear research. A Ph.D qualification in nuclear physics and a period of relevant post-doctoral experience would be an advantage.

Higher Scientific Officer

Ref. DL/913

The successful applicant will be required to liaise with university research teams using the NSF. Candidates will also be expected to collaborate in nuclear research programmes and to play an active role in initiating and developing new programmes. Other duties will involve work on the design and development of major equipment through to commissioning, operation and maintenance. As a member of the in-house team of scientists he/she will be expected to provide expertise in the methods of experimental nuclear physics to aid and direct the technical and scientific support staff in operating the NSF and for diagnosing problems as they arise.

Applicants (male or female) should have a good honours degree (or equivalent qualification) in an appropriate discipline with a period of relevant post-graduate experience. A Ph.D degree in nuclear physics and a period of post-doctoral experience in experimental nuclear research would be an advantage.

The salary scales according to qualifications and experience for the Higher Scientific Officer and Senior Scientific Officer posts are £7,435 to £10,039 per annum and £9,329 to £12,050 per annum respectively. These salary scales are currently under review.

There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

CLOSING DATE: 30th June 1985.

For further information please write or telephone Dr. J. S. Lilley on Warrington (0925) 65000, Ext. 558.

Application forms may be obtained from and should be returned quoting the appropriate reference number to: The Personnel Officer, Science & Engineering Research Council, Daresbury, Warrington, WA4 4AD. (6624)A

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

Faculty Position available, tenure-track, in molecular genetics, with a special interest in membranes. Applicants should have research background in recombinant DNA technology (preferably also in somatic cell genetics) and working knowledge of membrane proteins. M.D. and/or Ph.D. in appropriate field required. Successful candidate will hold tenure-track appointment (Assistant or Associate Professor, depending upon qualifications) in Department of Physiology and Molecular Biophysics at Baylor College of Medicine. Joint appointments in relevant academic departments also possible. Laboratory space and basic equipment provided and initial laboratory support available. Successful candidate will devote majority of his/her effort to establishing and maintaining an independent research program related to the main interests of the Department. The Department is building a strong program in membrane biophysics with expertise in electrophysiology, membrane biochemistry, reconstitution, and modelling. **Send vita, a brief description of past and present research experience, and names of three or more references to**

Dr. A. M. Brown, Incoming Chairman,
Physiology and Molecular Biophysics Department

Baylor College of Medicine,
One Baylor Plaza,
Houston, Texas 77030.

An Equal Opportunity A/A Employer

(NW1814)A

SENIOR SCIENTIFIC PROGRAMMER

National Optical Astronomy Observatories
The National Optical Astronomy Obervatories

(NOAO) needs a senior level programmer for its Detector R&D Program in Tucson. Working with scientists and engineers, the position involves development of software to operate state-of-the-art infrared arrays and signal processing equipment for astronomical instrumentation. We require skills in the development of real-time software for data acquisition and process control applications and date reduction and analysis programs. Applicants should have experience in FORTRAN and one of the structured languages such as "C". Experience in a real-time programming environment and software signal procesing is required. Knowledge of FORTH, UNIX , and DEC computers is desirable. An Electrical Engineering or Computer Science degree and at least five years experience as a programmer are required. Other degree background will be considered with more extensive programming experience.

The NOAO, headquartered in Tucson, Arizona, operates Kitt Peak National Observatory near Tucson; National Solar Observatory sites at Sacramento Peak, New Mexico, and on Kitt Peak; Cerro Tololo Inter-American Observatory in Chile, and the Advanced Development Program in Tucson.

Offering forefront technology challenges, and unclassified workplace, and a Sunbelt location, we are an EEO/AA employer with an excellent compensation package, including 24 days annual vacation and, after one year, a fuly vested retirement plan.

Send résumé in confidence to: Judy Ruffino, Personnel Manager, P.O. Box 26732, Tucson, Arizona 85726.

Equal opportunity employer. (NW1804)A

MACROMOLECULAR CRYSTALLOGRAPHER

Three-year Postdoctoral Research Associate position. Seeking motivated, talented person with experience in experimental aspects of structure determination to conduct x-ray diffraction studies. Several ongoing projects at different stages under the overall theme of intermolecular interactions. Excellent facilities for data collection, reduction, and analysis: rotating anode generators area detector, oscillation/precession cameras, 2 VAX 11-750s, node to CRAY, color vector and raster graphics. Exciting work environment within a strong departmental structure group including six faculty crystallographers. Salary negotiable. Superb working setting in new building located on cliffs overlooking ocean. Send C.V. and three references to:-

Elizabeth Getzoff or John Tainer, Department of Molecular Biology — MB3, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037. (NW1799)A

THE ROYAL VETERINARY COLLEGE
University of London

Division of Preclinical Studies
Department of Physiology, Biochemistry and Pharmacology

Lecturer in Biochemistry

Applications are invited from veterinary, science, or medical graduates for a post involving both teaching and research which it is hoped to fill by 1st October 1985. An interest in metabolism and previous experience in lipid and lipoprotein biochemistry would be of advantage.

Salary Scale (under review): £8,753 to £16,158 including London Allowance. Universities Superannuation Scheme, Removal expenses up to £1,400 reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. (Tel: 01-387-2898).

Closing date for applications: 17 June 1985. (6630)A

UNIVERSITY OF NATAL
DEPARTMENT OF ZOOLOGY
ZOOLOGY

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin for appointment to the post of:

LECTURER IN BIOLOGY

Minimum qualifications would be a PhD in Zoology, Botany, Biology or a closely allied field and/or suitable academic or research experience.

The successful applicant will be responsible for teaching a first year introductory course, which is essentially a service for students in the Faculty of Agriculture. The four major areas covered by the course are Cell Biology, Plant Anatomy and Development, Animal Physiology, and the Ecology and Evolution of Animals and plants. No particular area of expertise is required beyond a sound and comprehensive knowledge of biology, but the appointee will be encouraged to actively participate in research and to supervise post-graduate students.

The appointment carries an attractive salary package, details of which are obtainable from the Staffing Section on request (telephone 0331-63320). The salary offered will be determined according to the qualifications and/or experience of the successful applicant.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, service bonus, staff bursary, housing loan, and subsidy schemes, long leave conditions and travelling expenses on first appointment, are available from the Registrar, University of Natal, PO Box 375, Pietermaritzburg, South Africa, 3200, with whom applications on the prescribed form must be lodged not later than 28 June 1985 quoting reference PMB 29/85.

Application forms for overseas applicants are available from the Secretary, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. (W1895)A

COMMONWEALTH AGRICULTURAL BUREAUX

THE LEADING WORLD AGRICULTURAL INFORMATION SERVICE FOR SCIENTISTS AND TECHNOLOGISTS

SCIENTIFIC INFORMATION OFFICER

REQUIRED AT THE
COMMONWEALTH BUREAU OF PLANT BREEDING AND GENETICS, CAMBRIDGE

Duties:
Preparing abstracts for the Journal *Plant Breeding Abstracts*, which covers world scientific literature in this field: indexing, and answering scientific enquiries.

Qualifications:
A degree in botany, genetics, or a related subject is essential, plus a reading knowledge of at least one European language (Slavonic languages preferred) and the ability to write good English. Applicants must be able to render the contents of scientific publications into concise, accurate and readable abstracts.

Salary:
In the scales £5,909–£8,153 p.a., £7,435–£10,039 p.a. Starting salary according to qualifications and experience.

Application forms and further particulars from
Commonwealth Bureau of Plant Breeding and Genetics
Department of Applied Biology
Pembroke Street, Cambridge CB2 3DX
Tel. No: Cambridge (0223) 358381, etxn.216.
Closing date for applications: 20th June, 1985.

(6689)A

Bureau of Mineral Resources
Geophysics Division
Canberra
GEOMAGNETISM SECTION MANAGER
A$34757 - A$37028

The Geomagnetism Section of the Division of Geophysics, Bureau of Mineral Resources, comprises a staff of 15. The Section is responsible for operating the magnetic observatories and conducting magnetic surveys in the Australian and Antarctic regions, and also runs a modern palaeomagnetic facility. Mission oriented research is carried out in the fields of solid-Earth geomagnetism and palaeomagnetism.

Duties:
The Manager, acting under the Section Head, is responsible for administration of the section, and the co-ordination of magnetic (including palaeomagnetic) observatory and survey operations. The person appointed will be expected to initiate and participate in aspects of the research program.

Qualifications:
An appropriate degree or equivalent, ability to manage a scientific team, experience in magnetic (including palaeomagnetic) observatory and survey operations, the ability to carry out original research in geomagnetism.

Conditions:
The BMR is part of the Australian Public Service. To qualify for permanent appointment, applicants must be Australian citizens. Non-citizens may be appointed under a 3-year contract, with the expectation that they seek permanent appointment. Conditions of service (pension scheme, leave entitlement, etc.) are those pertaining to the Australian Public Service. Moving and settling-in allowances are paid to persons, and their dependent families, appointed from overseas.

Canberra, the national capital, is located approximately 280km southwest of Sydney and has excellent educational, recreational and sporting facilities.

Applications, including a curriculum vitae, statement of research interests, and names of professional referees should be addressed to: Staff Section, Bureau of Mineral Resources, P.O. Box 378 Canberra, A.C.T. 2601, Australia.

Please quote reference: Position No. 2409, Geomagnetism. Applications should be submitted before 30th June 1985. (W1899)A

AUSTRALIA

Consejo Superior de Investigaciones Cientificas
INSTITUTO CAJAL
(Madrid, Spain)

Three permanent research positions will be available in 1985 at The Cajal Institute for Neurobiology. One of the positions is open for neurophysiologist and the other two for specialists in any other branch of Neuroscience. The appointments will be at the Research Associate level. Applicants must be Spanish citizens and have a M.D. or Ph.D. degree.

Further information may be obtained from:
The Director, Instituto Cajal, Velâquez 144, 28006-Madrid, Spain. (w1889)A

Postdoctoral or Research Associate Position

Available July 1, 1985. Project involves function of actin isoforms, post-translationally modified tubulin species, and microtubule-associated proteins. Biochemisty or molecular biology background is desirable; the lab uses these, as well as peptide antibody techniques, HPLC, cell culture, and immuno-staining. Salary depends on experience. Send curriculum vitae and the names of three references to:

Dr. J.C. Bulinski, Department of Biology, University of California, Los Angeles, CA 90024. (NW1805)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions:

Research School of Earth Sciences
POSTDOCTORAL FELLOW/RESEARCH FELLOW/ SENIOR RESEARCH FELLOW
NOBLE GAS GEOCHEMISTRY

Applications are invited for an appointment in the School's Geochronology and Isotope Geochemistry Group, the field of interest being noble-gas geochemistry. The appointee will work with Dr Ian McDougall, Senior Fellow, in developing research in this field, which is new to the School. The School wishes to make the appointment at the Research Fellow level, but will be prepared to consider applications at the level PDF or SRF.

Noble gas investigations are being initiated to assist in elucidation of the geochemical evolution of the mantle, the mantle-crust system, and the atmosphere. Research initially will concentrate on mantle-derived rocks, with subsequent development of studies on fluids in the crustal environment. Provision of a suitable high-resolution gas-source mass spectrometer is in hand, and construction of appropriate extraction lines is planned.

Persons with suitable backgrounds in the physical sciences, and with particular interests in geochemistry, are encouraged to apply. Experience in noble gas studies on geological samples would be a great advantage.

The appointee will assist in developing the laboratory facilities, and will be expected to undertake discrete projects within this broad field of study. Active collaboration with other workers in isotope geochemistry is envisaged. Enquiries may be directed to Dr I McDougall (062 49 4136).

Closing date: **19 July 1985.** Rf: ES/1.5.1.

Faculty of Science
DEPARTMENT OF ZOOLOGY
POSTDOCTORAL FELLOW

The successful applicant will work on a NH & MRC funded project involving gene cloning of sheep parasites particularly larval tapeworms and liver fluke. Particular interest will be centred on those genes which code for antigens that are involved either in resistance to reinfection or immunopathological phenomina. A principal objective will be to obtain expression of these antigens in prokaryotic systems. Experience in gene cloning techniques is essential and some knowledge of immunological procedures would be highly desirable. The successful applicant will work in the Centre for Recombinant DNA Research, Research School of Biological Sciences (Executive Director: Professor J A Pateman FRS). The position is funded for 3 years. Previous applicants need not reapply as all applications will be considered.

Further information is available from Dr M J Howell, (062) 49 3060 or (062) 49 4225.

Closing date: **15 July 1985.** Ref: FS/1.5.1

CONDITIONS OF APPOINTMENT: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow $A21,411-$24,528 pa; Research Fellow $A24,840-$32,634 pa; Senior Research Fellow $A34,699-$41,390 pa. Current exchange rate is $A1.00 = Stg 0.48.

Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. **Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be sent to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. General Information also available from the Secretary General, Association of Commonwealth Universities (Appts) 36 Gordon Square, London WC1H 0PF.**

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W1880)A

UNIVERSITY OF TORONTO
DEPARTMENT OF BOTANY
POSTDOCTORAL FELLOW

required to work in group studying molecular basis of metal-tolerance in micro-organisms and higher plants. Applicants should have experience in recombinant DNA techniques; knowledge of microbial physiology and plant ecology an advantage. Salary is in range of $21,500 Canadian. Position is available immediately. **Please send detailed letter and C.V., plus three letters of reference to Prof. T.C. Hutchinson, Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1.** (NW1809)A

VICE PRESIDENT FOR RESEARCH
THE UNIVERSITY OF GEORGIA

The University of Georgia invites nominations and applications for the position of Vice President for Research.

Located in Athens, Georgia, The University of Georgia is the nation's oldest chartered state university. The University ranks among the top 50 research institutions in the country and operates with annual research expenditures in excess of $80 million across its 13 schools and colleges.

The Vice President for Research will administer the University's research resources, serve as an executive officer of the University and report to the President. He/she will facilitate and coordinate a vigorous expansion of competitively-funded research programs, and seek new avenues for creative partnerships with the private sector and government agencies. She/he will exert strong leadership in the organisation and funding of new, interdisciplinary research programs, and will formulate reward and incentive systems for faculty participation in sponsored research.

Nominees/applicants should have a record of executive-level accomplishments, knowledge of current R&D environments encompassing national and international perspectives in private, public, and governmental sectors, and should possess a working knowledge of higher education, including the nature and scope of university research activities. Candidates should have the terminal degree or equivalent experience in their discipline, and credentials suitable for appointment at the level of professor in the University.

Nominations should include the nominee's address and telephone number. Applications should include a letter of interest, a curriculum vitae, and the names and addresses of 5 persons who can serve as references. The closing date for receipt of completed applications is **July 15, 1985.** The position may be occupied as early as January 1, 1986.

Address all nominations and applications to:

Vice President for Research Search Committee
c/o Dr Luis M. Proenza
Office of the President
The University of Georgia
Athens, Georgia 30602

The University of Georgia is an Equal Opportunity/Affirmative Action Employer

(NW1795)A

MAMMALIAN CELL BIOLOGIST

A Ph.D. with academic or industrial post-doctoral experience is sought for a position in a multidisciplinary group studying high density mammalian cell culture in the Biotechnology Laboratory of Central Research at The Dow Chemical Company. The successful candidate will have experience in handling multiple cell lines and associated analytical techniques and will have responsibility for the planning, organization and execution of a high priority program in the development of optimal culture conditions.

For further information, please contact:

Dr. Kerr Anderson
Central Research
Biotechnology Laboratory
1701 Building
The Dow Chemical Company
Midland, MI 48674

(NW1808)A

SCOTTISH CROP RESEARCH INSTITUTE
DIRECTOR

The Governing Body of the Scottish Research Institute invites applications for the post of Director which will become vacant on the retirement of Professor C E Taylor CBE in March 1986.

SCRI, which is funded by an annual grant from the Department of Agriculture and Fisheries for Scotland, is a major crop research institute within the Agricultural and Food Research Service. It's research programme requires a wide range of scientific disciplines associated with the production and breeding of arable and horticultural crops.

Candidates should have an established record of scientific leadership and the capability to manage an organisation with over 300 staff.

Salary; £26,909 per annum non-contributary superannuation scheme.

The Institute is an equal opportunities employer.

Further particulars can be obtained from Mr N D Anderson, The Secretary to the Governing Body.

Applications accompanied by a full curriculum vitae and the names and addresses of three referees should be lodged with the Secretary to the Governing Body, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA.

Closing date 12 July 1985. (6679)A

DROSOPHILA DEVELOPMENT

University of Arizona

Two postdoctoral positions available to work on molecular aspects of pattern formation and morphogenesis in Drosophila. One position state-funded at the level of Visiting Lecturer, with opportunities for teaching. Salary commensurate with experience. Send CV, statement, and names, addresses, and telephone numbers of at least three referees to: Dr. Danny Brower, Department of Molecular & Cellular Biology, Biosciences West Building, University of Arizona, Tucson, AZ 85721, USA. Position to begin Fall 1985. An equal opportunity/affirmative action employer. (NW1801)A

TWO RESEARCH ASSOCIATE POSITIONS

Available immediately. Experience in proteins and recombinant DNA. Salary $18,000 to $22,000.
Send CV and three references to Dr. O.P. Bahl, Dept. of Biological Sciences, SUNY at Buffalo, Cooke Hall 347, Buffalo, NY 14260. Equal Opportunity/Affirmative Action Employer. (NW1811)A

Post Doctoral Fellow Experimental Pathology

At Smith Kline & French we wish to appoint a Post Doctoral Fellow within a new laboratory of our expanding Pathology Department.

The work will be to apply cell and organ culture techniques to the investigation of cell pathology related to new and model compounds. Integrated projects with histopathologists, EM and clinical pathologists will also be involved.

A PhD and post doctoral experience in the use of *in vitro* techniques applied to experimental pathology is required. Experience in bone marrow CFU and lymphocyte culture would be an advantage.

We offer a competitive salary and benefits for this two year fixed term appointment.

Please write for an application form or send a detailed CV outlining your qualifications and experience quoting reference number N/123/JW to John Ward, Personnel Officer, Smith Kline & French Research Limited, The Frythe, Welwyn, Herts AL6 9AR.

SK&F

UNIVERSITY OF MANITOBA
Department of Plant Science

POSTDOCTORAL POSITION PLANT CYTOGENETICS

Applications are invited for a position available September 1 to carry out research on wheat-rye chromosome substitutions in triticale. These studies concern the use of chromosome banding techniques for the identification and establishment within an isogenic background, disomic substitution lines involving individual chromosomes of rye and their homoeologues from wheat. Experience with chromosome banding techniques and electrophoretic analysis of plant proteins in desirable. The appointment is initially for one year with a maximum of two years. Starting salary from $18,500 to $20,500 per annum according to experience.
Send curriculum vitae with names and addresses of two referees to:- **Dr. E.N. Larter, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.**(NW1807)A

DEVELOPMENTAL BIOLOGIST

CORNELL UNIVERSITY MEDICAL COLLEGE

Applications are invited for a senior or junior tenure-track faculty position. Applicants should have at least two years of postdoctoral experience and maintain a research program in early development or morphogenesis, using genetic, recombinant DNA, biochemical, immunological, and/or cell biological tools to explore mechanisms of pattern specification and determination in a vertebrate or invertebrate system. The position includes supervision of graduate students and teaching in a related area.

Send curriculum vitae, research summary, and three letters of reference to: Dr. Rosemary Bachvarova, Developmental Search Committee, Department of Cell Biology and Anatomy, Cornell University Medical College, 1300 York Avenue, New York, NY10021. An Equal Opportunity/Affirmative Action Employer. (NW1767)A

POSITION OPEN

RESEARCH ASSOCIATE POSITION

available immediately to study the dynamics of membrane phase transitions using a novel volume-perturbation kinetic calorimeter which we have developed. We are seeking a person with a Ph.D. in Biochemistry, Biophysics or related field for a Research Associate position to work on this project.
Our calorimeter measures the thermodynamic phenomenon associated with phase transitions in membrane systems via temperatures and pressure changes and optical properties. The person we seek must be able to synthesize artificial lipid membranes in pure form and mixed with other constituents, and be able to reconstitute protein recombinant systems so the proteins retain their native functions. This person will conduct experiments, do some hardware modification, and modify and use computer software for data acquisition and experimental analysis. The individual must have a working knowledge of membrane systems. He/she must also be experienced with computer hardware and software methods for data acquisition, instrument control, data analysis and modelling studies.
Send Curriculum Vitae and names of referees to Dr. Michael L. Johnson, Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908. An Equal Opportunity/Affirmative Action Employer. (NW1813)A

CELL BIOLOGIST

UNIVERSITY OF ALABAMA AT BIRMINGHAM

Applications are invited for two senior, tenured positions in the Department of Cell Biology at the level of Associate or Full Professor. Candidates must have achieved international recognition in cell or molecular biology, have a productive and well funded research program and be willing to move the program to Birmingham. Preference will be given to individuals employing molecular techniques to study the structure and function of cell components, growth control, regulation of gene expression, cell development or motility. Successful candidates will be offered nationally competitive salaries, excellent laboratory space and facilities, and an outstanding research environment in a rapidly expanding medical center. Ample opportunities exist for teaching in the Schools of Medicine and Dentistry including gross anatomy, neuroanatomy, histology or in a new graduate program in cell biology.

Applicants should send a curriculum vitae and a brief description of past and present research experience and the names of three or more references by July 1, 1985 to: B. R. Brinkley, PhD, Professor and Chairman, Department of Cell Biology and Anatomy, Schools of Medicine and Dentistry, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294. An Equal Opportunity/ Affirmative Action employer. (NW1785)A

Southampton THE UNIVERSITY

Department of Chemistry

Production and properties of Silicon-on-Insulator materials

A Postdoctoral position will be available from October 1985 for a Physical Chemist or Physicist to participate in a fundamental study of the production of new semiconductor materials. The project will employ a novel combination of techniques, including electrochemistry and in-situ ellipsometry applied to porous silicon substrates, which are being evaluated as the basis of the next generation of fast devices.

Previous experience in electrochemistry or spectroscopy would be an advantage, and some knowledge of computer programming or computerised instrumentation is necessary. The salary will be at a point on the SERC 1A scale commensurate with the age and experience of the appointee.

Applications together with the names of two referees should be sent as soon as possible to: Dr. R. Greef, Chemistry Department, The University, Southampton SO9 5NH, UK, from whom further information can be obtained. (6670)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE (UNIVERSITY OF LONDON)

DEPARTMENT OF MEDICAL PROTOZOOLOGY

MOLECULAR BIOLOGIST

A post-doctoral position will shortly become available in this Department for a 3-year period for a suitable individual who wishes to join a team investigating the mechanisms of drug-resistance in malaria, under the direction of the Head of the Department, Professor W. Peters. The project will be carried out in close association with the members of the Wolfson Unit of Molecular Medical Microbiology and Parasitology who are based in this Department. Applicants must have training and experience in recombinant DNA technology and will work closely with other members of the team who are investigating the very practical problem of drug-resistant malaria from other points of view, including the evaluation in depth of promising new antimalarial compounds. The appointment, which will be for up to three years in the first instance, carries a starting salary in the range £8,543 to £13,173 per annum.

Candidates should send, as soon as possible, a full curriculum vitae describing their previous training, research experience and research interests, together with the names of three referees, to the Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT. Please quote reference MP2.

(6632)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

Positions are available for a temporary lecturer, postdoctoral worker and SERC-CASE student. All posts are tenable from the 1st of October, 1985.

The temporary lectureship is available for 3 years and results from an MRC-replacement scheme award to Dr H C Nimmo. The appointee would be encouraged to undertake research and to collaborate with existing groups within the Department.

The postdoctoral position is available for 2 years to work with Professor Houslay's group on guanine nucleotide regulatory proteins and their interaction with adenylate cyclase. This appointment is funded by the MRC and will be made within the first three points of the 1A salary scale for research workers.

An SERC-CASE studentship award for the study of a PhD is available to work jointly with Professor Houslay at Glasgow and Dr Baxter at Glaxo. This project is aimed at understanding the physiological importance of the tyrosine kinase activity associated with the insulin receptor. The conditions of this award are as generally applicable to research council studentships.

Further information may be obtained from, and informal enquiries may be made to, Professor M D Houslay, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, to whom application (3 copies), should be made by 30th June, 1985. (6691)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ZOOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE IN NEUROBIOLOGY

Applications are invited for a postdoctoral Research Associate to work with a small group, supported by the SERC investigating the physiology and structure of insect brain neurones. Interests are both in the operation of synapses which identified visual neurones make amongst each other, and in the function of these neurones in the control of behaviour. In particular, we plan to compare the membrane properties and synaptic structure of different neurones, with the aim of understanding more about the roles of spikes in regulating transmitter release from neurones. Applicants should have experience in ultrastructural or in electrophysiological techniques, and are required either to have or to be close to completing a PhD. The appointment is for 3 years tenable from 1st July 1985 (deferable for up to 6 months).

Salary will be up to £8,450 per annum on the Range 1A scale (£7,520 - £12,150) according to age, qualifications and experience.

Applications (2 copies of curriculum vitae and research experience), plus names of 2 referees, or enquiries to: Dr P J Simmons, Department of Zoology, University of Newcastle upon Tyne NE1 7RU not later than 13th June 1985. (6627)A

NATURE CONSERVANCY COUNCIL

(Commissioned Research —)

(Contract Post)

DEPARTMENT OF ENTOMOLOGY

HIGHER SCIENTIFIC OFFICER DATA HANDLING/ ENTOMOLOGIST

Applications are invited for a one year contract post funded by the Nature Conservancy Council, to be based in the Entomology Department, Rothamsted Experimental Station, Harpenden, Herts. The successful applicant will be responsible for analysing light trap data for moths gathered by the Rothamsted Insect Survey. The project will investigate whether any long term trends are apparent for the polulations of particular moth species, or for the fauna of selected sites. The RIS moth data is stored on computer file and so previous computing experience is essential, and some familiarity with the British moth fauna would be an advantage.

Qualifications. Candidates should have a first or second class honours degree, or equivalent, in computing studies or in zoology and with at least two years relevant postgraduate experience.

Salary depending on age and qualifications. Higher Scientific Officer £7,435 rising to £10,039 per annum.

Application forms and further details are available from Miss B A Meredith, Personnel 1, Nature Conservancy Council, Northminster House, Peterborough PE1 1AU.

The closing date for receipt of completed application forms is 21 June 1985. The Nature Conservancy Council is an equal opportunity employer. (6653)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY PATHOLOGY

MOLECULAR VIROLOGY UNIT

A molecular virology unit has been established to develop novel vectors for the expression of viral antigens in mammalian systems. This unit works in close collaboration with virilogists and molecular biologists in this department and at the Beatson Institute.

Three positions, supported by the National Institutes of Health, are immediately available. Applications are invited for 2 post-doctoral fellows (salary range £8,450 to £10,720 per annum) and a research assistant (£6,600 per annum). The research assistant may apply to register for a higher degree.

The applicants should have experience in recombinant DNA technology and a background in virology would be an advantage. Applications should be sent to, and further particulars may be obtained from, Professor W F H Jarrett, FRS, University of Glasgow Veterinary School, Bearsden, Glasgow G61 1QH (telephone number 041-942 2301). (6692)A

UNIVERSITY OF LONDON THE CARDIOTHORACIC INSTITUTE

DEPARTMENT OF MEDICINE

NEW BLOOD APPOINTMENT

Applications are invited for a "New Blood" appointment in the Department of Medicine. The successful candidate is likely to be a clinical or non-clinical scientist with experience in the application of immunological and cell biological methods to study of the pulmonary macrophage. He or she will be expected to be sufficiently experienced to contribute new basic scientific ideas in this area and to carry these through experimentally in the current programme of research being conducted in the Department of Medicine into the cellular, biochemical and host defence aspects of pulmonary disease. A clinical appointee would be expected to co-ordinate the programme of laboratory research as it is applied to in-patients and out-patients, and to participate in the teaching activities of the Department.

Candidates should not hold a permanent University appointment in the UK. Appointment will be on the Clinical or non-Clinical Lecturer scale. A London Allowance is payable.

Applicants should apply by letter, enclosing 8 copies of their curriculum vitae, a statement of not more than 500 words on why they are applying for this post, and naming 3 referees to The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP from who further particulars can be obtained. Closing date for application 21 June 1985.(6645)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING

POSTDOCTORAL AND POSTGRADUATE RESEARCH ASSISTANTS RESEARCH TECHNICIAN

Applications are invited from biochemical and chemical engineers, biochemists and microbiologists for four posts in the Department of Chemical and Biochemical Engineering to work on two research programmes.

(1) Biocatalytic reaction involving water-insoluable organic compounds (supported by SERC).

(2) Extended use of biocatalysts (supported by DTI and seven companies through The Institute for Biotechnological Studies).

Appointments for three years starting as soon as possible will be made on the 1A and 1B scale for research assistants, and grade 3 scale for the technician.

Applications including CV and names and addresses of two referees should be sent to Professor M D Lilly, Department of Chemical & Biochemical Engineering, University College London, Torrington Place, London, WC1E 7JE. (6652)A

THE NATURE CONSERVANCY COUNCIL

SENIOR SCIENTIFIC OFFICER

Co-ordinator for remote sensing of the environment.

The successful candidate will be required to assist with the management and co-ordination of the National Countryside Monitoring Scheme. The duties will include advising NCC staff on digital cartography and the development of NCC's use of satellite imagery for monitoring purposes. Other responsibilities will include liaising with the Department of Environment, Countryside Commission, Countryside Commission (Scotland), Institute of Terrestrial Ecology and other organisations concerned with the monitoring of countryside feature through remote sensing.

Candidates should have a degree in an appropriate subject and at least four years post graduate experience. Essential experience will include digital cartography, photogrammetry, statistical analysis and sampling strategies involving remote sensing, the ability to write and edit the necessary computer software and management experience of remote sensing programmes.

Salary range: £9,329 to £12,050.

For further details and an application form, please contact Mr S Woodfield, Personnel 1, Nature Conservancy Council, Northminster House, Peterborough PE1 1AU to whom completed forms should be returned by the 19 June 1985.

The Nature Conservancy Council is an equal opportunity employer (6654)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GEOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT/ SENIOR RESEARCH ASSISTANT/ RESEARCH ASSOCIATE

to work on a study of fault geometry and mechanics using data from colliery plans and records. Applicants should be graduates in a branch of Earth Sciences and have a good grounding in either structural geology or geophysics. An interest in computing and/or quantitative methods in geology would be an advantage. There will be no opportunity to register for a higher degree. A candidate currently registered for a higher degree will only be considered if thesis submission is imminent.

Appointment will be from 1st July, 1985, initially for one year. Initial salary will be within the range £6,600 — £9,390 per annum.

Applications, together with the names of three referees, should be received not later than 14th June, 1985, by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained.

Quote ref RV/294/N. (6662)A

VICTORIA UNIVERSITY OF WELLINGTON

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURER IN BIOCHEMISTRY

Applications are invited for the above position, which is available from 1 February 1986. Applicants should have recently obtained a PhD in contemporary biochemistry. The most desirable areas of expertise are those of molecular biology (in particular recombinant DNA technology), molecular enzymology or protein chemistry. The research interest of the Department lie in mechanistic studies in biochemical pharmacology and toxicology with a bias towards projects of agricultural significance. The appointeee will be expected either to redirect his or her expertise into existing projects or to initiate new research compatible with departmental interests. Experience in relevant fields such as biochemical pharmacology or insect biochemistry or toxicology would therefore be advantageous.

Salary will be the range NZ$23,622 — NZ$27,928 per annum. The appointment will initially be for a period of two years. Extension for a third year may be possible depending on the outcome of a review of the Life Sciences at Victoria University.

Conditions of Appointment and method of application are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or the Administrative Assistant (Appointments), Victoria University of Wellington, Private Bag, Wellington, New Zealand, with whom applications close on 26 July 1985. (W1901)A

THE SCHOOL OF PHARMACY

University of London

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

POSTGRADUATE Research Opportunities in Pharmaceutical Chemistry

Applications are invited for a number of positions for postgraduate research leading to higher degrees. Candidates should have or expect to have a good honours degree in pharmacy, chemistry, biochemistry or biophysics.

Research Council and industrially sponsored projects on offer include.

(a) Diffusion and light scattering of biopolymer systems.
(b) Applications of fast atom bombardment mass spectrometry in pharmaceutical and biological chemistry.
(c) Chemical and biological NMR spectroscopy; and
(d) Synthesis and structure of natural products and peptides.

Applications should indicate area(s) of interest, giving names of two academic referees and two copies of CV and, to be sent to: Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX. (6674)A

MICROBIOLOGIST

Molecular Immunology Division

The Molecular Immunology Division of Allelix Inc. is seeking a microbiologist with experience in the culture, maintenance and isolation of clinically important infectious agents for the Product Development Group. The successful candidate will work in a team developing rapid diagnostic immunoassays for the diagnosis of infectious diseases. This individual will possess a Ph.D. in microbiology and at least two years' post-doctoral experience in clinical microbiology. Experience in immunoassay and protein chemistry techniques will be advantageous.

Allelix provides the opportunity to learn from and interact with experienced staff scientists, in addition to the immediate project leader, in a well-equipped laboratory. Salaries and benefits are at fully competitive levels.

Interested candidates are asked to send complete resumés, including three references and quoting file number MI001, to:

Dr. J.G.R. Hurrell

Allelix INC

6850 Goreway Drive
Mississauga, Canada
L4V 1P1

(NW1810)A

UNIVERSITY OF LEICESTER

DEPARTMENTS OF ZOOLOGY AND OBSTETRICS AND GYNAECOLOGY

SENIOR DEMONSTRATOR IN REPRODUCTIVE PHYSIOLOGY

Applicants are invited for a post of Senior Demonstrator in Reproductive Physiology. This will be a joint appointment in the Department of Zoology and Obstetrics and Gynaecology. Applicants should have experience in tissue culture and/or the culturing of mammalian embryos, and should preferably be post-doctoral. The appointee will be required to assist in collaboration with the two departments in developing a programme of extracorporeal fertilisation. There will also be a teaching commitment in the area of cell and developmental biology in the Department of Zoology. The appointment will be for two years.

Initial salary will depend on age and experience on the scale £7,520 to £12,150 (under review).

Further particulars from the Registrar (Appointments), University of Leicester, University Road, Leicester LE1 7RH (telephone 0533 554455 ext 233), to whom applications on the form provided should be sent by 17 June 1985. Informal enquiries can be made to Professor H C Macgregor, Department of Zoology. Telephone 0533 554455 ext 115. (6642)A

UNIVERSITY OF THE WEST INDIES

St Augustine, Trinidad

DEPARTMENT OF MECHANICAL ENGINEERING, INDUSTRIAL ENGINEERING PROGRAMME

LECTURER/ ASSISTANT LECTURER

Applications are invited for the post of Supernumerary Lecturer/Assistant Lecturer in the Department of Mechanical Engineering, Industrial Engineering Programme, specialising in Operations Research and Production/Investment Planning. The appointment will be two years.

Salary scales: Lecturer: TT$54,708 × 1,908 – $66,156 (Bar) × 1,908 – $77,604; Assistant Lecturer: TT$45,480 × 1,908 – $49,296. FSSU. Unfurnished accommodation if available at 10% or furnished at 12% or housing allowance of 20% of pensionable salary. Up to five full economy passages on appointment and on normal termination. Study and Travel Grant.

Detailed applications giving qualifications and experience and naming three referees to the Campus Registrar, University of West Indies, St Augustine, Trinidad, West Indies. Details of post sent to all applicants, but also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1902)A

CANCER RESEARCH

The AMC Cancer Research Center seeks to expand its laboratory and clinical research programs. Individuals at all levels, from Post-doctoral Fellows to Department Chairmen, will be considered. Current interests include tumor cell biology, tumor immunology, experimental hematology, monoclonal antibodies, cell kinetics, nucleic acid metabolism, nuclear magnetic resonance imaging, as well as clinical and national community research and control programs. Scientists (Assistant Professor equivalent) will be expected to vigorously develop independent research. Senior Faculty must be of national prominence and hold significant external funding. Opportunities exist for collaboration and growth. Facilities, equipment and initial research support are available. Clinical resources on campus include a 32 bed inpatient unit, outpatient clinics and services. Individuals with interests bridging laboratory and clinical cancer research are particularly sought. Send Curriculum vitae and other pertinent information to:

Dr. Philip Furmanski
Associate Director
AMC Cancer Research Center
1600 Pierce Street
Denver, Colorado 80214

(NW1798)A

UNIVERSITY OF THE WEST INDIES MONA, JAMAICA

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the following posts in the University of the West Indies

LECTURER/ASSISTANT LECTURER

It is desirable for teaching purposes that the successful applicant should have a broad training in Biochemistry. Certain areas of research interest are particularly welcome, eg plant biochemistry, toxicology, nutrition, immunology, biotechnology. Duties to be assumed as soon as possible.

Salary scales (under review): Lecturer (non-medical) J$23,688 × 768 - 28,296 (B) × 825-33,246. Assistant Lecturer (non-medical) J$20,379 × 717-21,813.

Up to five full economy class passages plus baggage allowance up to US$900 on appointment and normal termination. Special allowance up to US$900 on appointment and normal termination. Special allowance up to US$300 for shipment of academic books and teaching/research equipment on appointment. Unfurnished accomodation at 10% of pensionable salary or housing allowance of 20% of pensionable salary. UWI contribution of equivalent of 10% of salary to Superannuation Scheme. Subsidized Health Service. Annual Book Allowance. Annual Study and Travel Grant for self, spouse and three children. Transportation allowance to persons who own and operate a motor vehicle.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status, and the names and addresses of three referees should be sent as soon as possible to the Campus Registrar, UWI, Mona, Kingston 7, Jamaica. Applicants in the UK should also send one copy to the Assistant Secretary General (Appointments), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. Further details obtainable from either source. (W1905)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
London W1P 6DB
(University of London)

DEPARTMENT OF ANATOMY AND BIOLOGY AS APPLIED TO MEDICINE

POST-DOCTORAL RESEARCH ASSISTANT 1A

Applications are invited for this SERC supported post, tenable from October 1, 1985 (or earlier), to work for 3 years on cell contact and movement using advanced fluorescence methods.

Written applications, including the names of two referees, should be sent to Professor D Gingell at the above address. (6650)A

TECHNICAL UNIVERSITY OF GRAZ, AUSTRIA

The Chemistry Department of the Technical University of Graz invites applications for the position of a

FULL PROFESSOR OF PHYSICAL CHEMISTRY

at the Institute of Physical and Theoretical Chemistry. Applicants are expected to be internationally recognized researchers having appropriate teaching experience to teach the full course program of Physical Chemistry. Willingness to cooperate with existing research groups at the institute (crystal structure, theoretical chemistry, spectroscopy and magnetochemistry, solid-state chemistry, quantum chemistry) and interest in interdisciplinary team work is expected. Candidates are asked to send their resume (curriculum vitae, teaching experience, list of publications etc) and reprints of five of their most important publications.

Application deadline is September 16, 1985. Submit your application and resume to: Dekan der Technisch-Naturwissenschaftlichen Fakultät der Technischen Universität Graz, Petersgasse 16, A 8010 Graz, Austria, where also additional information may be requested. (W1891)A

UNIVERSITY OF CALIFORNIA

DEPARTMENT OF PHYSICS

ENDOWED CHAIR PROFESSOR OF PHYSICS

The UCLA Physics Department has an open Endowed Chair position. An appointment will be considered in any field of experimental or theoretical physics. The successful candidate will have a regular University of California tenured faculty position; the endowment income will support the candidates' program.

The successful candidate will be expected to lead an internationally recognized research program, and to teach at the graduate and undergraduate level.

Applicants should send a curriculum vitae, a brief statement of past, present, and future research interests, and the names of 5-10 referees to C Kennel, Chair, Department of Physics, University of California, Los Angeles, CA 90024.

The department is especially interested in identifying women and minority candidates.

The University of California is an affirmative action/equal opportunity employer. (NW1435)A

UNIVERSITY OF SOUTHAMPTON

CHAIR OF OCEANOGRAPHY

Applications are invited for the Chair of Oceanography which will become vacant on the retirement of Professor H Charnock, FRS, on 30 September 1986. Applicants should have research interests in the physically-related marine sciences and the ability to foster work in a multi-disciplinary field.

Further details may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH, to whom applications (10 copies from persons in the UK) should be sent before 12 July 1985. (6626)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF PHYSIOLOGY

RESEARCH ASSISTANT

Applications are invited for the above post, tenable for up to three years, to work on a project concerned with thermal changes in the immersed human body. This phase of the work is a theoretical study and will involve mathematical modelling. Applicants should have, or expect to get, a good honours degree, ideally with some experience of modelling biological systems but ability and enthusiasm could offset lack of experience.

Salary within range £6,600 — £7,520 on the 1B Scale for Research and Analogous Staff, with appropriate placing (scale under review).

Further particulars and application forms from The Secretary, The University Aberdeen, with whom applications (2 copies) should be lodged by 28 June 1985. (6622)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following position:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF DEVELOPMENTAL BIOLOGY

POSTDOCTORAL FELLOW - MOLECULAR ANALYSIS OF DEVELOPMENT PROJECT

A Postdoctoral Fellow is required in the Department of Developmental Biology for a project on genetic analysis of events in the cell cycle of Chlamydomonas. A background in genetics, biochemistry and ultrastructural analysis would be advantageous. The position will commence on 1 January 1986.

Further particulars can be obtained from Professor B Gunning, Head of Department (062) 49 2372, or from Dr P C L John (address until 31 July 1985, Department of Botany, Queen's University of Belfast, Belfast BT7 1NN, UK).

Closing date: 31 July 1985. Ref: BS 15/5/1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow $A22,614 - $25,905 pa.

Appointment, unless otherwise stated, will be: Postdocotral Fellow normally two years, with the possibility of extension to maximum of three years.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when requesting further particulars, and when submitting applications, which should be sent to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. Further information also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an equal opportunity employer. (W1904)A

POSTDOCTORAL POSITION

Is available immediately to study functional role of insulin in the brain at the cellular level with Drs. D.W. Clarke and M.K. Raizada. Experience in cell culture and biochemical techniques is a disirable. Experience with immunocyto-chemistry would be helpful. **Contact Dr. Mohan K Raizada, Dept of Physiology, J274, Univ of Fla Gvl, FL 32610 or telephone 904 392-3791.**

(NW1802)A

AFRC FOOD RESEARCH INSTITUTE — BRISTOL

LANGFORD,
BRISTOL BS18 7DY

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER (85/17)

ASSISTANT SCIENTIFIC OFFICER (85/19)

SHORT TERM CONTRACTS (3 YEARS)

Applications are invited for 2 short term contracts (3 years) to examine gaseous forms of preslaughter anaesthesia in pigs and poultry. The project is aimed at resolving some of the humanitarian problems associated with the slaughtering of livestock.

Salary SO/HSO £5,909 — £10,039. ASO £4,456 — £6,315.

Qualifications SO/HSO (Post 85/17) Degree in a Biological Science with relevant post-graduate research experience.

ASO (Post 85/19) HNC in Biology.

Further particulars and an application form from the Personnel Officer at the above address.

The Agricultural and Food Research Council is an equal opportunities employer. (6649)A

UNIVERSITY OF GLASGOW

DEPARTMENTS OF BIOCHEMISTRY AND PHARMACOLOGY

WELLCOME LECTURESHIP IN MOLECULAR PHARMACOLOGY

Applications are invited for a Wellcome Lectureship in Molecular Pharmacology to be held jointly in the Departments of Biochemistry and Pharmacology. The appointment will be for three years: it may be renewed for a further two years subject to satisfactory progress. Applicants should not be more than 35 years of age and persons having a permanent University post in the UK are ineligible.

The successful applicant will contribute to the teaching in both departments and in research will become part of an active interdepartmental group. The departments are in the process of appointing a 'New Blood' lecturer to the same group.

The salary will be within the range of £7,520 — £14,925 per annum of the Lecturer's scale, with placement according to age, qualifications and experience.

Those wishing to be considered should send eight copies of a statement of their qualifications and experience, including a list of publications and the names and addresses of not more than three persons to whom reference may be made, to Professor R M S Smellie, Department of Biochemistry, University of Glasgow, Glasgow, G12 8QQ, not later than 21st June, 1985, from whom further particulars may be obtained. The applications must be accompanied by a summary of not more than 500 words outlining the proposed research. (6666)A

HEINRICH-PETTE-INSTITUT FÜR EXPERIMENTELLE VIROLOGIE UND IMMUNOLOGIE

AN DER UNIVERSITÄT HAMBURG

Applications are invited for the Tenure Position of

HEAD OF DEPARTMENT OF MOLECULAR BIOLOGY

Candidates with extensive scientific experience are expected to lead a research group in the general field of molecular biology. Budget and tenure positions are available. The Heinrich-Pette-Institut is an independent research establishment, active in the fields virology, immunology, and cell biology. Administrative experience and willingness to participate in running affairs of the institute are required. Teaching at the University of Hamburg is on voluntary basis. The salary is according to BAT I and depends on age and family status.

Applications including curriculum vitae and list of publications should be sent: An das Direktorium, Heinrich-Pette-Institut, Martinistraße 52, D-2000 Hamburg 20, Federal Republic of Germany. Closing date 31 August 1985. (W1892)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

DEPARTMENT OF ENTOMOLOGY

LECTURER OR SENIOR LECTURER

Applications are invited from medical entomologists for a tenured post in the Entomology Department. Preference will be given to applicants combining tropical experience with an outstanding record in research, preferably in biochemistry, ecology, population dynamics, or insect behaviour, but experience in any field within entomology applied to human health will be considered.

The person appointed will be expected to develop active research programmes, with overseas connections, and the extensive facilities of the London School will be available for this.

Appointment will carry a salary in the range £8,753 to £18,938 (inclusive of London Weighting) on the Universities' Lecturer or Senior Lecturer scale according to age, qualifications and experience, with membership of USS.

Further information may be obtained from Professor A N Clements (01-636 8636 ext 348). Applications with full curriculum vitae and the names of three referees should be sent to the Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

Closing date: 5 July 1985. (6635)A

We are a major Italian pharmaceutical company with a growing interest in biotechnology.
We are looking for an experienced

BIOCHEMIST/ IMMUNOCHEMIST

For a newly created laboratory of biochemistry and immunology, located in the urban area of Milan, Italy.

Potential candidates should have a doctorate degree and post-doctoral training. Previous experience in hybridoma technology for diagnostic purposes would be considered an advantage.

We Offer:
- career opportunities based on performance;
- a competitive salary in accordance with experience and qualifications;
- a stimulating, dynamic work environment;
- assistance in relocation expenses.

Candidates from EEC Countries are encouraged to apply.

Please contact in confidence:
P.O. Box 10119
Personnel Dept.
20110 Milan, Italy (W1898)A

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY

(University of London)

HAMMERSMITH HOSPITAL

RESEARCH ASSISTANT

Applications are invited for the post of research assistant for up to 3 years to work on arachidonic acid metabolism in fetal growth retardation. Candidates should have or expect to obtain a 1st class or upper 2nd class honours degree in Biochemistry. The successful applicant will be expected to register for a PhD in London University.

Further information from and applications to Professor M G Elder Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London W12 0HS. (6633)A

THE UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

BIOCHEMISTRY LABORATORY

WELLCOME LECTURESHIP IN BIOCHEMISTRY

Applications are invited for the Wellcome Lectureship in Biochemistry. Applicants should have research experience, preferably in the area of the biochemistry of secretion of polypeptide hormones, growth factors or neuropeptides. Those holding tenured positions are not eligible for consideration, and applicants should not normally be over 35 years in age.

Appointments will be for 3 years in the first instance, renewable for a further 2 years, subject to a satisfactory review. The salary will be on the University Lecturer scale (£7,520 to £14,925 pa).

Send self addressed envelope (6" x 9") for further particulars and an application form to Mrs L Vivian, Personnel Office, Sussex House, The University of Sussex, Falmer, Brighton BN1 9RH, Sussex. Closing date for applications 10th June 1985. (6629)A

UNIVERSITY OF BATH

SCHOOL OF CHEMISTRY

POST DOCTORAL RESEARCH OFFICER

Applications are invited for a Research Officer to work with Dr S Mann for three years beginning October 1985 to investigate the structural and mechanistic properties of magnetic solids formed in bacteria. Applicants should have a strong interest in structural inorganic chemistry and have some experience in electron microscopy and electron diffraction.

Starting salary up to £7,980 pa (under review).

Application forms from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting ref no: 85/79. Closing date: 13/6/85. (6619)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY PATHOLOGY

MOLECULAR VIROLOGY UNIT

A molecular virology unit has been established to develop novel vectors for the expression of viral antigens in mammalian systems. This unit works in close collaboration with virologists and molecular biologists in this department and at the Beatson Institute.

Three positions, supported by the National Institutes of Health, are immediately available. Applications are invited for 2 post-doctoral fellows (salary range £8,450 to £10,720 per annum) and a research assistant (£6,600 per annum). The research assistant may apply to register for a higher degree.

The applicants should have experience in recombinant DNA technology and a background in virology would be an advantage. Applications should be sent to, and further particulars may be obtained from, Professor W F H Jarrett, FRS, University of Glasgow, Veterinary School, Bearsden, Glasgow, G61 1QH (telephone number 041-942-2301). (6658)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals, Houndmills, Basingstoke, Hants. RG21 2XS, UK

or

Paul Siman, Bio/Technology, 65 Bleecker St., New York, NY 10012.

MOLECULAR BIOLOGIST

Applications for an assistant professorship are invited from individuals with major commitment toward research and whose interests are in the structure, regulation or expression of viral, or mammalian, genes. Expertise in genetic engineering of enzymology-protein chemistry, an asset. Knowledge of or readiness to learn French essential. Send applications to: Dr Pierre Bourgaux, Directeur, Département de Microbiologie, Faculté de Médecine, Centre Hospitalier Universitaire, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.

(NW1790)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL

(University of London)

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

required to investigate the organisation of Campylobacter DNA, particularly the structure and expression of toxin genes. Previous experience, preferably postdoctoral, in prokaryotic molecular genetics and recombinant DNA techniques is essential. Experience with expression vectors is desirable. The successful applicant will join a group working on various applications of molecular biology to clinical problems.

The post is available immediately for a fixed term period of up to three years. Salary will be on Scale 1A for Research and Analogous staff, (£7,520 — £12,150 pa) at a point appropriate to age and experience plus £1,233 pa London Allowance.

For further information concerning the project or the department, telephone Dr L Archard, 01-741 1858.

Applications on forms obtainable from The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London, W6 8RP, to be submitted within three weeks of the appearance of this advertisement.

(6625)A

UNIVERSITY OF LONDON

RANK CHAIR OF PHYSIOLOGICAL BIOCHEMISTRY TENABLE AT IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

The Senate invite applications for the above Chair, tenable in the Department of Biochemistry at Imperial College of Science and Technology.

Candidates should have demonstrated an interest in cellular biochemistry and/or control of integrated physiological processes. They should have significant research accomplishments and an interest in the teaching of these aspects of biochemistry in advanced undergraduate courses.

Applications (10 copies) must be received not later than 15 July 1985 by the Academic Registrar (N.), University of London, Malet Street, London WC1E 8HU, from whom further particulars should first be obtained.

(6631)A

NEUROENDOCRINE RESEARCH GROUP

DEPARTMENT OF ANATOMY

UNIVERSITY OF CAMBRIDGE

We are seeking a postdoctoral physiological psychologist, or relevantly qualified zoologist or clinical graduate to join a multidisciplinary research tea investigating the neuroendocrine basis of social behaviour and anxiety in an experimental setting. The position is funded by the MRC and available immediately; the initial appointment is for 1 year, renewable for up to 5 years in total.

Please send applications (including CV) to Mrs J Penton, Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY by July 1st, and arrange for references to be sent direct from 2 referees.

(6686)A

THE ANIMAL VIRUS RESEARCH INSTITUTE

POST-DOCTORAL MOLECULAR BIOLOGIST

Applications are invited for a 3 year appointment to work on analysis of the genome structure of classical swine fever (hog cholera) virus and its relationship to bovine virus diarrhoea. The work will involve molecular cloning of the genome RNA and nucleotide sequence determination. The successful applicant will work with Dr Charles Penn in the Division of Molecular Biology. He/she should have a degree in biochemistry, microbiology or a related subject and a PhD. Experience in gene cloning would be an advantage.

The salary will be in the scale of £7,435 - £10,039 (Higher Scientific Officer grade) pay award pending. Sick leave and non-contributory superannuation schemes, 22 days annual leave. Accomodation may be available. The AVRI is an equal opportunity employer.

Application to include a curriculum vitae and the names of two referees should be sent as soon as possible to the Secretary, Animal Virus Research Institute, Ash Road, Pirbright, Woking Surrey GU24 0NF. Tel: 0483 232441. (6640)A

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURESHIP

A vacancy exists for a Temporary Lecturer as a result of Dr J R Burton being awarded a five-year Research Fellowship by the Lister Institute. Candidates with research interests in any area of Biochemistry are eligible to apply for preference may be given to those with experience of recombinant-DNA technology or immunology. It is expected that the successful candidates will be appointed for a period of three years with the possibility of extension for a further two years.

Salary within the range £7,520 — £11,675 on the non-clinical lecturers salary scale.

Applications including the names of three referees should be sent to the Personnel Department (Academic Staffing), the University, Sheffield S10 2TN by 14 June 85 from whom further particulars are available. Quote ref: R275/G (6617)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

RESEARCH ASSISTANT

Applications are invited for the above post, tenable immediately for up to two years, to work on the isolation of cNDA clones for the structural glycoprotein present in human aorta. A range of microbiological, immunological and gene cloning techniques will be involved. Applicants should have an honours degree in an appropriate discipline.

The appointment is funded by the Maitland Ramsay Trust. Starting salary within the range £6,600 to £7,980 per annum (under review) according to age and experience, on the 1B scale, plus USS.

Further information can be obtained by writing or by telephone from professor Serafini-Fracassini (tel 0334-76161, ext 275).

Applications (2 copies preferably in typescript) enclosing a full curriculum vitae and the names of 2 referees should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ to arrive not later than 20 June 1985.

(6661)A

UNIVERSITY OF OXFORD

DEPARTMENT OF NUCLEAR PHYSICS

APPOINTMENT OF RESEARCH OFFICER

The department will shortly appoint a Research Officer in Particle Physics. Whilst mainly a research appointment, the appointee will be required to undertake seventy-two hours a year of laboratory demonstrating and he/she will be encouraged to give graduate lectures. The research programme of the experimental particle physics group includes the Soudan II search for proton decay, a major contribution to the DELPHI detector for LEP (CERN), preparation for an experiment at HERA (DESY), and development of an indium detector for solar neutrinos.

The appointment will be for three years with possible extension to five years. Salary will be on the Lecture scale £7,520 - £15,930 (under review).

Applications with the names and addresses of two referees should be sent to Mr M S Gautrey, Department of Nuclear Physics, Keble Road, Oxford from whom further particulars may be obtained. The closing date is September 5th 1985.

(6676)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY DIVISION OF IMMUNOLOGY

SCIENTIST

A Scientist with a post-graduate qualification in Biochemistry or Organic Chemistry is required to join in a project investigating the development of immunoassay systems using red cell-labelled antibodies. Experience in protein chemistry or immuno-chemistry desirable. The appointment is for two years in the first instance, possibilities of an extension.

Enquiries to Professor R R A Coombs, Division of Immunology, New Adenbrooke's Hospital, Hills Road, Cambridge. Tel Cambridge 0223 245171 ext 336. Age up to 30

Salary range: University scales age related.

Formal applications together with curriculm vitae and the names and addresses of two referees to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. (6667)A

UNIVERSITY OF LEICESTER/DARESBURY LABORATORY

POST-DOCTORAL RESEARCH ASSOCIATE IN EXPERIMENTAL X-RAY DIFFRACTION AT HIGH PRESSURES

Applications are invited for the above post, funded by SERC, tenable from 1st October 1985, or as soon as possible thereafter, for up to three years.

The successful candidate will be expected to pursue research at the Synchrotron Radiation Source at Daresbury into structural changes in solids at very high pressures, on the newly commissioned Power Diffraction Station on the Wiggler beam line. The project involves application of novel high-resolution powder diffraction techniques to materials at very high pressures, and is backed by a major theoretical programme of molecular dynamics simulations.

Applicants should hold a PhD in experimental science. Experience with X-ray or neutron crystallography, and computing would be an advantage. The starting salary will be in the range £7,520 to £8,450 according to age and experience.

Applications, together with a full curriculum vitae and the names of two referees to Dr D M Adams, Department of Chemistry, University of Leicester, Leicester LE1 7RH by 15th July 1985. (6682)A

SANDWELL HEALTH AUTHORITY

The Midland Centre for Neurosurgery and Neurology

DEPARTMENT OF PATHOLOGY

ELECTRON MICROSCOPIST

in charge of Section

(Basic/Senior Science Graduate)

required on a Locum basis (available grading up to Senior as defined by Whitley Council PTA regulations). Well housed department with two other staff, two Siemens microscopes, several ultramicrotomes. Successful applicant will be required to continue and develop service in Muscle, Central and Periperal Nervous System Pathology, to Hospital and Region. Collaboration with current research projects will be expected and there is an excellent opportunity to develop a personal research programme on the readily available material.

Intending applicants are welcome to visit by appointment with Dr M V Salmon (Neuropathologist) - 021-558 3232 ext 314.

Job description and application form from the Administrator, Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick, Warley, West Midlands B67 7JX. Tel: 021-558 3232 ext 251. (6677)A

Cell Biologist/Biochemist/ Neurobiologist

Postdoctoral research position is immediately available for PhD with experience in cell biology, biochemistry, or neurobiology to investigate hereditary neuromuscular disorders. Initial research will focus on mechanisms of regulation of cell growth and the developmental expression of surface proteins in nerve, muscle, and related tissues. Applicants should have a PhD and experience in: cell biology and biochemistry; culture of nerve, muscle and fibroblasts; immunological methods for the study of cell proteins. Extensive possibilities exist for collaboration with expert neuromuscular morphologists and with active molecular genetics/recombinant DNA laboratory. Excellent support; salary commensurate with experience. Direct inquiries to: Dr. Robert H. Brown, Jr., Day Neuromuscular Research Center, Massachusetts General Hospital Warren 370/374, Boston, MA 02114. (NW1792)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

ENDOCYTOSIS

A post-doctoral research assistant is required to work on a cell-free assay for studying the pathway of adsorptive endocytosis. The aim is to understand the molecular basis of this process. Preference will be given to candidates with training in membrane biochemistry and/or enzymology.

This project is funded by the MRC and the position is for three years commencing 1st January 1986. The salary will be in the range £7,520-£12,150 per annum.

Applications, including a curriculum vitae, research summary and the names and addresses of three referees, should be sent to the Personnel Officer, The University, Dundee, DD1 4HN, Scotland as soon as possible. Informal enquiries concerning this project should be addressed to Professor Graham Warren, who is presently at the European Molecular Biology Laboratory, Postfach 10 2209, 6900 Heidelberg, West Germany. Tele phone number 010 49 6221 387324. Please quote Ref EST/66/85J. (6466)A

RESEARCH ASSOCIATE POSITION

Opportunity exists to work on a project concerned with the biochemical mechanisms of hormone (insulin) action to control phosphorylation and redox state. Applications are invited from qualified candidates with a Ph.D. or M.D. degree. Submit a resume of research interests, curriculum vitae and three letters of recommendation to Dr. Joseph Larner, Department of Pharmacology, Box 448, University of Virginia School of Medicine, Charlottesville, Virginia 22908. An affirmative Action/Equal Opportunity Employer. (NW1812)A

AGROGEN BIOTECHNOLOGIES SENIOR SCIENTISTS — PLANT TISSUE CULTURE

Agrogen Biotechnologies Inc., a Vancouver, BC based Canadian company interested in plant tissue culture, micropropagation of woody ornamentals and forest tree species and related biotechnologies is seeking Senior Scientists in plant tissue culture to provide project leadership and research support to its micropropagation program.

The positions require a PhD with a strong background in plant physiology, cell biology, genetics or biochemistry with several years research experience in plant tissue culture. Agrogen offers the opportunity to join an energetic interdisciplinary research team, excellent research facilities, competitive salary and fringe benefits.

Please forward your curriculum vitae and other pertinent information in confidence to: Dr. D. Paul Webb, Director of Research, Agrogen Biotechnologies, Inc. 1710-1066 West Hastings Street, Vancouver, BC V6E 3X2. (NW1793)A

THE UNITED MEDICAL AND DENTAL SCHOOLS

ST THOMAS'S CAMPUS

RESEARCH ASSISTANT

(post-doctoral)

required to work on project on the role of Coxsackie-B Viruses in the pathogenesis of insulin-dependent diabetes mellitus (type 1).

Sound virological experience essential; experience with such techniques as ELISA, monoclonal antibody production and PAGE an advantage. Funding is being sought for one year appointment from 1 September 1985 in the first instance. Salary not less than £7,520 plus £1,233 LWA pa (grant application pending).

For further information, contact Professor J E Banatvala, Dept of Virology, tel: 01 928 9292 ext 2405.

Applications giving full cv and names and addresses of 2 referees to Staffing Officer, Dean's Office, St Thomas's Hospital Medical School, London SE1 7EH by 13 June 1985. (6664)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF GEOLOGY

POST-DOCTORAL/ POST-GRADUATE RESEARCH ASSISTANT IN SEISMOLOGICAL COMPUTING

To work on the acquisition, digitisation processing and interpretation of the data from an international seismic refraction experiment to be carried out in Kenya in August 1985. The data processing will require the development, maintenance and operation of Fortran 77 programmes on VAX 11/70-8600 system. The appointment will be for two years from July 1985 with a salary of £6,600 — £7,055.

Applications, including a CV and the names of two referees, should be sent by June 7 to Dr M A Khan, Department of Geology, The University, Leicester LE1 7RH from whom further particulars may be obtained. (6641)A

UNIVERSITY COLLEGE LONDON

RESEARCH TECHNICIAN

Grade 4

Cell Biology

Applications are invited for the above post to assist in the development of a new project on the intracellular transport of membrane proteins, funded for 3 years by the MRC.

The person selected to join our small friendly group will have an interest in research and be qualified to at least HNC standard. Experience with tissue culture, cell fractionation or protein purification would be an advantage.

Salary £6,106 — £6,837 + £1,250 London Weighting.

Applications, including CV and the names of 2 referees should be sent to Personnel Officer (Technical Staff GE6), University College London, Gower Street, London WC1E 6BT. (6651)A

ROYAL HOLLOWAY AND BEDFORD COLLEGES

RESEARCH ASSISTANT

Applications are invited from Physicists, Engineers or Applied Mathematicians, to join a team engaged in a range of projects on fundamental aspects of Electromagnetic Antennas for Millimetric Wavelengths. This project involves both theoretical work (using analytical and computational techniques) and supporting experimental studies in one or more of the following areas:-

(1) Electromagnetic Theory
(2) Numerical Control Engineering
(3) Applied Optics

Candidates should have an aptitude for research and a good degree or a postgraduate qualification in a relevant subject. Salary scale £9,863 — £11,093 (under review).

Applications (2 copies) with curriculum vitae and names and addresses of 2 referees should be sent immediately to the Personnel Officer, Royal Holloway and Bedford Colleges, Egham Hill, Egham, Surrey TW20 0EX. (6648)A

FACULTY POSITION

The Psychology Department of the Massachusetts Institute of Technology announces the opening of a faculty tenure-track position in neuroscience. Applicants with a PhD or equivalent degree are sought with training and research background in mammalian brain-morphology and function.

Interested applicants should send a letter of inquiry with curriculum vitae and three references by July 31, 1985, to:

Dr. Peter H. Schiller
Psychology Department
Search Committee
Massachusetts Institute of Technology, E25-634
77 Massachusetts Ave.
Cambridge, MA 02139.

Qualified minorities and women are encouraged to apply. MIT is an affirmative action/equal opportunity employer. (NW1791)A

MIT

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

WELLCOME LECTURESHIP

Applications for the above post are invited from candidates in the field of neuroscience with expertise and interest in pursuing research involving the use of an in-vitro brain slice. Candidates wishing to visit the department or make provisional enquiries should contact Professor A M Sillito (0222)-44211 ext 2062.

Candidates should be less than 35 years old and must not currently hold a permanent university appointment. The post is tenable for five years (subject to a review after three years) and should commence on the 1st October 1985, although this date could be negotiated for a suitably qualified date. Appointment will be made at the appropriate point on the lecturer scale (scale is £7,520-£14,925).

Applications (three copies) with curriculum vitae, 500 word synopsis of the proposed research and the names and addresses of three referees should be forwarded to the Vice-Principal (Administration) and Registrar, University Coilege, P O Box 78, Cardiff CF1 1XL from whom further particulars may be obtained. Closing date 24th June 1985. Reference: 2967. (6678)A

UNIVERSITY OF DUBLIN
Trinity College

TEMPORARY LECTURER IN ZOOLOGY

Applications are invited for the above post in the Department of Zoology at Trinity College, Dublin. The appointment will be tenable from 1st October, 1985 for one year, and may be renewed for a further year. Preference will be given to candidates having interests in Invertebrate Reproduction.

Salary: IR £9,302 per annum.

Application forms and further particulars relating to this appointment may be obtained from.

Establishment Officer
Staff Office
Trinity College, Dublin 2
Tel: 772941 Ext 1775

to whom completed application forms should be returned by **not later than Tuesday, 11th June, 1985.** (6638)A

UNIVERSITY OF ZUERICH
DEPARTMENT OF PHYSIOLOGY
A MOLECULAR BIOLOGIST

is required for a three year postdoctoral position, to work on the regulation of mRNA by oxygen availability in cultured kidney cells. Applicants should have PhD or equivalent in Biochemistry/molecular Biology and a minimum of two years of postdoctoral experience in these areas. Knowledge in cell culture techniques would be an advantage. Salalry in range sFr. 47.000-60.000/yr, depending on experience and qualification. The position is available now, starting date to be agreed.

Application with the names and addresses of two referees should be sent to Professor C. Bauer, Dept. of Physiology, University of Zuerich, Winterthurerstr. 190, 8057 Zuerich, Switzerland. (W1894)A

PROFESSOR & HEAD
Biological Sciences
Carnegie-Mellon University

The Department of Biological Sciences invites applications from qualified individuals to serve as Professor and Chairperson. We seek an individual with an established reputation in any field of biological research and an ability to provide leadership in the continuing growth of this department. We expect to appoint the successful candidate by 30 June 1986 or before

Applications or inquiries should be sent to Professor W.R. McClure, Chairman, Search Committee, Carnegie-Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213.
Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer.
(NW1765)A

CONFERENCES and COURSES

UNIVERSITY COLLEGE, LONDON:
SCHOOL OF MEDICINE

PART TIME COURSE IN TOXICOLOGY

A course in toxicology consisting of lectures and practical classes will be given weekly for 30 weeks, on Wednesdays at 15:45-19:00 hrs, starting on 2nd October 1985.

A broad treatment of topics in toxicology will be presented, including basic histopathology, biochemical and genetic aspects and methodology. Regulatory aspects of toxicology will also be discussed. The course is intended (1) for graduates with some experience in a relevant field; (2) for postdoctoral and other scientists wishing to broaden their knowledge of toxicology.

The fee for the 30 week course is £600. Numbers will be restricted to a maximum of 25 participants. Some assisted places where all or part of the course fees are waived are available to suitable applicants whose fees are not paid by their employer (e.g. persons working in Universities or similar institutions and holding non-tenured posts). Further details and application forms may be obtained from: —

Dr. I.C. Shaw
Toxicology Laboratory
Department of Clinical Pharmacology
University College London
5 University Street
London WC1E 6JJ, England
(01) 388-2411 Ext 212

Closing date for applications — 1st July 1985. (6656)C

Royal Postgraduate Medical School
(University of London)

A specialist scheme of study for graduates in medicine and biology leading to the award of:

DIPLOMA in ENDOCRINOLOGY

This is a one academic year, full-time, intensive laboratory-based training course open to graduates in medicine and biology who wish to develop skills in modern endocrinological investigative methods. The selected applicants, who must be experienced in light or electron microscopical morphology, biochemistry or a related discipline, will undertake a series of research projects during their training period in recognised laboratories within this Medical School. Award of the Diploma will depend on successful completion of an examination or by thesis.

Further details and application forms may be obtained by writing to:

The School Office, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS.

Completed application forms must be received before 31 July 1985 for registration on the 1985-86 Diploma course (6681)C

GRANTS and SCHOLARSHIPS

Research Award in Immunoregulation

Centocor, Inc., will award a $10,000 unrestricted research grant for the most outstanding human Interferon-Gamma scientific paper published (or accepted for publication) between 1/1/85 and 6/30/86.

For details, write to Centocor customer service.

CENTOCOR
244 Great Valley Parkway
Malvern, PA 19355
Fax (215) 644-7558
Telex 834823

(NW1726)H

WORKSHOP

ST JOHN'S COLLEGE CAMBRIDGE

α-1-ANTITRYPSIN MOLECULES AND MEDICINE

8-11 September 1985

Guest Chairman Professor C-B Laurell Sweden.

An international workshop to sumarise progress and define goals in research and clinical management.

Enquiries: Professor R W Carrell St John's College, Cambridge CB2 1TP UK. (6477)V

FELLOWSHIPS

THE UNIVERSITY OF SHEFFIELD

Faculty of Medicine

J G GRAVES MEDICAL RESEARCH FELLOWSHIP

Applications are invited for the above Fellowship tenable for a period of three years from 1 October 1985. The Fellow will be required to undertake full-time medical research in one of the medical or dental departments or hospital. Initial salary according to qualifications and experience within the range £7,520 — £8,920 a year, a higher salary may be offered to a candidate with appropriate experience and seniority. Additional funds may be available for the Provision of laboratory assistance and materials.

Further particulars from the Personnel Department (Academic Staffing), the University, Sheffield S1Q 2TN to whom applications (10 copies), including the names and addresses of three referees, indicating the line of research proposed), should be made by 17 June 1985. Quote refs R269/G. (6665)E

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

supported by the British Gas Corporation. The work will be concerned with "Development of Membranes with Improved Selectivity" and will be directed mainly towards synthesis of novel polymers suitable for fabrication into membranes showing improved selectivity in gaseous diffusion processes combined with high stability at elevated temperatures.

The post will initially be for one year with a salary of up to £8,920 per annum depending on age, qualifications and experience. Superannuation is under USS conditions.

Applications are invited in the form of a curriculum vitae (3 copies) including the names and addresses of two referees, and should be sent to the Personnel Office (JLG), University of Surrey, Guildford, Surrey GU2 5XH as soon as possible, quoting reference 75. (6646)E

Please mention

nature

when replying to these advertisements

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Cell Culture of Marine Algae

Applications are invited for a Post-Doctoral Research Fellowship funded by the University of Aberdeen to work with Drs S B Wilson, F B Williamson and W F Long. The project will involve the development of continuous cultures of marine red algal cells primarily to produce materials with commerical value.

The post has initially been funded for one year but scope exists to obtain further financial support giving an appointee with flair and imagination a chance to share in the longer term future of this exciting new field. Applicants should preferably have a PhD and some experience in cell culture.

Salary on Range 1A Scale for Research and Analogous Staff up to £8,920 per annum, with appropriate placing (scale under review).

Further particulars and application forms from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by 30 June 1985. (6668)E

POSTDOCTORAL FELLOWSHIP

In Molecular Biology/Biochemistry Position available Sept. 1, 1985 in Department of Microbiology at SUNY Stony Brook to study adeno-associated virus genetics, vector construction immunology or DNA replication. Send CV and references to Dr. Nicholas Muzyczka, University of Florida, College of Medicine, Box J-266 JHMHC, Gainesville, Florida 32610. (NW1797)E

POSTDOCTORAL FELLOWSHIP POSITIONS

Molecular Biology

Well-established cardiovascular research laboratory studying the molecular biology of lipoproteins and receptors is seeking molecular biologists with experience in cloning and DNA sequencing.

Candidates with a Ph.D. in molecular biology or biochemistry are eligible.

Send curriculum vitae and three letters of reference to: Personnel, c/o Dr. Brian McCarthy, The Gladstone Foundation Laboratories for Cardiovascular Disease, University of California, San Francisco, P.O. Box 40608, San Francisco, CA 94140-0608.

(NW1803)E

POSTDOCTORAL FELLOWSHIPS

California Biotechnology Inc. invites applications from recently qualified Ph.D.'s to join its postdoctoral research program. CBI is an established biotechnology company located in the Palo Alto area forty miles south of San Francisco. The company, which has about eighty scientific employees, is dedicated to applying the techniques of molecular biology, protein chemistry and cell biology to develop novel human and veterinary pharmaceuticals. It currently has major research programs in hypertension, atherosclerosis, inflammation, lung disease, reproductive biology, growth factors and polypeptide hormones. Recent graduates, who have experience in areas such as recombinant DNA technology, protein and peptide chemistry, cell biology and pharmacology, would join established research teams in one of the above areas. The postdoctoral fellowship would for a minimum of two years.

(NW1800)E

Please send applications, including a complete curriculum vitae to:

CBI

Dr. John Shine
Director of Research
California Biotechnology Inc.
2450 Bayshore Frontage Road
Mountain View, CA 94043

ESF PROGRAMME OF RESEARCH FELLOWSHIPS IN TOXICOLOGY

Short-term (up to 6 months) and Long-term (6-12 months) Research Fellowships (deadline: 30 September 1985) and Short-term (up to 1 month) Visiting Fellowships (no deadline) are offered to scientists working in European countries. No formal condition of age, level or field of training, provided the project contributes to furthering research in TOXICOLOGY in Europe. Information and application forms can be obtained from: Dr John Smith, European Science Foundation, 1, quai Lezay-Marnésia, F-67000 STRASBOURG, France; tel (88) 35 30 63; telex 890440. (W1890)E

EMBO
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
Autumn 1985 — Awards
Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany.

(W1893)E

STUDENTSHIPS

THE POLYTECHNIC OF CENTRAL LONDON

Faculty of Engineering & Science

RESEARCH POSTS IN THE SCHOOL OF BIOTECHNOLOGY

A. GENERAL AND MOLECULAR GENETICS OF FILAMENTOUS MICROORGANISMS

Post 1. SERC Research Studentship
Post 2. Research Assistantship

B. MICROBIAL PHYSIOLOGY/ BIOCHEMISTRY

Post. 3 SERC Biotechnology Research Studentship for a project entitled "Beta-lactum biosynthesis in free and immobilized Streptomycetes.

Post 4. Research Assistantship — candidates should have an interest in analytical biochemistry and particularly HPLC techniques.

Posts 1, 2 and 3 are tenable for three years from 1st October 1985. Applicants should hold or expect to gain a first or upper second class honours degree in a relevant discipline. The successful candidates will be expected to register for a PhD Degree.

Post 4 is available immediately or as soon as possible and applicants should hold or expect to hold a degree in an appropriate subject and is tenable for a minimum of three years. For suitably qualified and motivated candidates there may be an opportunity to register for an M Phil degree.

Salaries for posts 1 and 3 will be according to SERC current regulations. Salaries for posts 2 and 4 will be according to University Research Assistant Scales since the project "Extended Use of Biocatalysts" is undertaken by PCL jointly with University of Kent at Canterbury and University College London under the auspices of the Institute for Biotechnological Studies. The programme is financially supported by the Department of Trade and Industry and seven major industries.

Please send a curriculum vitae and the names of two referees to the Personnel Officer, The Polytechnic of Central London, 309 Regent Street, London W1R 8AL, as soon as possible.

PCL is an equal opportunities employer. (6615)F

OXFORD UNIVERSITY

Research Laboratory for Archaeology

RESEARCH STUDENTSHIP (SERC)

for thermoluminescence, laser-based optical dating, radiocarbon dating using a tandem accelerator or archaeomagnetism using a SQUID cryogenic magnetometer. Suitable for physicist, chemist or earth scientist anticipating good honours degree.

Details: Dr M J Aitken, FRS. 6 Keble Road, Oxford OX1 3QJ, telephone (0865) 55211. (6659)F

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF NEUROLOGY

POSTGRADUATE RESEARCH STUDENTSHIP

Applications are invited for a research studentship tenable in the Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital. The successful applicant will read for the degree of PhD. Two topics are available. (i) intermediary metabolism in skeletal muscle and the origin of metabolic myopathies; (ii) the use of cytochemical techniques to study the molecular differentiation of the muscle cell surface during development and regeneration of the neuromuscular junction.

Candidates should be qualified in an appropriate discipline (biochemistry, physiology, etc) and should possess or expect to obtain a First Class or Upper Second Class Honours degree.

Application should be made within two weeks of the appearance of this advertisement in the form of a curriculum vitae, and candidates should include the names of two academic referees. The project for which application is being made should also be identified. All correspondence should be sent to Professor J B Harris, Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, from whom further information may be obtained. (6643)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GEOGRAPHY

POSTGRADUATE STUDENTSHIP

Applications are invited for a postgraduate studentship to work with Dr Ann Henderson-Sellers on Synthesis of cloud climatologies.

This project builds on previous work in the department and is a contributory part of the International Satellite Cloud Climatology Project (ISCCP). The student will be expected to have, or aquire, a sound knowledge of meteorology. Much of the data analysis is computer-based so that previous experience of computer programming would be useful. The project is funded by a grant from the US Air Force and will involve the student collaborative work with US and European scientists. It is anticipated that applicants will hold, or hope to gain, at least an upper second class honours degreem in physics, mathematics or physical geography or a similar science subject.

Applications, together with the names of two referees should be received not later than 20 June, 1985, by The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote ref RV/291/N. (6620)F

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

Applications are invited from Chemistry graduates, or those about to graduate for the following research studentships which will become available as from 1st October, 1985.

(1) A CASE studentship in conjunction with ICI Pharmaceuticals to study A Thermodynamically-Based Scale of Hydrogen Bond Basicities. (Dr M H Abraham).

(2) A CASE studentship in conjunction with the Imperial Cancer Research Fund to study Mechanisms of Aromatic Hydroxylations. (Dr J R Jones).

(3) A Ministry of Defence sponsored studentship to study Aromatic Amine Cure of Epoxy Resins. (Drs G J Buist and J R Jones).

(4) A CASE studentship in conjunction with ICI to study The Synthesis of Polysulphones from Arylsulphonic Acids and PCl_5. (Professor J B Rose).

Interested persons should send their curriculum vitae and the names and addresses of two referees to Dr J R Jones, Chemistry Department, University of Surrey, Guildford GU2 5XH, as soon as possible.(6647)F

GEORGE HENRY LEWES STUDENTSHIP

Applications are invited for this Studentship to pursue original investigations in Physiology. Appointment may be from 1 October 1985 for one year with a possibility of renewal for up to two further years. The award will provide an annual maintenance grant in line with the maintenance element of a Research Council research studentship; and the trustees will pay University and College fees for the holder at the rates charged to science research students from the UK.

Candidates should by 28 June 1985 send (a) a statement of their qualifications and their need for pecuniary help, (b) the subject of their proposed research, and (c) the names and addresses of two referees, to Professor R D Keynes, Physiological Laboratory, Downing Street, Cambridge CB2 3EG, from whom further particulars may be obtained. (6639)F

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF MICROBIOLOGY

RESEARCH STUDENTSHIP IN MOLECULAR BIOLOGY

Applications are invited from persons possessing or expecting to obtain at least an Upper Second Class Honours degree in a Biological Science.

The successful applicant will join a group involved in the study of microbial pathogenesis using molecular genetic techniques.

Further details may be obtained by contacting Dr I N Clarke on Southampton 0703 777222 ext 3853. Please quote reference number AJS/85/37/N. (6671)F

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

SERC RESEARCH STUDENTSHIP

Photosensitization of Tumours

Applications are invited for the above studentship, tenable from October 1985, to study fundamental biochemical aspects of photochemotherapy, a new approach to cancer treatment, involving photosensitization of tumours by porphyrin-containing drugs.

Candidates should have, or expect to obtain, a good Honours degree in Biochemistry or an appropriate related discipline.

Further information may be obtained from Dr S B Brown, Department of Biochemistry, University of Leeds, LS2 9JT (0532 431751 ext 7500), to whom applications (including the names of two referees) should be sent. (6663)F

UNIVERSITY OF SHEFFIELD

DEPARTMENTS OF BIOCHEMISTRY AND MICROBIOLOGY

SERC EARMARKED STUDENTSHIP FOR PROTEIN ENGINEERING

A studentship is available for three years from October 1985 to work for a PhD on in vitro mutagenesis of a bacterial glutamate dehydrogenase. The student would join an interdisciplinary team headed by Professor J R Guest (DNA manipulation), Dr P C Engel (enzymology) and Dr D W Rice (crystallography) but would primarily be involved in the DNA aspects of the project.

Applicants should have or expect to obtain a First or Upper, Second Class Honours Degree in Biochemistry or Microbiology.

Applicants naming two referees should be sent to Dr P C Engel, Department of Biochemistry, University of Sheffield, Western Bank, Sheffield S10 2TN by 14 June 1985. Quote ref: R247/G. (6616)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY

SERC CASE STUDENTSHIP

Applications are invited for a SERC CASE Studentship to work in collaboration with Dr F Y Liew (Wellcome Research Laboratories, Beckenham) on the protection potential of T cell responses in mice to genetically engineered specific HSV glycoprotein peptide fragments.

Applicants should have at least 2.1 Honours degree in Biochemistry, Biology, Immunology or Genetics.

Applications, together with the names of 2 referees should be made by 30 June 1985 to Dr W L Chan, Dept of Zoology and Comparative Anatomy, University College London, Gower Street, London WC1E 6BT. Tel: (01) 387 7050 ext 428. (6675)F

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY

SERC STUDENTSHIP

Applications are invited for a 3 year studentship to work on the structure of squid rhodopsin and its role in activation of the photoreceptor enzyme cascade, using electron microscope image analysis and biochemical methods. Applicants should have, or expect to obtain, a good Honours degree in Biophysics, Biochemistry or related subjects.

Applications with a curriculum vitae and names of 2 referees should be sent as soon as possible to Dr H R Saibil, Department of Zoology, South Parks Road, Oxford OX1 3PS, to whom further enquiries may be made (Tel (0865) 56789 ext 317). (6657)F

UNIVERSITY OF READING

SUB DEPARTMENT OF HORTICULTURE

SERC CASE STUDENTSHIP IN PLANT PHYSIOLOGY

Applicants are invited for a CASE studentship tenable from 1 October 1985 to study genetic and environmental effects on the physiology and biochemistry of pod development in cocoa, in collaboration with Cadbury Schweppes plc.

Candidates should possess, or expect to obtain, a first or upper second class honours degree in Plant Sciences, Biochemistry or a closely related subject.

Further details may be obtained from Dr P Hadley, Sub Department of Horticulture, University of Reading, Earley Gate, Reading RG6 2AU. Applicants should send a curriculum vitae with the names of two referees not later than 10 June 1985. (6618)F

IMPERIAL COLLEGE (University of London)

CENTRE FOR BIOTECHNOLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited from candidates, preferably with a background in molecular biology, who hold, or expect to be awarded, at least two: one honours in biochemistry or biology for SERC research studentship. The award is for three years commencing October 1985. The project will involve the supression of gene expression in plants by anti-message RNA.

Informal inquiries may be made to Doctor Conrad Lichtenstein, Tel: (01) 589 5111 Ext 4112/7094.

Applications (cv and two academic referees) should be sent to me no later than June 30th 1985.

To: Centre for Biotechnology, Imperial College, London SW7 2AZ. (6690)F

UNIVERSITY OF ABERDEEN

DEPARTMENTS OF CHEMICAL PATHOLOGY AND THERAPEUTICS AND CLINICAL PHARMACOLOGY

POSTGRADUATE STUDENTSHIP

Applications are invited from graduates to work on a research project involving the use of isolated renal proximal tubular cells to investigate mechanisms of drug-induced nephrotoxicity. Candidates should have a good Honours degree in Biochemistry, Pharmacology or a related subject.

The studentship is initially for one year and the student will be registered for an MSc. Applicants, with a full curriculum vitae and the names of two academic referees, should be sent to Dr P Whiting, Department of Chemical Pathology, University Medical Buildings, Foresthill, Aberdeen AB9 2ZD as soon as possible. Further details are avalable from Dr Whiting (telephone 0224-681818, ext 2822) or Dr G Hawksworth (ext 2463). (6685)F

UNIVERSITY OF WARWICK

DEPARTMENT OF CHEMISTRY

SERC CASE AWARD

Solution Chemistry of Technetium : Redox and Extraction Properties

Applications are invited from graduates or those expecting to graduate this Summer with at least a class II-I honours degree in chemistry, for this studentship. The project, carried out in collaboration with British Nuclear Fuels plc, will be concerned with kinetics of chain reaction of TcO_4^- with hydrazine and with the co-extraction of Tc with UO_2^{2+} ion. Normal SERC rates will be supplemented by £600 pa.

Further details may be obtained on application to the Personnel Office, University of Warwick, Coventry CV4 7AL. Please quote Ref No 42/A/85/O. Closing date for applications 21st June 1985. (6672)F

UNIVERSITY OF GLASGOW

BIOTECHNOLOGY UNIT

SERC CASE STUDENTSHIP — ICI

To clone and study expression of resistance and production genes from Streptomyces for an antibiotic of interest to ICI. The project is supervised jointly by Dr I S Hunter (Glasgow) and Dr P Groves (ICI, Corporate Lab, Runcorn).

Applicants should have, or expect to obtain, a first or upper second class honours degree in a related biological science. Interviews will be conducted towards the end of June.

Applications (CV and names of two referees) should be sent to Dr I S Hunter, Institute of Genetics, Church Street, Glasgow G11 5JS, before June 13th, 1985. (6596)P

AWARDS

ROSENSTIEL AWARD IN OCEANOGRAPHIC SCIENCE

Nominations are invited for the 1985 Rosenstiel Award in Oceanographic Science.

This award, administered on behalf of the Rosenstiel Foundation by the Rosenstiel School of Marine and Atmospheric (RSMAS) at the University of Miami recognizes outstanding contributions to marine science, including oceanographically relevant aspects of atmospheric science, and fundamental developments in ocean engineering. The award consists of a cash prize, currently $5,000, and a medal. The recipient of the award will be invited to spend a week at RSMAS for discussions with faculty and students. The award will be presented at that time.

To accumulate the multidisciplinary value of oceanographic science, the award recognizes, on a rotating basis, achievements in four broad disciplinary areas. In 1985 the emphasized discipline will be physical oceanography and air-sea interaction. The achievements recognized may consist of contributions towards the development of ocean science in general, or of more focussed individual research of recognized impact on our understanding of the marine environment.

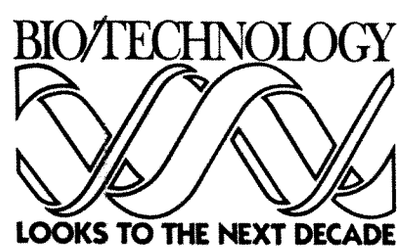
Nominations for the 1985 award for outstanding achievement in physical oceanography or air-sea interaction should be directed to Dean Alan Berman, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149-1098, before August 15, 1985. Nominations should include a brief justification together with relevant reference. The selection panel would especially welcome nominations of outstanding younger scientists, whose early contributions suggest a continuing role of leadership in the field. (NW1796)N

CONFERENCES and COURSES

ATTENTION SCIENTISTS AND RESEARCHERS

Posters are now being accepted for BIO/TECHNOLOGY's premier conference

BIO/TECHNOLOGY



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January 20-22, 1986, Clarion Hotel, New Orleans

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BIO/TECHNOLOGY Conference
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Ideas suitable for support from corporate or venture funding are particularly welcome.

Conference Themes:
Day 1: The Molecular Biology of Plants & Animals
Day 2: Commercial Production Processes
Day 3: Innovations in Pharmaceuticals & Immunology

ASSOCIATESHIPS

Research Associateship in Experimental Nuclear Physics

There is a vacancy for an experimental physicist to join the nuclear physics group as a Research Associate at the Daresbury Laboratory, an establishment of the Science and Engineering Research Council situated in the north Cheshire countryside.

The group is involved in a research programme on the Nuclear Structure Facility, a large tandem accelerator which currently is operating at up to 20 MV on terminal.

Currently, the principle areas of study are: direct reactions with light and heavy ions, production of exotic nuclei far from stability, nuclei with very high angular momentum, nuclear breakup and fragmentation and studies of isotope shifts and hyperfine structure using laser-induced resonance fluorescence. Experimental equipment includes a Q3D magnetic spectrometer, advanced gamma-ray and neutron detection apparatus, and a 1m diameter scattering chamber. An isotope separator with an associated dilution refrigerator and a beam line for laser studies has recently been brought into full operation, a recoil separator is in an advanced commissioning stage, and a heavy-ion polarised source is under construction.

Applicants (male/female) should possess a PhD degree or expect to obtain one during 1985.

An appointment will be made at a salary between £9,977 and £11,220 per annum depending on age, ability and experience. The post is available for a fixed term of three years and is superannuable.

CLOSING DATE: Sunday 30th June, 1985.

For further information please write or telephone: Dr. J. Lilley on Warrington (0925) 65000 Ext. 558.

Application forms may be obtained from and should be returned quoting reference DL/912 to: The Personnel Officer, Science & Engineering Research Council, Daresbury Laboratory, Daresbury, Warrington, Cheshire, WA4 4AD. (6623)O

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

CONGRESS

INTERNATIONAL SOCIETY OF DEVELOPMENTAL BIOLOGISTS
Announces the
TENTH INTERNATIONAL CONGRESS OF ISDB
"NEW DISCOVERIES AND TECHNOLOGIES"
AUGUST 4-9, 1985
The Westin Bonaventure Hotel
Los Angeles, California, USA

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Peter Albersheim, Merton Bernfield, Hans Bode, James Bonner, Jeremy Brockes, Donald Brown, Arnold Caplan, Pierre Chambon, Thomas Cline, Jonathan Cooke, Gerald Edelman, Robert Edwards, Judah Folkman, Scott Fraser, Vernon French, Richard Gardner, Walter Gehring, Benjamin Geiger, Norton Gilula, Paul Green, Suresh Goel, Clifford Grobstein, Elizabeth Hay, Ross Hodgetts, Ernest Jaworski, Howard Jones, Jr., Yoshihiro Kato, Klaus Kratochwil, Elias Lazarides, Nicole Le Dourain, Edward Lewis, Anne McLaren, Alberton Monroy, Aron Moscona, Drew Noden, Christiane Nüsslein-Volhard, Daphne Osborne, Masukichi Okada, Richard Palmiter, Martin Raff, William Rutter, Kenneth Ryan, Gordon Sato, Lauri Saxén, Jozef St. Schell, Robert Schimke, Howard Schneiderman, David Schubert, Yinghsien Shih, Allan Spradling, Timothy Stewart, Masatoshi Takeichi, Jean Paul Thiery, Robert Trelstad, Victor Vacquier, Sergei Vassetzky, Stephen Wachtel, Lewis Wolpert, Savio Woo, Carl Wood.

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Los Angeles, California 90089-0191
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(NW1787)Q

ASSISTANTSHIPS

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
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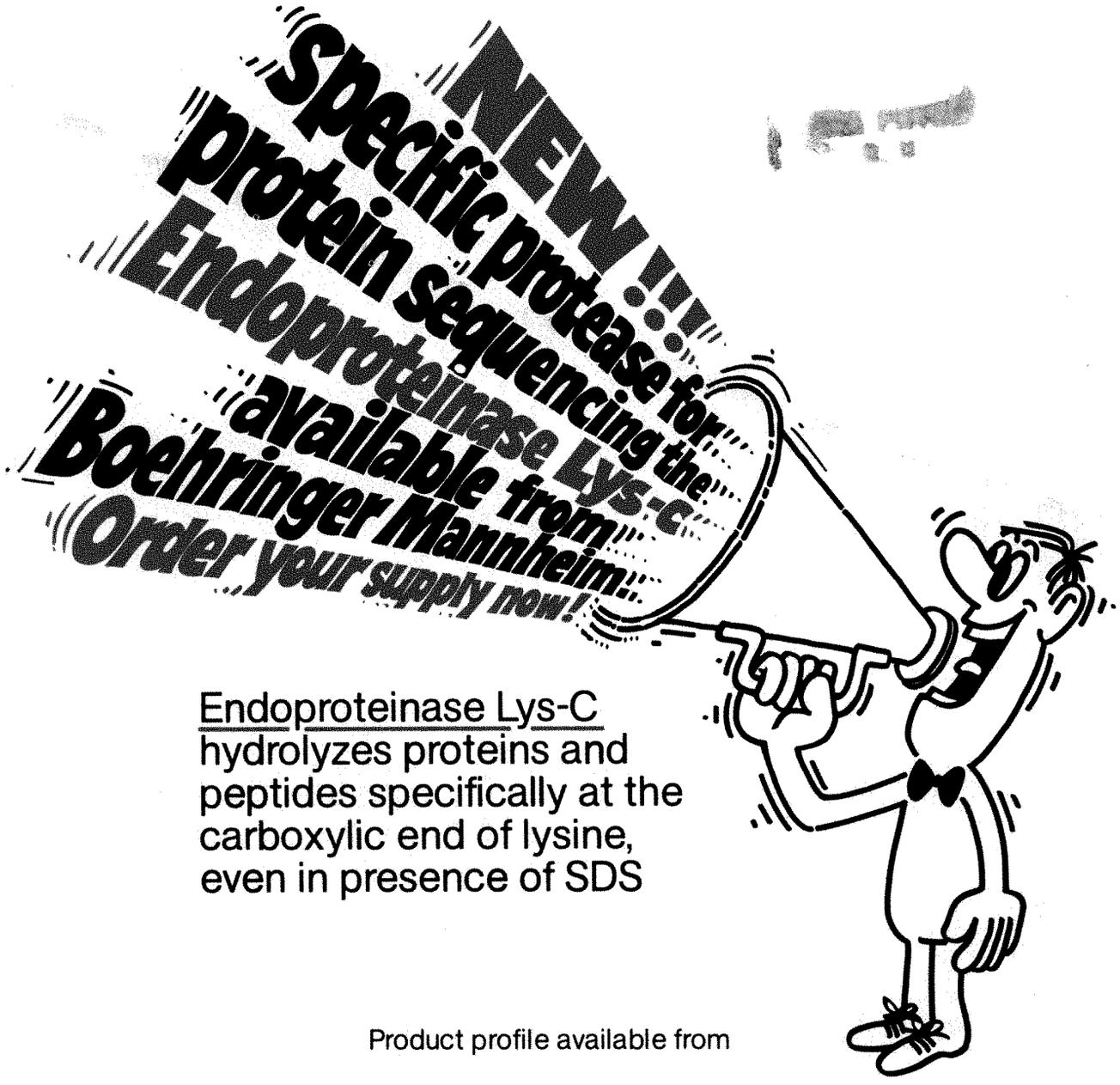


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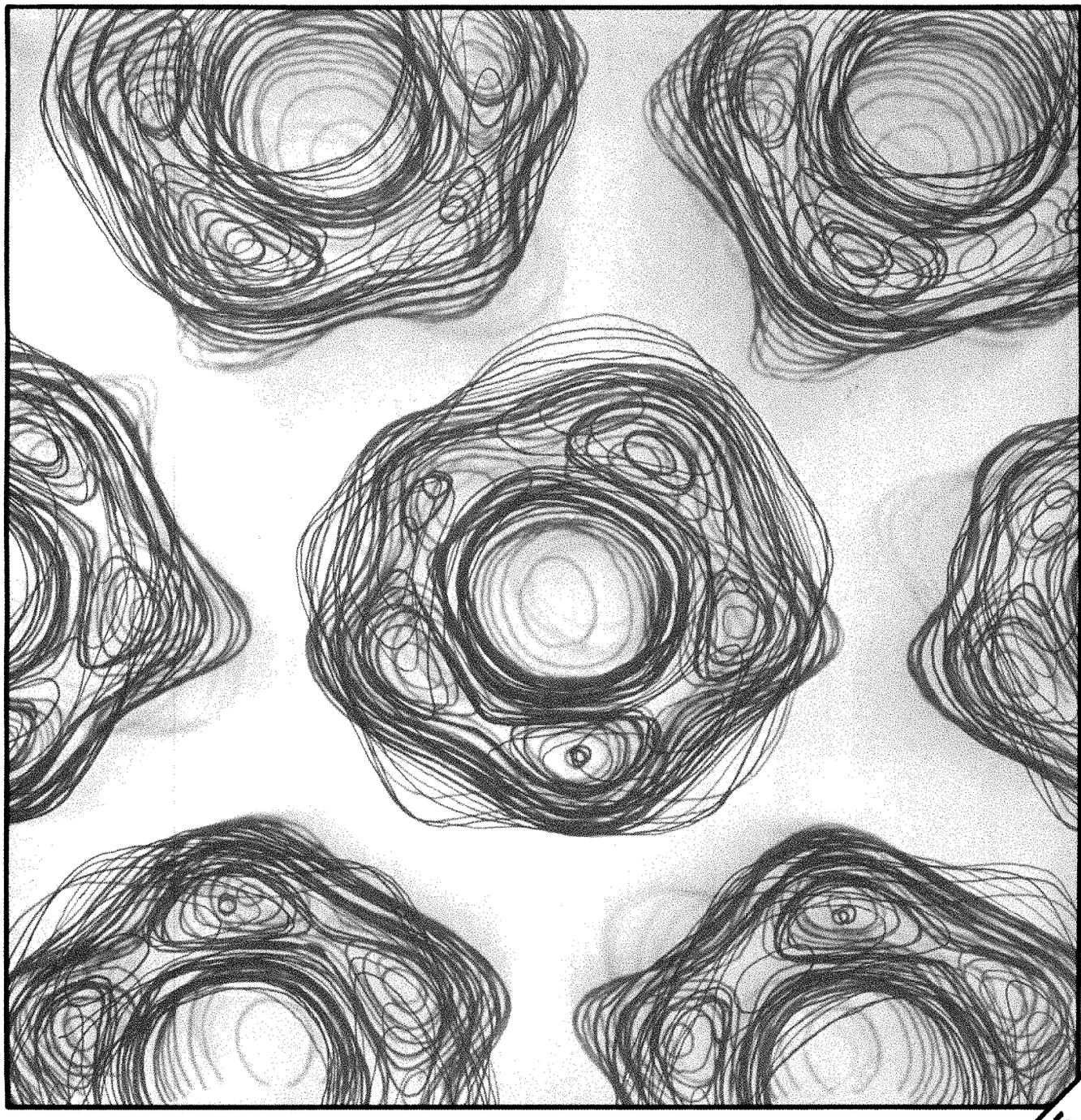
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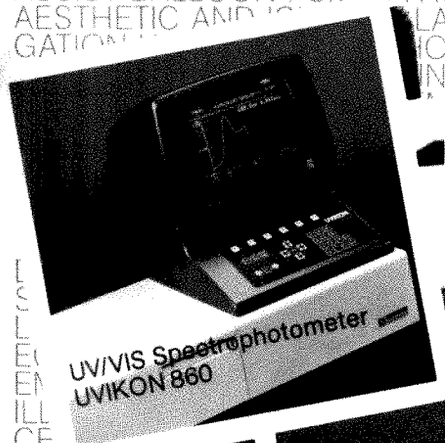
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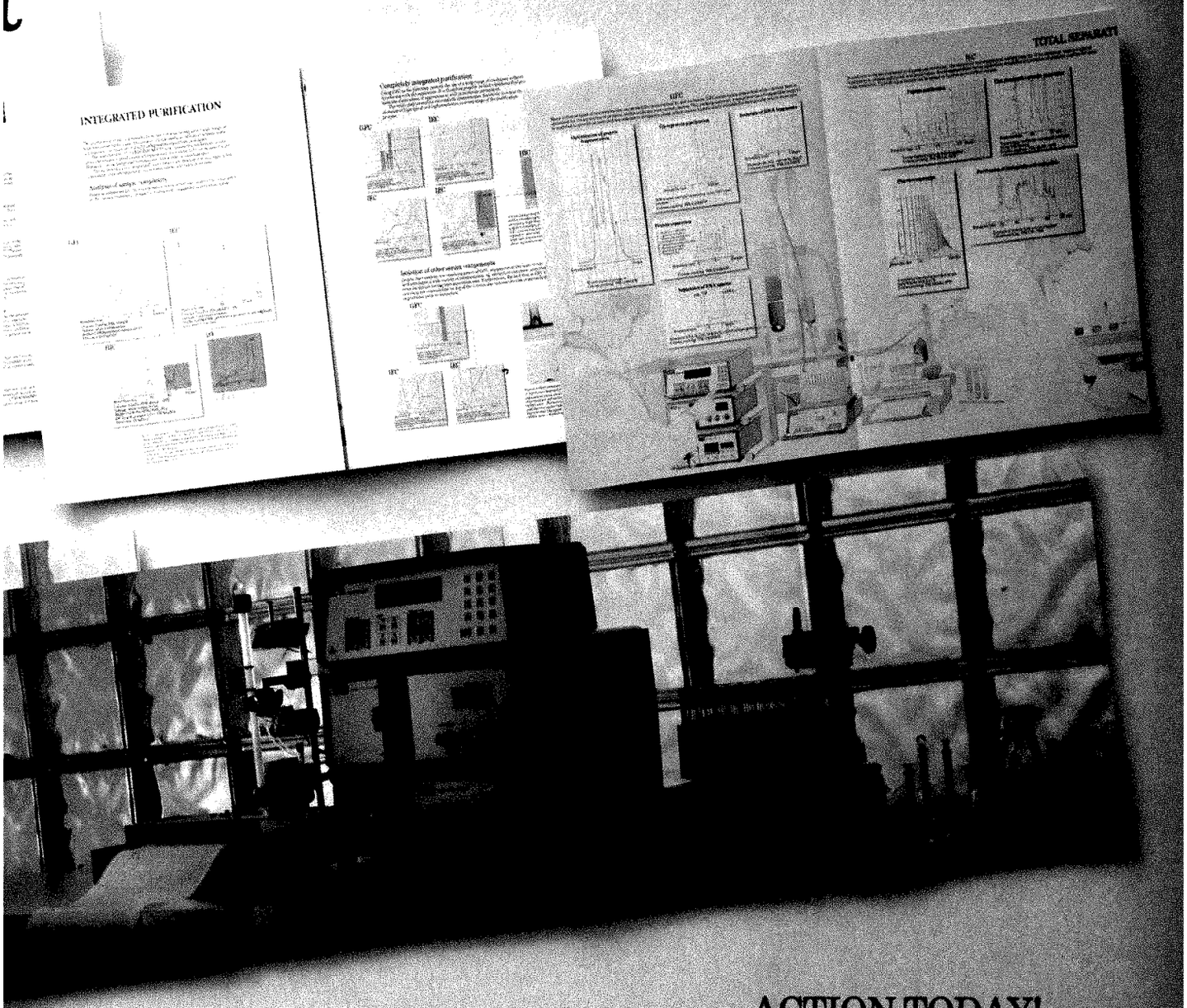
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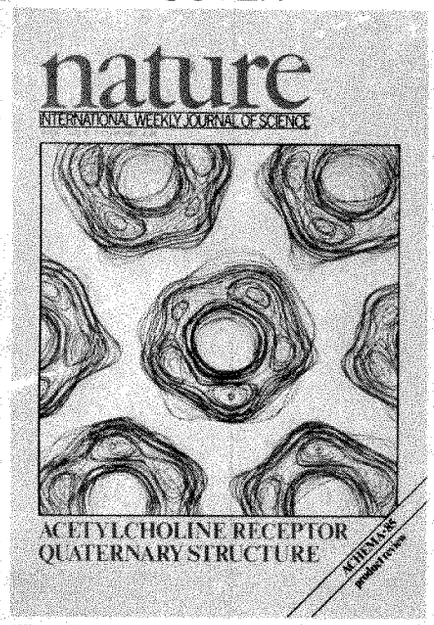
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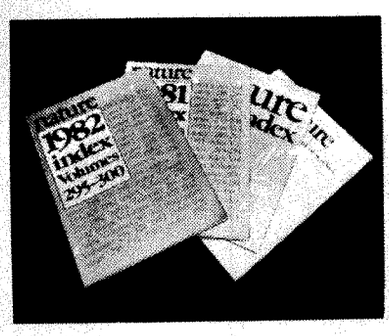
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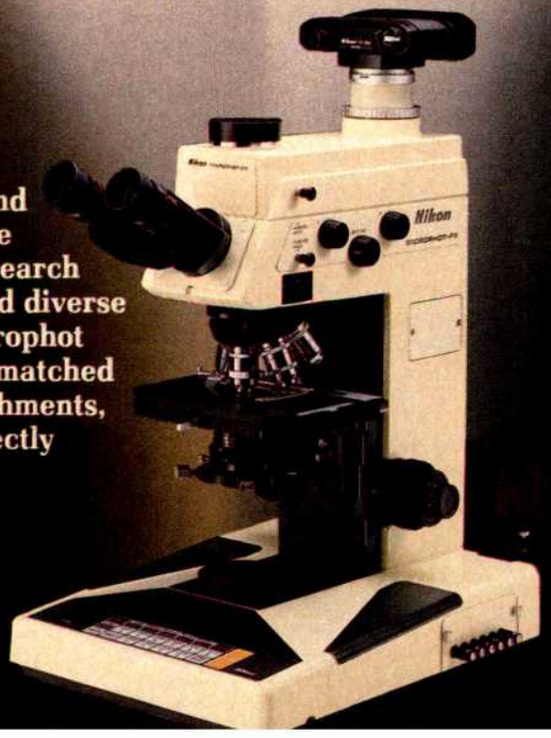
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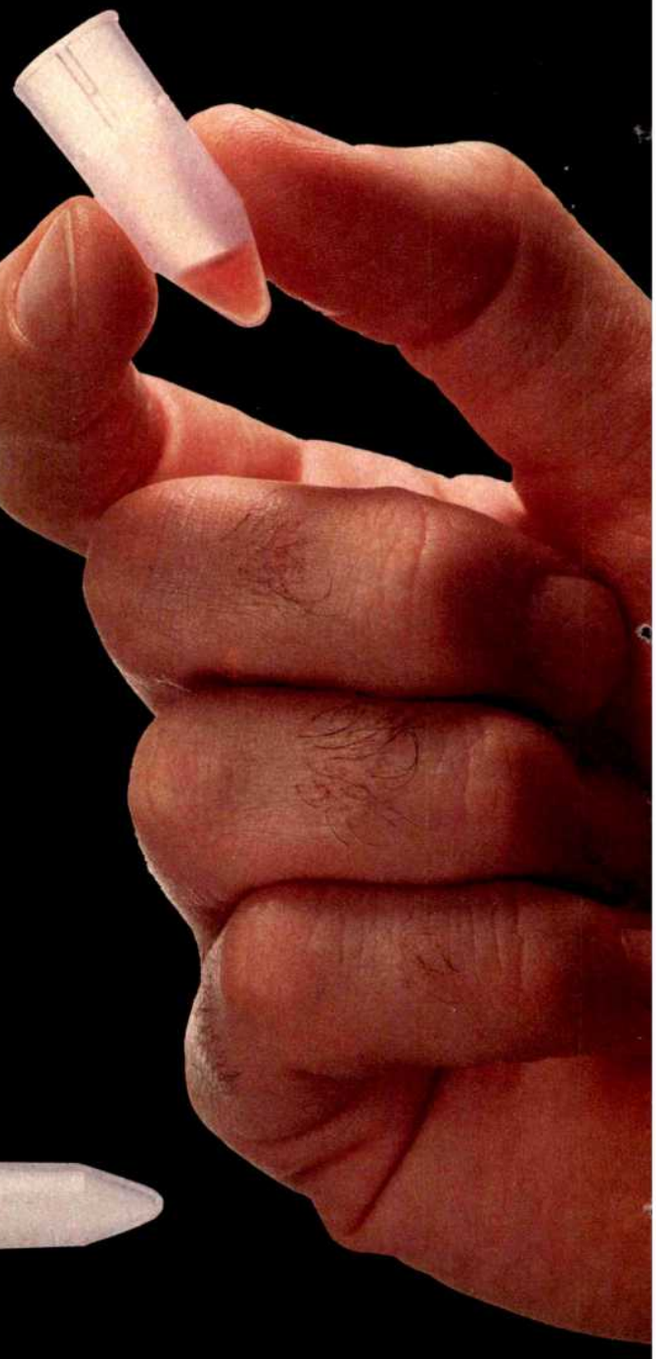
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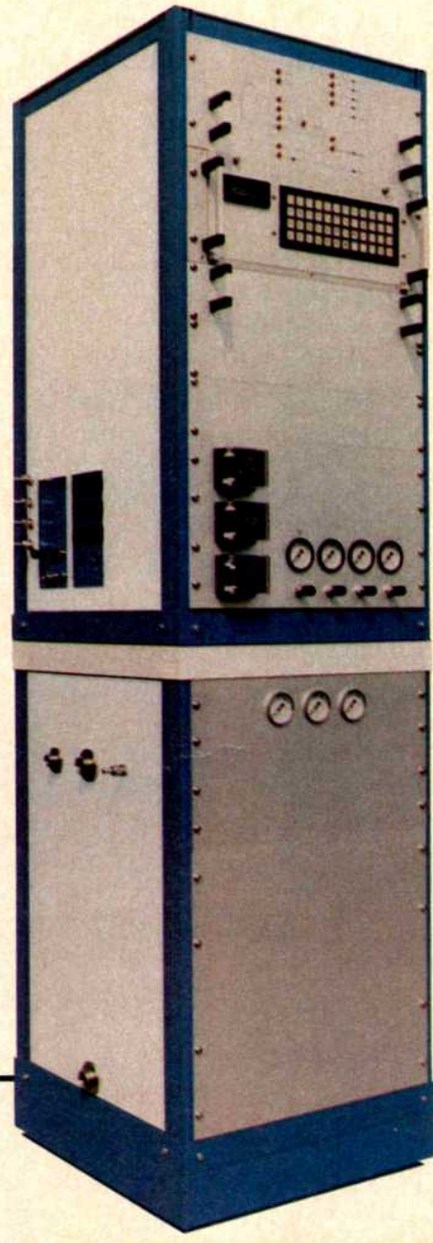
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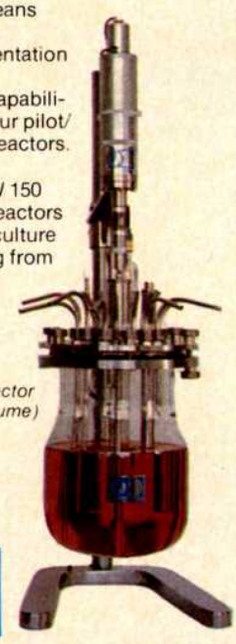
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nature

NATURE VOL 315 6 JUNE 1985

What will Eureka discover?

West European governments are warming to the French proposal for a combined venture in high technology. The motives are political, but the risks are high.

THE President of France, M. François Mitterrand, must by now be thoroughly exasperated by his European counterparts. Throughout the five years of his presidency, M. Mitterrand has been preaching the need for European collaboration in high technology. Every summit meeting of the heads of the industrialized nations has been treated to a homily on the subject, and has been met with a blend of incomprehension and scepticism. Almost as if to humour the French government, the others agreed four years ago to mount several attempts at coordination in specific fields, providing a frisson of panic for officials in advance of succeeding summit meetings, but which have not yet produced much of substance. So why should M. Mitterrand have expected more from this year's summit, at Bonn? Indeed, because the proposed collaborative programme, called Eureka, had been advertised in advance as an alternative to collaboration by West European governments in the US programme of research on ballistic missile defences, otherwise known as the Strategic Defense Initiative (SDI) or "star wars", he had every reason to expect another rebuff. That, indeed, is what happened at Bonn, but since then there has emerged a fondness for the brave concept of Eureka that is only partly diminished by the general ignorance of what Eureka is supposed to be, how it is to be organized and what it may accomplish. For the time being, the working principle seems to be that a good idea should not be crabbed simply because it is vague. More generously to all concerned, even professionally pragmatic governments such as the British now seem to be willing to consider what Eureka might have to offer (which has surprised the French).

Eureka

For fear that Eureka may get out of hand, it is important to be clear that government's motives are at best mixed. The idea of foreign collaboration in the SDI research programme seems to have cropped up first during the British prime minister's visit to Washington last December, and to have been a British suggestion whose implications were not fully appreciated at the time. Now, the US government seems to value collaboration for political reasons: governments that agree to collaborate on the research are likely to be stauncher allies in resisting the Soviet argument that even the research programme is an abomination (which it is not). Governments (Britain and West Germany, for example) at first attracted to collaboration saw two advantages: the opportunity to learn some high technology at no direct cost and the opportunity to influence the political decision that may eventually have to be made about deployment. They have since been given pause by the more careful calculation that legal difficulties would restrict the technical benefits they might hope to win and that junior partners in such a gigantic enterprise might in the end have very little influence. The French government, traditionally suspicious of US influence in Western Europe, is consistent in its unwillingness to collaborate. Politically, the interested governments should set their faces firmly against collaboration in SDI unless they can win terms that will assure their independence in the strategic arguments that must lie ahead. The more immediate question is whether Eureka is in any way the alternative it purports to be.

Eureka, essentially a French conception, is to some extent an exercise in continental (European) chauvinism. It is firmly in the tradition of previous French technological initiatives leading to the Concorde (an uneconomic supersonic passenger aircraft), the European Airbus (a range of successful commercial aircraft), the Ariane satellite launcher (probably a success) and Superphénix (a prototype fast reactor, still unproven). Although many of these and other projects have been (or have seemed) worthwhile in themselves, French enthusiasm for them has in part derived from the wish to narrow what used to be called the technology gap between the United States and Europe (not to mention France). It is also worth remembering that some projects with this objective, such as the plan in the 1970s to beat off the US computer invasion of France, have been costly failures.

Solutions

Successful or otherwise, however, these projects have in common the circumstance that, from the point of view of Western Europe as a whole, they are second-bests. If the European Communities were not functioning as an efficient common market, and in the process generating the wealth of which Europe is potentially capable of generating, the need for the artificial definition of projects that will increase industrial company spending on research and development would have long since faded away. Instead, European companies would be spending from their own resources on research and development on a scale commensurate with that of their rivals in the United States and Japan — and the pace of innovation would presumably be enhanced commensurately. Throwing public money at joint projects must therefore be counted a stop-gap expedient for encouraging industrial development in Europe. Governments will have to worry less when there is a common market, but this will come about only when the same governments abandon their restraints on European competition, especially their failure to follow the principle that public purchases of goods and services should be open to competition. And for the time being, even (or especially) in France, national chauvinism takes precedence over European chauvinism.

Throwing money at Eureka, at least while the project is as ill-defined as at present, could be worse than a mere waste of money. As so far described, the essential chacteristics of Eureka are that it should be a big project and that it should include the principal components of the SDI programme — information processing, high-powered lasers, particle beam technology, artifical intelligence and so on. For the time being, Eureka is a rag-bag of technology looking for a goal. No doubt more tangible objectives will emerge in the next few weeks. But as there is no reason to expect these to be related to market needs of any kind, the obvious risk is that Eureka will be a recipe for diverting

Biological manuscripts

Miranda Robertson, Biological Sciences Editor of *Nature*, is now based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees).

European research efforts from the more realistic goals that European companies have already set for themselves. Nobody will deny that in the course of such a programme of technological development, European technology would acquire new skills. European politicians are fond of insisting that "science for its own sake" is a waste of resources, which is debatable; what they forget is that technology, however marvellous, which is not harnessed to practical objectives has literally no purpose.

The lessons for the West European governments lately attracted by Eureka are therefore plain. Their first objective should be to sweep away the restraints on movement and competition which at present make European technology less effective than it might be. Second, they should be prepared to back with public money only those projects emerging from the discussion of Eureka that seem likely to be commercially successful as well as technically challenging. Third, they should persuade each other that technological chauvinism is not a game worth playing for its own sake, but that it may even divert scarce skill for more useful tasks. Finally, they should acknowledge among themselves (and tell their voters as much) that the technology gap, although not necessarily here to stay, cannot by its nature be quickly bridged. Impatience is a dangerous trap. □

Fraud prevention

Only colleagues can judge the value and authenticity of a person's work.

THERE is much in the belief that the pressure on people to publish research articles is part of the explanation for the cases of fraud in science that have become notorious in the past few years, but too much attention should not be paid to the suggestions from Los Angeles last week (page 447) that journals could do much to help by, for example, stamping out the tendency towards the multiple publication of the same data. Quite apart from the practical difficulties (identical manuscripts simultaneously submitted, for example), better regulation at this stage of the publication process amounts merely to the remedy of symptoms, not the underlying disease. In any case, if repeated publication helps the process of communication, by what test is that by itself a crime? Far better that reforms should aim at the abatement of the pressure to publish.

How does the pressure arise? Now that young people's promotion prospects in academic life are substantially determined by their record in research, it is natural to look for fraudsters among the rising stars. And this has partly been confirmed. But why, then, have the most conspicuous cases of fraud apparently arisen at some of the most distinguished laboratories? Perhaps work done in relative obscurity is scrutinized less carefully, while it is also possible that the pressure to seem to succeed is all the greater the more success there is about. But a further disturbing possibility suggested by last week's discussion in Los Angeles is that there may be a symbiotic relationship between those who advance their reputations by fraud and much more senior people whose reputations are apparently established. For however replete a person's resume may be with research papers, his present influence may depend to a large extent on being thought still active in research. Indeed, the success of a department or a whole school in winning research funds from outside may depend on its head's reputation as one who still beavers away in the laboratory.

In the circumstances, it is no great surprise that great men have been vulnerable to younger fraudsters. Sadly, these relationships are often indistinguishable from those between truly great men and younger talented protégés. The remedy is not easily found, for one of its components would have to be a mutual recognition by the scientific community and those among its leaders who have become inactive that it is still possible for an established scientist to run a creative laboratory even when his direct contribution to the work is small. Then, only close colleagues would be in a position to distinguish wheat from chaff — which, in any case, is the present state of affairs. □

Educational yardsticks

The British government should not attempt to measure institutional efficiency.

THE British government's empty discussion paper on the future of British higher education (see *Nature* 23 May, p.270) has predictably been condemned by academics and the organizations which represent them, but it is unusual that the chairman of the Committee of Vice-Chancellors and Principals, Dr Maurice Shock, should have so openly broken with the conventions of polite restraint. For academics and even the author of the discussion document, the Secretary of State for Education and Science, however, the most significant development in the past two weeks may be the volume of protest there has been from the House of Commons at the emptiness of the policy now made public.

A large part of the trouble of the universities in particular during the past five years has come about because they had allowed themselves to lose their political friends, once equally strong on both sides of the political spectrum. If the opposition parties now do no more than recognize that the management of higher education in Britain has become a stick with which to beat the government, higher education cannot be confident of benefiting.

But there is always a chance that the politicians will once again come to recognize the social importance of higher education, which is the only long-term guarantor of well-being.

Calculations

Meanwhile, universities and others would do well to watch out for a little-noticed appendix of the discussion document, which purports to be a first tentative attempt to calculate "performance indicators" for the objective measurement of efficiency in higher education. The idea is that there should be constructed a set of numbers measuring such characteristics of higher education as the cost of educating people in different kinds of institutions, or by means of a course of instruction, and culminating in an attempt to quantify the social value of graduates in different fields.

To be fair, the government's document emphasizes that the numbers tucked away in last month's appendix must be taken with several pinches of salt — but it then goes on to promise (or threaten) better numbers in the future. The snag, on the present showing, is that the fact that the numbers now published would not satisfy the requirements of examiners of beginning-economics courses is unlikely to diminish the influence they will have on public policy.

The most obvious defect of the calculations now attempted is that the average costs which are their chief product may be entirely misleading. It is not surprising that the average cost of educating students in British universities turns out to be higher, subject by subject, than in polytechnics, or that a medical degree is more expensive than one in engineering (which nevertheless is shown to be surprisingly higher than a science degree). But these numbers are irrelevant to the economic management of a system of higher education already in being, when the practical questions should be whether this or that change in the pattern of an institution's work would yield this or that benefit, and where the useful answers will be provided in terms of marginal, not average, costs.

All this has long since been recognized. In the present condition of British higher education, the first implication of a properly conducted study would probably be that there is too much educational plant which is under-used, or that the government could at this juncture buy a great deal of higher education cheaply, if it chose to follow that enlightened course.

Because there is every likelihood that the British government will persist with its economic analysis, and that the results will be used for a further shaping of the system as a whole, academics (and economists in particular) should do what they can to see that the job is done properly and independently. □

Nuclear testing

Size of Soviet tests disputed by geophysicists

Los Angeles

US geophysicists made angry accusations last week that the US government is "lying" over estimated yields of Soviet underground nuclear tests. Allan Lindh of the US Geological Survey said at a symposium on treaty verification organized by the American Association for the Advancement of Science (AAAS) that geophysicists agree there is no evidence that the Soviet Union has cheated on the Yield Threshold Test Ban Treaty (YTTBT), which places an upper limit of 150 kilotons on weapons tests. The Department of Defense (DoD) was accused of hiding the evidence from citizens behind a "veil of secrecy".

The treaty verification symposium was one of the more heated sessions at this year's AAAS meeting, which included discussions on several aspects of arms control. Sally Horn of DoD repeated President Reagan's charge that the Soviet Union has probably exceeded the 150-kiloton limit in the YTTBT, which went into effect in 1976. Other speakers, notably Colin Gray of the National Institute of Public Policy, argued that because the Soviets have cheated on "most if not all" arms treaties (except the Nuclear Non-proliferation Treaty), such treaties have been "harmful or irrelevant".

Although much of the information needed to judge Soviet compliance with arms treaties is subject to security classification, analyses of seismic records are published in the open literature, and have been the subject of long-running arguments. The dispute centres on the method used to estimate explosive yields from seismic data, and in particular the bias parameter incorporated to take account of the local geology of the Soviet test site at Semipalatinsk.

Yield estimates are derived from body P-waves at a frequency of about 1 Hz. The relationship between the magnitude of an event judged from P-wave amplitude and explosive yield has been thoroughly investigated at the Nevada Test Site in the southwestern United States. According to Jack Evernden of the US Geological Survey, confirmed data published more than 10 years ago (based on comparisons with other types of seismic waves) indicate that for the Semipalantinsk test site a value of 0.4 should be subtracted from magnitudes to estimate explosive yield. That judgement has recently been confirmed by, among others, the Air Force Technical Applications Center in Florida, which has a primary responsibility for yield estimates. Present US government estimates, however, use a bias value of only 0.2, resulting in yield estimates that are twice as high, as with the 0.4 value. Evernden says the decision to use a bias of 0.2 was "obscure", and claims there is unanimity among geophysicists that the higher value is correct. When the higher value is used, yield estimates converge on the 150-kiloton legal limit.

The DoD representative at the symposium was unable to shed any light on the matter. She professed unfamiliarity with the detailed arguments but admitted there was a "debate" on the question.

Seismic verification would be a crucial factor in any comprehensive test ban that might be negotiated in future; negotiations for such a ban were suspended in 1980. Evernden, who used to work for DoD, publicly rebutted a paper published in *Science* in January this year by Willard Hannon of Lawrence Livermore Laboratory, which questioned whether a politically plausible detection network could detect most low-yield explosions. In Evernden's view, almost all test explosions above 1 kiloton (the limit of military value) can now be distinguished from earthquakes by a simple network of non-array detection stations. Others at the conference argued, however, that the lack of progress towards a more comprehensive test ban resulted from a lack of political will rather than for technical reasons. **Tim Beardsley**

US research restrictions

Pentagon prefers export control

Los Angeles

THE US Department of Defense (DoD) has told the research community that it will in future make routine use of export regulations to restrict access of foreign nationals to scientific data which, while not classified, might be of military significance. As a result, many scientific and technical conference organizers will be obliged to put sensitive material in special "export-controlled sessions" restricted to US nationals and to foreign nationals only if validated by their embassies. Participants in such sessions must undertake to keep the restricted material confidential.

The new policy was spelled out at a meeting of the American Association for the Advancement of Science by Dr Sumner Benson, substituting for Richard Perle, Assistant Secretary for Defense for international security policy. In the past, some technical societies have voluntarily restricted access to conferences for fear of violating arms traffic regulations: the new policy, by giving control to DoD, will make such voluntary censorship unnecessary.

Export controls will be used only for scientific data that are not considered "fundamental". According to a DoD policy instituted last year by Richard DeLauer, then Under-Secretary of Defense for research and engineering, "fundamental" research on university campuses is controlled only by use of security classification.

The new policy outlined by Benson would implement authority in the 1984 DoD Authorization Act allowing DoD to restrict unclassified data with military or space applications. That authority was granted because of concern that the Freedom of Information Act might allow the Soviet Union access to sensitive research results, because data released under the Freedom of Information Act cannot be controlled by export regulations.

Benson's announcement clears up much of the confusion that has been rife since DoD enacted export-controlled sessions at an April meeting of the Society of Photo-Optical Instrumentation Engineers in Virginia. Then, it was unclear whether such sessions would become commonplace, but at at a recent private meeting, organized by the Institute of Electrical and Electronic Engineers DoD apparently declared itself happy with the formula. It remains to be seen whether learned societies will be happy to accept export-controlled sessions as a matter of course.

The new policy is a further development in the administration's continuing efforts to restrict access to sensitive technology. Benson said that reliance on security classification would be expensive and cumbersome but that export-controlled sessions would be more predictable and flexible, while allowing maximum access to restricted information consistent with national security. Benson said that the new arrangements would also have the advantage of not penalizing researchers at institutions which have set their faces against classified research on-campus.

The export regulations to which DoD is appealing are themselves being revised. According to the latest draft, information resulting from research contracts which embody restraints on free publication — even non-military contracts — would require a validated export licence to be transmitted to foreign nationals.

Boyd McKelvain of General Electric Co. made a strong plea at last week's meeting for moderation in the use of export regulations. McKelvain said he was unaware of any cost/benefit analysis supporting further restriction of data flowing to friendly nations, and warned that industrial laboratory managers are concluding that they will be forced to avoid employing foreign nationals. In McKelvain's view, unilateral action by the United States to prevent access by foreign nationals to US research could backfire and lead to reprisals, causing economic damage to US industry. **Tim Beardsley**

European defence programme

Favourable noises for Eureka

EUREKA, the French project for a European alternative to the American "star wars" or Strategic Defense Initiative (SDI) programme, seems to be in a much better state — politically speaking — than it was a few weeks ago. Or so French sources are making out, now that the British foreign minister, Sir Geoffrey Howe, has indicated to his French counterpart M. Roland Dumas that he favours Eureka, despite initial British reticence.

The new British position seems to be that a variable-geometry, high-technology European programme, run by what the French describe as a "light" administration that would include the European Commission but not be controlled by it, could well be effective. Sir Geoffrey, it is believed, would like Eureka to concentrate on the "coordinated exploitation" of basic research. This is a logical insistence, as it is exactly what Britain alone has for long failed to achieve.

However, while Paris is now happy with London's support, and while administrations in many European states are now behind Eureka, contradictory statements and confusions about the project still abound, both between nations and between senior ministers of single nations, and particularly between France and Germany. Originally, German Chancellor Helmut Kohl had embraced SDI research in collaboration with the United States while French President François Mitterrand had rejected it, describing it as mere "subcontracting" and fearing a drain of technology and brains to the United States. But two weeks ago the West German foreign minister Herr Hans-Dietrich Genscher had visited the French capital and expressed doubts about star wars and enthusiasm for the Eureka project. Hopes were high for an agreement of last week's summit meeting between Kohl and Mitterrand at Lake Constance in Bavaria. In the event Kohl refused to abandon his support for SDI research, and failed to give any explicit backing to Eureka.

Afterwards, *Le Monde*, normally France's most respectable newspaper, launched an extraordinary front-page, and apparently politically inspired, attack on Kohl, describing him as provincial and vaccillating, and complaining that deep issues went completely over his head. But Mitterrand on Friday lowered the temperature greatly by describing Eureka as "a Franco-German idea", by claiming that it was of "vital necessity for West Germany" and that Germany agreed with this. Thus the waters muddy, and the true Eureka is hard to find.

At least the French view is becoming sharper. For example, M. Hubert Curien, the research minister, has now clearly confined M. Mitterrand's "non" to star wars to government actions and government support, and left industry free to decide for itself whether SDI involvement would pay. In practice, then, as countries that have said "yes" or "ja" are unlikely to be enthusiastic about SDI research if it involves the spending of large amounts of government money (unless that government is American), there may be little difference between the French and other positions.

In further clarification, Curien last week made the longest public statement yet on the precise nature of Eureka. He claimed it was well received in Rome, Copenhagen and Berne as well as in London (and Bonn), and promised the first "one or two" real programmes by the end of the summer. He was at pains to emphasize the need for industrial involvement in defining programme content and objectives, and said that such consultations were well under way. As to what the programmes will be, Curien remained curiously silent.

Robert Walgate

In vitro fertilization

Travemünde

MOST participants at this year's meeting of West German physicians (Deutscher Ärztetag) believe that the absence of legal guidelines in modern reproductive medicine should be made good by their own initiative. Fertilizations outside the mother's womb now occur in a "legal vacuum".

The physicians resolved to adopt professional rules (*Richtlinien*) to decide between correct and incorrect behaviour. Their unanimous opinion was that such rules should not be left solely to politicians and lawyers. Although it was also clear that many physicians have no wish to play a decisive role in *in vitro* fertilization, their demand was refused that there should be a stop to the programme until the inherent complications are understood.

The final communique was described by some as a presumptuous attempt by physicians to force their morals on the rest of society, particularly the restriction of the new technology to married couples.

Supporters of the restriction, however, hold that marriage and the family are the more relevant legal and ethical values, and that exceptions should be allowed only on the recommendation of a specially founded commission. Both sides agreed that the "rent-a-mother" (*Leihmutter*) concept is unacceptable, if only because of the risks of commercialization.

The physicians have taken this initiative now because the federal government is as slow and indecisive in this context as in that of recombinant DNA technology. One aim of the meeting was to put pressure on the government to clarify the legal position so that physicians are not left to decide alone. Jurgen Neffe

European defence programme

Ambitions of Heriot-Watt

WHILE European governments consider how to respond to American Secretary of State Casper Weinberger's invitation to join in star wars — otherwise the Stategic Defense Initiative (or SDI) — research, the SDI organization in Washington is not letting any grass grow under its feet. Last week, an SDI representative in England visited Europe's leading laboratory in optical computing at Heriot-Watt University in Edinburgh, and all but made an offer of a $150,000 grant, with a promise of several times that amount to come. In return Professor S. Desmond Smith and his team, who lead an eight-university European group created by the European Commission in Brussels to design the first all-optical computer, will join an American network working for SDI.

The Heriot-Watt group leads the world in this technology. In the past few months Smith and colleagues were able to demonstrate the first thee-element loop of optical switches operating at visible wavelengths at room temperature. This shows that "an indefinite extension of optical logic circuits is possible" said Professor Smith on Monday. Optical computing may offer not only speed but also enormous parallel processing capability, and is of much interest to SDI proponents, as it might make possible the rapid computations needed to identify, track and destroy multiple targets.

Is this the kind of loss of European high technology to the United States that the French had in mind in putting forward the Eureka programme? Not quite. "I'm here to build Europe", says Smith, "and if this grant is not going to help them I'm not going to use it".

For Smith, the lesson of this first SDI grant in Europe, which he described as "peanuts" compared with his collaboration's existing £2 million from British and European sources over the next three years, is that the US Department of Defense seems to spend not just for defence, but for the whole of American industry. This is exactly how US industry get into new technology so fast, Smith feels. By contrast, in seeking UK support to build prototypes of a working optical parallel processing system, Smith is finding that his requirement falls neatly between the Department of Education and Science, the Department of Trade and Industry and the Ministry of Defence. But in an exchange of letters with the Prime Minister, her chief scientific adviser, Sir Robin Nicholson, and others, Smith finds there is considerable support for an improvement in interdepartmental cooperation. Meanwhile, he is keeping closely in touch with the British government about the American approaches.

Robert Walgate

Research fraud

New ways of shading truth

Los Angeles

PRESSURE to publish and a lingering "pre-med syndrome" among medical researchers contribute not only to the relatively rare cases of outright scientific fakery, but also to various much more frequent abuses of scientific publication, according to medical researchers and journal editors speaking at the annual meeting of the American Association for the Advancement of Science.

Dr Edward Huth, editor of *Annals of Internal Medicine*, said that journals and scientific societies should consider adopting a precise definition of authorship to prevent one of the most frequent abuses, "false authorship". Other common abuses that he had encountered were the practice of slicing one study into a series — "salami science" — and repetitive publication.

Huth said false authorship usually occurs when a department chairman, laboratory technician or anyone not directly responsible for the intellectual content of the paper is listed as an author. Huth told of a case in which a researcher learned only by accident that his name had been included among the authors of a paper submitted to the *Annals*. While the researcher had provided some technical advice to the main author, at another institution, he had contributed neither to the design of the experiment nor to its execution, and had not even seen the final paper or any drafts. In fact, he had specifically declined an offer to be listed as an author.

Another common deception is statistical manipulation of data. Dr John Bailar, a statistical consultant to the *New England Journal of Medicine*, said that the deliberate misuse of statistics should be part of an "expanded definition of fraud". Inappropriate statistical tests have often been used to exaggerate the significance of the results presented. In some cases, authors seem to have tried several statistical tests, reporting only that which gives the best results. Hypotheses suggested by the data are often reported with *P*-values, which have meaning only for hypotheses formulated independently of data.

Huth suggested several steps that could be taken to curtail these abuses, which, he said, because of their "frequency and ubiquity", make them "more worth our attention than fakery". Although little can be done to prevent "salami science", repetitive publication could be checked by specifically asking reviewers to bring prior publications of the same material to the attention of editors.

False authorship may be more difficult to eliminate. The *Annals* requires each author to sign a form acknowledging that he has seen the final version of the paper and agrees to its publication. The shortcomings of even this approach were revealed by the case Huth mentioned above: the eight signatures on the forms bore a striking similarity.

Dr Robert Petersdorf, dean of medicine and vice-chancellor for health sciences at the University of California at San Diego, suggested that the problem lies deeper. Science has become "too big, too competitive, too entrepreneurial, too bent on 'winning' ". Federal research grants to medical schools have grown from $436 million in 1960 to $2,351 million in 1981, increasing proportionately much faster than enrolment.

Petersdorf said that the "pre-med syndrome" of intense competition and resorts to cheating carries through to competition for grants and for tenure, "eroding the moral fibre" of physicians.

The deputy editor of the *New England Journal of Medicine*, Dr Marcia Angell, suggested that grant-making agencies and tenure committees could help eliminate these abuses at source by refusing to consider more than the three articles a candidate considers to be his best in a given year.

Whether pressures to publish are responsible for the more serious cases of fraud remains unknown. Patricia Woolf, at the sociology department at Princeton University, noted that the number of papers published per scientist per year has changed little but in one group she has studied in detail (chemists), the number of "super-productive" researchers has clearly grown.

The National Institutes of Health (NIH), which investigates cases of fraud involving federal research grants, say that they receive about two reports a month of fraud or scientific misconduct, a minute fraction out of the 20,000 research projects active on any given day. Dr William Raub, deputy director for extramural research and training, said that NIH will nonetheless issue rules this summer requiring institutions that receive federal funds to report any cases of suspected fraud. **Stephen Budiansky**

Arianespace

Sky-high insurance unfair

ARIANESPACE, the largely French company that now runs Europe's Ariane space launcher on something approaching a commercial basis, is fed up with the huge insurance premiums that are being asked of satellite makers for launch on Ariane. According to M. Frédéric d'Allest, president of Arianespace, his company is being forced to pay for the losses made by insurers of the ill-fated Palapa B-2 and Westar-6 satellites, mis-launched from and then recovered the US space shuttle, and for recent failure of Syncom IV-3 and the earlier TDRS-A, also both shuttle-launched. Insurance premiums are now so high, 20 per cent of the capital cost of Arabsat, recently launched by Ariane, for example, that the growth of the satellite communications business itself is under threat.

According to Arianespace officials, before the shuttle setbacks premiums were set at around 6 per cent for a shuttle launch and 9–11 cent for Ariane. This was after Ariane had suffered two early failures, and reflected a feeling among the insurers that because the shuttle was manned, there should be less risk of uncorrectable trouble with a shuttle launch. But this did not take into account the large, multi-million dollar booster rockets needed by shuttle-launched satellites to take them from the low orbit of the shuttle up to geostationary heights, says Arianespace. This is the technology in which the shuttle has faced difficulties, while Ariane, which launches into an elliptical "geostationary transfer" orbit from which only a small "apogee motor" is needed to reach geostationary orbit, has gone from strength to strength. Ariane has now successfully launched 13 commercial satellites in its last eight flights, and it is time the insurers recognized the fact, says d'Allest.

But the insurance companies are reckoned to have lost almost $200 million on Palapa and Westar alone, despite their recovery, and another $85 million for Syncom, and they are fighting shy. Premiums are now around 18 per cent, with higher figures for those the companies think can pay, such as the Arabs. The premiums are the same for the shuttle and for Ariane, claims Arianespace, and they come as a package, making no distinction between the launch to first orbit (which the shuttle has accomplished perfectly) and the boost phase. M. Deschamps, director-general of Arianespace, would like the insurers to make a distinction by charging the same to Ariane and the shuttle for the first injection, and then more to shuttle customers for the boost, thus reflecting the apparently higher risk. At present the terms are "a brake" on growth, says Deschamps.

And Arianespace is certainly looking for growth, with 25 future satellites in the queue, an order book totalling FF 6,500 million (£600 million), a second launch pad at the launch site in Kourou, French Guiana, nearing completion and a third planned, apart from French space agency work on advanced and more powerful forms of Ariane, including a mini-shuttle, Hermes. And while President Reagan is attempting to shift more of the real costs of shuttle launches onto the customers, raising launch fees, Arianespace is planning to follow suit to raise profits, but not so much as to threaten the 30 per cent of the world market for satellite launchers the company hopes to be taking from 1987 on. Here is one field in which Europe feels at last that, for the time, it is holding one or two trump cards. **Robert Walgate**

European molecular biology

Computing facilities for EMBL

Brussels

PLANS to expand databank services, increase in-house and collaborative research and improve access by researchers to software and data at the European Molecular Biology Laboratory (EMBL) in Heidelberg have been given a first airing.

The idea for a European biocomputing facility, which would give the laboratory a greater coordinating role in Europe, was proposed by its director, Lennart Philipson, and was welcomed by scientists and science policy advisers, not only from Europe but also from the United States and Japan at a meeting to discuss developments in biosequencing in Italy last month.

EMBL's biocomputing programme involves the development and application of computing methods and a large-scale public nucleotide sequence databank, set up in 1981, which exchanges information regularly with the Los Alamos databank in the United States. The preliminary proposal outlined by Philipson would mean an overall expansion of computer operations and the setting up of a software library to enable remote users from all over Europe to screen data at EMBL, and to download them, together with the software, for local analysis. A data library is also envisaged which would collaborate with other databanks to supply protein, microbiological and gene-mapping data. The aim is not, however, to centralize all remote user computation but to act as a source of data and portable software for remote users. Computation would be mainly on a query and search basis, with EMBL providing a package of utility programmes for data screening, searching and manipulation. A software and data link-up with large national centres could also occur.

The facility would also carry out sequence analysis, developing methods and algorithms to interpret nucleic acid and protein sequences, basic research in the field of protein folding and engineering, modelling of proteins, protein-nucleic acid interactions and other more complex structures, biological graphics, image analysis and the development and maintenance of a user-oriented system and database for the analysis of two-dimensional electrophoresis. Finally, the facility could constitute a forum both for research and for the elaboration of software policy.

The expansion would require EMBL to increase staff and invest in more high-level hardware. The laboratory already has plans to install a VAX 8600 cluster core by the end of this year to which further VAX computers could easily be added. The added functions would, says Philipson, require an extension of hardware. Preliminary estimated costing of the proposal puts capital expenditure at DM 7.3 million and annual operating expenses at DM 3.2 million.

According to Philipson, the needs could be covered by a 10 per cent increase in the current budget. At present the 12 to 14 countries participating in the laboratory (including non-EEC countries such as Sweden, Finland and Israel) contribute a total of DM 38 million to EMBL's annual budget of DM 45 million.

Extra funds for space and hardware increases could be found in two ways: by contracts involving some of the interested countries or from the European Community, says Philipson. While some countries need and welcome European cooperation in this field, others, such as the United Kingdom, have been less keen to contribute in the past, preferring to build up activities on a national level. Whether the venture will be taken up in the laboratory's second scientific programme, which will run from 1987, will depend very much on several meetings that have been planned by EMBL's Scientific Advisory Committee and, ultimately, the EMBL Council. The next meeting of the advisory committee is scheduled for three weeks' time.

Anna Lubinska

US research support

Defence money no panacea

Los Angeles

UNIVERSITIES in the United States are in for a disappointment if they expect to reap a windfall from a new Department of Defense (DoD) initiative to support academic research. The "University Research Initiative" proposed in this year's Pentagon budget is, in fact, largely a symbolic step even in the eyes of Pentagon research chief Leo Young; Young's predecessor, George Gamota of the University of Michigan, speaking at the meeting of the American Association for the Advancement of Science, noted that when DoD began a similar programme to support university instrumentation three years ago, expectations ran so high that the $30 million a year programmes received $600 million worth of proposals.

Young said that the new initiative would help to overcome a "perception" problem dating from the passage of the so-called Mansfield amendment in the late 1960s. This rider on the defence appropriations bill requires all DoD to research support the mission of the agency, and has often been blamed for dwindling DoD support of basic research in the 1960s and 1970s and for continuing to prevent DoD from entering into a closer partnership with universities. Gamota, however, believes that the amendment has been used as an excuse within the Pentagon for not increasing basic research funds — particularly by those who wish to see other programmes funded.

The new initiative would provide $25 million, evenly divided between the Army, Air Force and Navy research offices and the Defense Advanced Research Projects Agency, for graduate fellowships, instrument purchase and young investigator awards. The House Armed Services Committee has recommended increasing that figure to $200 million, but the Senate has approved the lower figure. The Pentagon is proposing a total basic research budget for next year of $971 million. Historically, about one-half to two-thirds of that money goes to universities.

Existing programmes that support some 300 graduate fellowships, as well as the $30 million a year instrumentation programme, would continue even without the new initiative — and despite the Mansfield amendment. Young noted that the current version of the amendment provides a wide loophole by allowing research with a "potential" for supporting the Pentagon's mission "in the opinion of the Secretary of Defense".

Stephen Budiansky

Soviet pay rises

SOVIET scientists, designers and production engineers will from next year receive higher wages and more generous bonuses. But the resolution of the Community Party Central Committee, the USSR Council of Ministers and the All-Union Trade Union Confederation, which established the new pay scales in mid-May, made it clear that the purpose of the reform is to reduce the time spent in research and development, and in the implementation of new technologies, the endemic bottle-neck of Soviet civil industry.

Under the new scheme, managers of enterprises and organizations will be empowered to pay supplementary salaries and bonuses to persons responsible for "creative contributions" to new technology. Bonuses will be more closely linked to the "economic benefit" produced by the new technologies, and several measures have been proposed to improve the system of granting "author's certificates" (the Soviet equivalent of patents) and of making the attendant payments to scientists and technologists. Proposals to establish two new honorific titles: "Meritorious Designer" and "Meritorious Production Engineer", were also approved.

Although the new pay system seems to be aimed initially at applied scientists and technologists, it will almost certainly produce some spin-off for those working in basic research. During the past decade, there has been increasing emphasis on the need to strengthen links between academic research and production, and on the coordinating role of the Academy of Sciences in ensuring that research results are quickly implemented.

Vera Rich

University of California

Oil disquiet at Santa Barbara

Los Angeles

THE University of California at Santa Barbara (UCSB) is about to build 65 new faculty houses with spectacular views of the Pacific Ocean, nearby beachfront and modern conveniences, all within walking distance of campus. But university officials are worried that professors attracted to their seaside campus might be disappointed. The reason is that UCSB has rich oil deposits in its front yard. An oil company plans to erect two giant drilling platforms in shallow water only a mile and a half away from campus and from the new faculty housing. And it is not just the view that is being threatened: the university's marine scientists fear that a marine study-preserve in the waters off campus could be harmed by the mud produced in drilling.

UCSB chancellor Robert A Huttenback seems reconciled to development of some kind, but says that the way it is done may be crucial. "If the air gets mucked up, the research environment could be ruined. The university could lose its appeal to students and faculty."

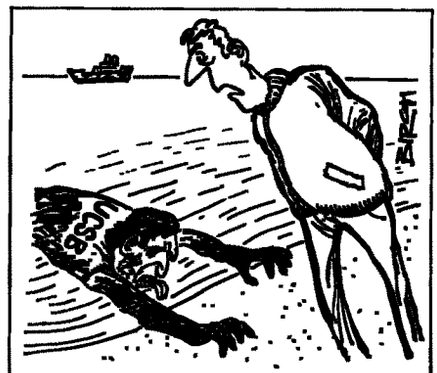
Everyone agrees the project will go ahead. The state of California is eager to lease the offshore tract to Atlantic Richfield (Arco), a Los Angeles-based oil company, because the state expects to earn between $600,000 and $1 million a day when the facility reaches peak production in 1992.

Arco has held its lease on the tract since 1945; but has only recently discovered the major new oil deposits. The Santa Barbara Channel and nearby Santa Maria Basin, most of which are under federal control, are expected to become the second largest offshore oil development in the Western hemisphere. Six other oil companies hold leases off Santa Barbara's coast but only Arco's is in the university's front yard.

The project calls for two double platforms to rise about 14 stories above the ocean floor and will produce from 80,000 to 500,000 barrels of oil a day. Plans for treating and transporting the crude oil and natural gas are under discussion.

On campus, likely winners include the structural and ocean engineers who will participate in designing new offshore platforms. Most worried are the marine scientists. Those affected by the project bring in over $5 million a year to the university in outside grants.

Aquatic biology has 1,450 undergraduate majors and 148 graduate students, making it the nation's largest marine sciences programme for undergraduates. Pioneering work has been done on abalone culture, bioluminescence, kelp reproduction and sediment transport. A specialized seawater system pumps water into laboratories and classrooms.



Not another oil-slicked Marine Biologist!

Because of their stake in the waters off campus, UCSB scientists are trying to convince state authorities that the usual legal requirements for protecting the environment are not enough to safeguard the area for research purposes.

Will toxic drilling muds damage the marine habitat? A preliminary study, paid for by the chancellor's office, indicates that such muds are detrimental to juvenile but not adult organisms. There is worry the muds might kill young abalone and other species and also foul the seawater intake system To quell such fears, Arco has said it might barge drilling wastes ashore to avoid contamination.

The university also fears there will be a housing shortage caused by the influx of workers. Rents are already high and the school will have to increase financial aid so that students can afford to live nearby.

But aesthetic concerns top the list. The campus is only two miles from the Santa Barbara airport and some professors are already bothered by helicopters taking off every 15 minutes or so as part of the overall oil development taking place in the region. Oil platforms, no matter how well built, strike many people as ugly. And their operations do sometimes emit foul-smelling odours that even now (from other oil rigs seven or more miles offshore) waft across campus a couple of times a month.

The views of the university are important to Arco, according to Robert Flaherty, a company vice-president. Nobody in the industry would produce oil and gas at the price of damaging UCSB's educational and research activities, he says. "We can't see the remotest possibility of these terrible effects."

Sandra Blakeslee

Franco-Japanese voyage

Hitch mars start for *Nadir*

Tokyo

NO sooner had the French research vessel *Nadir*, with the deep-sea submersible *Nautile* riding piggy-back, set sail on 1 June on the second phase of the Franco-Japanese deep-sea project Kaiko than mishap struck with an accident that injured two French crewmen, one of them seriously They were struck by flying metal from a ring that broke when testing the crane used to lower *Nautile* from the mother ship. Two relief crew are being flown out to Japan. Once they are on board, the *Nadir* will immediately set sail again on its three-leg cruise to explore the plate boundaries running along the deep-sea troughs and trenches off the coast of Japan.

The accident aboard the *Nadir* must add to the worries of Kazuo Kobayashi, Japanese co-director of the project, whose worst nightmare is that the *Nautile* will collide with a ship when she surfaces. The seas off the Pacific coast of Japan are criss-crossed by some of the busiest shipping lanes in the world and, near the junction of the Japan and Kuril trenches, where the cold southward-flowing Oyashio current meets warm air masses from the south, fog is frequent. As the *Nautile* has a cruising speed of only one knot, extensive surveys of currents in the study areas have been carried out before the survey to avoid unnecessary "drift" of the submersible during its planned two-hour descent to 6,000 m. Transponders placed on the bottom will allow navigation with pinpoint accuracy and the submersible can be directed by telephone to spots of interest by the mother ship. Time on the bottom will be about five hours at 6,000 m during which the submersible can traverse about 9 km of sea floor The three-man submersible will carry only one scientist at a time, so French and Japanese researchers will take turns to descend.

The temporarily delayed voyage is due to finish on 19 June after exploring the Tenryu Canyon and Zenisu Ridge which flank the Nankai Trough — a sediment-filled basin that straddles the boundary between the Eurasian plate on which Japan rides and the subducting Philippine plate which plunges beneath Japan.

The second leg, from 22 June to 10 July, will traverse the Suruga and Sagami troughs that cut into the Japanese mainland at the leading edge of the subducting Philippine plate. *Nautile* will then investigate Dai-ichi Kashima Kaizan, a Fuji-sized seamount that is being sliced and swallowed up by the Japan Trench (*Nature* 313, 433; 1985). On the final leg, from 13 July to 1 August, a seamount in a similar predicament will be investigated at the junction of the Japan and Kuril trenches, and it is hoped to make the deepest dive to nearly 6,000 m.

Nautile may also find subduction-zone analogues of the "vent communities" of giant tube worms and clams found at spreading centres, but here associated with cold seeps of methane rather than hot sulphur-rich waters (see *Nature* 313, 339; 1985). No biologists, however, will participate in the dives, which will be manned by geophysicists, geologists and geochemists.

David Swinbanks

Polish education

Threat of repressive new law

THE current clampdown on the academic community in Poland could seriously imperil Polish hopes of regaining Most Favoured Nation status (MFN) with respect to the United States. Loss of MFN, as part of the package of sanctions imposed by the United States when General Jaruzelski imposed Martial Law in December 1981, has cost an estimated $50 million in Poland's foreign trade earnings. Alleged government plans for the deployment of graduates, recently described by the underground Solidarity journal *Tygodnik Mazowsze* could well render this loss permanent.

According to *Tygodnik Mazowsze*, new legislation is being drafted jointly by the Ministry of Science and Higher Education and the Ministry of Education to ensure that all "graduates" work for ten years in the nationalized sector of the economy. "Graduates", the paper explains, apparently quoting from a leaked draft of the proposed legislation, includes all "Polish citizens who have completed basic vocational schooling, secondary vocational schooling of all types, higher education or who have taken PhDs abroad". If such persons fail to work in the nationalized sector for at least ten years after graduation, they may be asked to return to the treasury the cost of their education. As a corollary, "graduates" who have not completed this ten years' service, if going abroad, will be required to deposit the cost of their education as a guarantee of their return.

The last requirement appears to contravene the terms laid down in the US Senate by the Jackson-Vanik amendment (law 504A) of 1974, under which the US government is forbiden to grant MFN to any other government which requires intending emigrants to repay the cost of their education. According to the extracts of the draft quoted in *Tygodnik Mazowsze*, implementation of the proposed law is expected to bring into the treasury several hundred million zloty a year from the contributions of graduates to the nationalized sector, as well as forfeited "guarantees".

In addition to the MFN problem, the implementation of the law could seriously damage Poland's international image. The new law would apply for 15 years after the end of their studies. This would seriously hamper the prospects of younger Polish scholars wishing to travel abroad for, even if they intended to return, they might not be able to pay the deposit. Linkage of higher education with employment contravenes the international human rights law.

The extension of the term "graduate" to cover the trade-school sector could hit not only small craftsmen, but also the run-down private agricultural sector. Since 1980, the Polish government has given various grudging guarantees to the private farmers (who account for some 75 per cent of all Polish agriculture) that no attempts will be made to force them into the system of state and collective farms. The government has reluctantly agreed the principle of a scheme, proposed by the Catholic Church, to raise agricultural capital abroad and, according to Fr Alojzy Orszulik, the press spokesman of the Polish Catholic hierarchy, the government negotiators are about to "finish their work" on the plan. The rumoured new law would, however, mean that graduates of agricultural academies and agricultural secondary schools could return to their family's farms only in middle life. The foreign patrons of the Church scheme (who include the governments of the United States and the European Communities) may well be given pause.

Vera Rich

Failure for West German Greens

LAST month's election in Nordrhein-Westfalen (NRW), the most populous state in the Federal Republic, was a great success for the Social Democratic Party (SPD). It comes exactly halfway through the legislative period of the Christian Democratic (CDU) Liberal (FDP) coalition government in Bonn and is widely regarded as a vote against the coalition policies. Equally important was the failure of the Green Party to obtain the 5 per cent vote necessary to win seats in the NRW parliament.

The Green Party fared less well than it had hoped in the election in Saarland three months earlier, where it obtained only 2.5 per cent of the votes. The 4.6 per cent in NRW was not a significant improvement on the result five years ago, when the Greens were a completely unknown factor.

Coalition with the SPD was made impossible by the Greens' determination that NRW should withdraw completely from the nuclear industry. But, like the CDU/FDP in Bonn, the SPD in NRW has a strong interest in maintaining, among other nuclear power stations, the nuclear research centre at Jülich and the fast breeder reactor at Kalkar. It seems the FPD has now adopted enough of the political standpoint of the Green Party to weaken its appeal to ecologically minded voters.

Immediately after the election, an old quarrel broke out within the Green Party between the fundamentalists and realists. The former oppose all political compromises, while the latter strive to exert direct political influence. The difference between the two factions is so basic that it may splinter the Green Party. The three established parties may henceforth need take less notice of new radical ideas.

Jürgen Neffe

Indian cooking

Chulhas win fair wind

New Delhi

CHULHAS (wood stoves) that do not emit smoke and use less wood are fast replacing traditional cooking stoves in rural households, ushering in a "smokeless revolution" in India's countryside.

Some 800,000 smokeless chulhas have so far been installed and 5,000 villages have become smokeless in 15 months since the project was started by the Department of Non-conventional Energy Sources (DNES). The project has exceeded expectations, says DNES secretary Maheshwar Dayal, whose department has fixed a target of five million improved cooking stoves by 1990.

About 40 per cent of all the energy in India is used for cooking food. The traditional woodstoves made of mud and brick in India's 112 million homes annually burn 133 million tonnes of firewood and over 200 million tonnes of cow-dung cake. But the stoves are so inefficient that rural women spend long hours collecting firewood. The improved cooking stoves, with a combustion efficiency of more than 15 per cent, have drastically cut down consumption of firewood. And because these stoves are designed to burn the fuel completely, the kitchens are smoke-free. Dayal says that smokeless chulhas introduced have saved 600,000 tonnes of firewood. By 1990, the saving would be 4.2 million tonnes, valued at Rs 2,940 million (£210 million).

Some 29 models of improved cooking stoves have been developed and introduced by social organizations and research institutions such as the Indian Institute of Technology (IIT) in Delhi, the Central Power Research Institute (CPRI) and the Indian Institute of Science (IIS) in Bangalore. The cooking stoves vary according to the region, type of food cooked and fuel used. But all new designs are tested and certified by testing and training centres at IIT, CPRI and the Punjab University of Agriculture in Chandigargh.

While fixed-type chulhas are installed by government agencies and voluntary organizations, portable models are marketed at prices ranging from Rs 38 to Rs 63 (£3 to £5) by 12 private companies that have sprung up to cash in on the smokeless revolution. As an incentive to buyers, the government has abolished excise duty on fuel-efficient wood-burning stoves.

DNES has also developed community chulhas capable of cooking for up to 200 persons, with fuel efficiency as high as 35 per cent, recommended for hostels, hotels and canteens. The Tamil Nadu state government is planning to install the improved community chulhas in the 33,000 schools whose midday meal project is being threatened by firewood shortage.

K.S. Jayaraman

Oceanography

Sino-Japanese collaboration

Tokyo

IN the wake of successful cooperation between the United States and Japan in oceanography, Japan's Science and Technology Agency has recently revealed plans for joint research with China in a multi-million dollar long-term study of the East China Sea and the Kuroshio, a huge warm ocean current in the Western Pacific that affects fishing, weather and shipping.

Although details are still under negotiation, the project will start in March 1986 and run for seven years The chief participants will be Japan's fishery, meteorological and maritime safety agencies and China's National Bureau of Oceanography.

The project, which was proposed by China in "vague" terms two years ago and put into more concrete form by Japan, will follow on from a 10-year study of the Kuroshio sponsored by the Science and Technology Agency That survey, the Kuroshio Exploitation and Utilization Research (KER) project, is due to end next year but has only covered a small area of the East China Sea, a politically sensitive region for oceanographic research because of its potential oil reservices.

The Kuroshio (black stream, black because of its low reflectivity) is a 100-km wide "river" of clear warm water originating east of the Philippines that flows north past the eastern coast of Taiwan and then winds its way along Japan's Pacific coast before turning abruptly out into the Pacific on meeting the southward-flowing cold Oyashio current. At this bend in the Kuroshio, the Kuroshio Extension, huge swirling eddies of warm water extending to depths of hundreds of metres break off from the main stream and head slowly north, often persisting for periods of more than a year before finally being absorbed into the Oyashio or reattached to the Kuroshio.

With a speed of 3-4 knots and a volume transport of 30-60 tons per second the Kuroshio represents a tremendous potential source of energy, a point not missed by energy-hungry Japan; one purpose of the KER project is to assess the viability of harnessing the Kuroshio's power. But the real power of the Kuroshio lies in its influence over Japan's fisheries.

In the East China Sea, the current creates one of the world's largest fishing grounds and is an important spawning area for several commercial fish such as sardines. According to a KER report published in March, vast numbers of fish eggs are spawned next to the temperature/salinity front of the Kuroshio and eggs and larvae are picked up by the current and transported hundreds of kilometres downstream to nursery grounds off the Pacific coast of Japan. In addition, fish such as tuna and bonito feed along the length of the Kuroshio and the warm eddies north of the Kuroshio Extension can cause switches in the migration routes of several commercial fish such as Pacific saury Japan's vast fishing industry thus has a tremendous vested interest in understanding the vicissitudes of the Kuroshio. In fact some large tuna fishing vessels are already equipped with automatic picture transmission equipment for receiving and processing real-time infrared satellite images of the Kuroshio. As such equipment is beyond the resources of all but the largest fishing vessels (a terminal costs about $65,000) the Japan Weather Association (JWA) introduced a satellite data service in October last year which provides HRPT (high resolution picture transmission) infrared imagery data in photographic or floppy disk form for as little as $4.60.

Satellite-monitoring both of drifting buoys and sea-surface temperature will also form an important part of the joint project with China, according to Dr Hideo Nishida of the Hydrographic Department of the Maritime Safety Agency. The annual budget for Japan's contribution to the project will be similar to that of KER, he said, more than Y100 million ($0.4 million).

At the same time as the Science and Technology Agency's project gets under way in the East China Sea, the Ministry of Education-affiliated Ocean Research Institute (ORI) of the University of Tokyo intends to tackle the other end of the Kuroshio, focusing attention on the warm core rings north of the Kuroshio Extension. Professor Takahisa Nemoto, head of the Plankton Division at ORI, announced at an international seminar here in Tokyo this week that a cruise of the institute's RV *Hakuho Maru* will study Kuroshio warm core rings in September 1987, while the institute's smaller research vessel, the *Tansei Maru*, will carry out preliminary studies in the same area in 1986.

David Swinbanks

France

Future of encyclopaedia in doubt

THE creation of a great new French encyclopaedia, mostly of the sciences, was announced by the present French government in 1982. It was to use all available new technologies (databanks, electronic access, video) as well as trhe more traditional ones (print) and it was to be the 20th century's answer to the *Encyclopédie Diderot*, the great enterprise of the Enlightenment to encompas all knowledge in a few fat volumes. Where it is now? Appealing, it seems, to the French public for a few sous. A new advertisement has appeared in Paris asking for "patrons" to invest in the new encylopaedia, which, until recently at least, has been unable to attract a French publisher willing to carry any financial risk.

The publishers North-Holland, it is hoped in Paris, may produce an English version, but the *Commission Diderot*, which is in charge of the 20th century masterwork, needs to raise another FF 10 million (around £1 million) to bring this "encyclopaedia of living questions" to the French. It will be interesting to see if the French will be willing to pay. So far the commission has a grant of some FF 750,000 from the ministries of education, research and culture, and has been promised a further FF 4 million from industry. That not being enough, the appeal appears to have been the only way forward.

So will the encyclopaedia, the brain-child of present education minister Jean-Pierre Chevènement (who was research minister at its launch), be worth a minimum of FF 100 investment? The French market is not large and the publishers' judgement should be respected. But FF 100 to be associated with a great project?

The encyclopaedia is certainly great. 200 volumes are expected by the end of 1989, and the first 20, involving 200 authors, are promised by April 1986. But its structure is novel and even contradictory. The principle is that "knowledge" is not fixed but changing; and moreover that at any time there can be many opinions on a subject. While this may be true of many ideas, there are also many of which it is not true. And it is difficult to see how a flux is to be fixed in encyclopaedic form.

In practice, the *Commission Diderot*'s strategy was to send out a nation-wide "appeal for questions (this came before the appeal for funds), an appeal which led to a list of 350 (such as "will carbon-dioxide emissions alter the climate and by how much?") out of which the commission has selected some 50 for the first volumes. Some 80 per cent of the questions received were in the physical or biological sciences or in mathematics, and only 20 per cent in the social sciences (which surprised the commission secretariat). Answers to these questions by the 200 authors will form a resource, it is said in Paris, from which many kinds of publication may be drawn. A great deal depends, therefore, on these authors, who must produce texts which are "intelligible to all" and address all the fears and hopes raised by the subjects they cover.

According to Dominique Lecourt, shortly after he was appointed director of the project, the encyclopaedia is to be "a powerful instrument of liberation". It will also "reject any attempt to assemble the sum of all knowledge or to present it according to an apparently rational order" It is a mountainous and confusing task, and it must be asked if even France is large enough to face it — even with a few more sous.

Robert Walgate

Lobbying of NSF denied

SIR — In "US engineering research: Six special centres founded", (*Nature* 11 April, p.488; 1985), Tim Beardsley reports on several grants made by the National Science Foundation (NSF) to establish major engineering research centres. At the end of the article, Beardsley implies that political influence was involved in the selection of one of the awardee institutions. This letter's erroneous implication could cause great potential harm.

Beardsley states: "At least one US senator had lobbied NSF to locate an engineering research centre in his state, apparently successfully. But Bloch [Erich Bloch, director of NSF] insists that siting decisions had been made exclusively on the basis of peer review of the 142 proposals from 106 institutions."

The only communication about this matter that NSF received from the senator concerned was a form letter asking for information about the proposal on behalf of a constituent. That would hardly seem to constitute "lobbying" even by the most rigid of definitions.

Beardsley apparently reached his conclusion after reading a press release issued by the senator's office that seemed to imply a greater involvement. Other writers attending the press conference about the awards correctly identified this as a case of a political figure being overzealously associated with an event beneficial to his constituency. Beardsley's statement beginning, "But Bloch insists " does not help matters greatly.

NSF goes to great lengths to ensure that decisions on all proposals are made on the basis of scientific merit and that the scientific and engineering communities are aware of and confident in that fact. In the case of the engineering research centre proposals, the review involved a four-stage process in which a number of competent persons participated, including highly respected engineers from academia and industry. Let me state clearly that politics was not and is not part of the process. The implication in the Beardsley article to the contrary is most unfortunate.

RAYMOND E. BYE JR
Office of Legislative and Public Affairs,
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Washington, DC 20550, USA

Tim Beardsley writes: While there is no evidence that NSF's reviewing was influenced by political pressure, the form letter referred to, from Senator Alfonse D'Amato of New York, was sent to NSF in response to a request for support from Mr Gregory Fusco, vice-president of government relations at Columbia University. The senator's involvement was readily confirmed by his office at the time the story was written. Senator D'Amato asked NSF to keep him informed of the progress of Columbia University's application and asked that Mr Fusco's request "be given every consideration". Some might disagree with Mr Bye's assertion that this does not constitute lobbying (*Oxford English Dictionary*: "to seek to influence"). Only the over-sensitive, however, would object to the word "insists" in connection with Bloch's denial that political factors influenced the selection process; insisting that one is innocent carries no implication that the opposite is true.

Embryo research

SIR — In their attempt to establish a status for the *in vitro* fertilization embryo, which denies the scientific understanding of the human embryo and the medical code of ethics in regard to it, Drs Evans and McLaren make some remarkable points[1].

They castigate Professor Lejeune because he ". . omits to say that research on the detection and prevention of these genetic defects... in some instances can only be done at this early (embryonic) stage" while omitting to point out themselves that most developmental genetic research work can be and is being done on ethically suitable animal and insect models. Indeed, *Nature* is organizing an international conference ("Genes and Systems in Development", San Francisco 7-9 October 1985) "to address what is perhaps the central unanswered question of biology today: How is the process of development directed by genes?" Most of the papers are based on animal models.

When the normal developmental processes of pattern formation are understood, then the abnormal processes can be solved — for how can one hope to understand the pathological without a proper understanding of the normal? In regard to the use of insect embryo models for understanding genetic development, David Ish Horowicz has suggested a relatively short time span: "It is conceivable that within 5 years it will all be over. We will have solved the universal biological problem of pattern formation"[3]. Allowing a further five years to solve the question of abnormal pattern formation (genetic defects), the answers should be available before the turn of the century. In the meantime, we can console ourselves in the knowledge that infants with genetic defects comprise less than 1 per cent of live births and 993 out of every 1,000 embryos, which implant with genetic defects, will not result in a live birth[4].

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Women students

SIR — In his article, "Women in science and engineering", (*Nature* 2 May, p.84), Richard Pearson suggests that the cause of the surprisingly low representation in the "less traditionally heavy engineering" field of electrical engineering and electronics may be that "the competition for places is much harder in these subjects".

Surely this implies that women engineers are simply not as able as men in the field of electrical engineerng and electronics, and therefore cannot compete for these jobs?

I find this an extraordinary suggestion from a member of the Institute of Manpower Studies, but comparable to the engineering careers consultant at my old university who advised all female engineers to become accountants.

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SIR — In Richard Pearson's article on sexual discrimination (*Nature* 2 May, p.84) there is an important difference between what is purported to be the percentage of medical students in the United Kingdom who are women, and what the government believes the figure to be. Pearson's Table 1 gives 49 per cent as the percentage of first-year women medical students in 1982 (a figure he attributes to the Universities Central Council on Admissions). The Department of Health and Social Security publication *On the state of the public health for the year 1983* (HMSO) gives the percentage number of women entrants in UK medical schools in 1982-83 as 44 per cent, and for the previous year 43 per cent. Whom do we believe? Medicine being what it is, I imagine the DHSS figure is nearer the mark.

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Liberation all round

SIR — We were appalled to read the report from R.A. Tatouille (*Nature* 28 March, p.323) on the formation of the Vegetable Liberation Front. This is elementism of the worst kind. We have formed the Association for the Liberation of Long Oppressed Ingots to counter this oppression, and to lobby support for non-carbon based objects.

Remember the rallying cry of ALLOI "Metals of the World unite — you have nothing to lose but your purity".

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Avoiding recurrent catastrophes

The death of perhaps 15,000 people in Bangladesh last month was caused by cyclonic and tidal forces in concert. Prevention may be impossible, but better warning is essential.

THE tragic loss of life in the Ganges delta of Bangladesh two weeks ago will never be accurately quantified. Even in normal circumstances, the numbers of people eking out a living in the delta can only be guessed at; the numbers of those swept away by the tidal wave that filled the Bay of Bengal on 25 and 26 May will be counted only imperfectly. But tragedies like this recur at frequent intervals along this low-lying coast. What can be done to prevent them, or at least to mitigate their effects? Understanding the phenomenon must be a first step, which is why there is interest in a calculation of the interaction between wind-driven surges and the natural tide in the Bay of Bengal, published in the *Philosophical Transactions of the Royal Society* (**A313**, 507-535; 1985) by B. Johns of the University of Reading and associates from the Indian Institute of Technology at New Delhi: A.D. Rao, S.K. Dube and P.C. Sinha.

In the long run, accurate prediction of these tidal waves must be a prerequisite for avoidance action. Two weeks ago, the meteorological services did warn of impending trouble along the low-lying coast, although the argument continues whether the warnings might have been fuller. Too fatalistically, people also ask what value broadcast warnings may be to people too poor to own radio receivers.

The essence of the phenomenon that killed about 15,000 people in the Bay of Bengal is that by which a cyclonic pressure may create a surge of water elevation as it moves through shallow water onto the shore of an enclosed tidal basin. The first expectation is that the increase of water level and thus the risk of flooding will be greater when the arrival of a cyclone coincides with high tide. But by now it is accepted that the interaction between the tides and the wind-driven surge is not linear. The whole may be greater (and more damaging) than the sum of the parts.

Johns *et al.* have provided a valuable snapshot of where the modelling of tide/surge interactions now stands, and also of what remains to be done. The paper sets out to model retrospectively the tidal wave that hit the low-lying coastal strip of the Indian state of Orissa, on the west of the Bay of Bengal on 3 and 4 June 1982. When more is known of the track of the cyclone that drove last month's tidal wave onto the coast of Bangladesh, the same mathematical apparatus may be used to reconstruct that sequence of events as well.

The technique is in principle straightforward. First, the bottom topography and the coastline of the bay are represented by smoothed curves. This is the idealized but still realistic basin in which the water is driven by the forcing of tidal oscillations in the Indian Ocean, and by the surface winds of cyclones. But because of the large volume of water in the several mouths of the Ganges, one elaboration of the model puts a series of 200-km stretches of water at the head of the bay.

The hydrodynamic equations for the motion of the water in the bay are simplified to the case of a flat surface, although the Coriolis forces associated with rotation are allowed for. The coupling between the Indian Ocean and the bay is supposed to be determined primarily by the astronomical tides, whence arises the most immediate difficulty of the model: by what conditions should the interaction be represented on the boundary, taken as an imaginary line running east from the Sri Lankan coast? In principle, if there were enough tidal stations around the bay, it would be possible to determine this external forcing function accurately, simply by running the model and comparing its predictions of the tides with observations of them in normal circumstances. In the event, the data are so sparse that Johns *et al.* can do no more than represent the tidal effect of the Indian Ocean on the bay by means of a regular sinusoidal variation of height with constant phase along the whole length of the arbitrary boundary. If catastrophic surges in the bay are to be predicted accurately, this needless simplification must be avoided, from which it follows that much better tidal data must be collected from many more stations in the bay.

Incorporating cyclones in the model is also an empirical process. The chief physical effect is that of cyclonic winds on the sea surface. Johns *et al.* represent the Orissa cyclone by a wind-field modelled to allow for decreasing velocity with increasing distance from the centre, but fitted with the crude meteorological data available for the cyclone. By their account, the predicted height of an induced surge in the Bay of Bengal will depend sensitively on the wind-field pattern out to 100 km or so from the centre of the storm, which points to another obvious difficulty: because there is at present no way of inferring precise wind-speeds from synoptic meteorological data, real-time predictions are, for the time being, uncertain.

In the circumstances, it is remarkable that the model functions as well as it does. By forcing the movement of the mass in the bay by the crude representation of the effects of the external tide, as well as by the Orissa cyclone following its measured track, Johns *et al.* conclude that the average height of the tidal wave in June 1982 may indeed have reached 8 m at some parts of the coastline. But given the unavoidable arbitrariness with which the constants specifying the wind-field must be chosen, the real proof of the pudding is that the model seems to have reconstructed the sequence of events correctly.

The Orissa inundations of 1982 turn out to have been interesting in their own right (but perhaps they all are). According to the model calculations, not much seems to have happened to the level of water off the Orissa coast (except for a gradual rise totalling 2 m in a day) until the centre of the cyclone made its landfall on the afternoon of 3 June. Thereafter, however, the computer calculations bear out such anecdotal evidence as there is that the largest elevation of sea-level occurred some hours later, further to the north-east. During this interval, the model shows, it is almost as if a tidal wave was moving steadily along the coast from the point of landfall, at first increasing with height. The tidal and cyclonic effects are not additive, which is what would be expected of solutions to a set of equations which are inherently nonlinear, but the result is that some odd things can be expected. The times of maximum inundation may differ by several hours from those of high tide. Parts of the coast far from the point of landfall may be inundated twice.

Directly, none of this has much to do with last month's tragedy. Indeed, the physical scale of these phenomena is so great that outright avoidance is clearly beyond reach. The only realistic way of mitigating recurrent damage on last month's scale is that there should be more accurate warnings, and that better use should be made of them. Much might be done by running the model of Johns *et al.* not merely on those cyclones which turn out to be damaging, but on the much larger number of meteorological depressions in the Bay of Bengal which turn out to be relatively benign, for then it might be possible to build up a kind of taxonomy of dangerous cyclones. But for such an exercise to be worthwhile, better tidal observations are essential.

John Maddox

Neurosciences

Modulation of some deadly sins

from Charles F Stevens

SEVERAL recent articles, three of them elsewhere in this issue, shed light on neuromodulation, the shadowy side of brain function. We understand quite well in terms of cellular mechanisms about half of what the brain does — the integration and transmission of information. But the other half — the neuromodulatory systems that seem to mediate, for example, glutony, sloth, lust and anger, if not more of the seven deadly sins — is only now beginning to be elucidated.

The well understood part of brain function has been revealed through a line of research initiated by Hodgkin, Huxley, Katz, Kuffler and Eccles. Integral membrane proteins, known as channels, control the permeability of the neuronal membrane to ions, and thus regulate the brain's electrical activity. The channels change their conformation in response to synaptically released neurotransmitters or variations in membrane voltage. These conformational changes cause the channels' transmembrane pores to open and permit ionic current to flow across the neuronal membrane. The neurotransmitter, the first messenger, thus triggers the current flow that acts as a second messenger by producing changes in membrane voltage in remote parts of the neurone, where the behaviour of other channels is in turn altered. The number of distinct kinds of channels is quite large: neurones have at least a dozen species of channels that sense membrane voltage, and more than a dozen types that open their pores after binding specific neurotransmitter molecules such as acetylcholine, dopamine or serotonin.

Neurotransmitters are released at the brain's 10^{13} synapses, and part of their function is to cause channels to open. But some neurotransmitters also have a quite different function that is not yet fully understood. They bind to receptors on the neuronal surface and employ chemical second messenger systems, rather than current flow, to produce myriad effects. A typical second messenger is cyclic AMP, produced by neurotransmitter-activated adenylate cyclase. Second messengers in the brain have a wide variety of effects, ranging from the ability to alter the rate of transcription of specific genes (see, for example, Barinaga, M. *et al. Nature* **314**, 279; 1985) to the modification of channel properties, as described below. The hallmark of these modulatory effects is that they involve covalent modification of proteins rather than simple conformational changes, and thus occur on a time scale of seconds to minutes or longer, whereas the typical channel opening and closing that is responsible for the nerve impulse, for example, occurs in the order of milliseconds.

The three papers on neuromodulation in this issue involve three different types of channels that respond to membrane voltage by opening pores selective for potassium ions; more than half a dozen distinct types of potassium channels are known. On page 501, Aghajanian reports that the neurotransmitter noradrenaline causes the suppression of one species of potassium channel ('A' channels) in neurones of the dorsal raphe nucleus. Because the normal function of this channel is to slow the production of nerve impulses, the suppression results in an increase in the rate of firing. Stanfield, Nakajima and Yamaguchi report on page 498 that the neuropeptide substance P suppresses a second type of potassium channel (the inward rectifier), so increasing the excitability of neurones cultured from the globus pallidus. Finally, on page 503, Ewald, Williams and Levitan demonstrate the mechanism by which cAMP increases the activity of a third type of potassium channel (the calcium-activated potassium channel). Using patch clamping of isolated membrane patches from nerve cells of the snail *Helix* and channel reconstitution in artificial lipid bilayers, they find that the action of the catalytic subunit of cAMP-dependent protein kinase increases the probability that the channel will open. Their observations demonstrate that the substrate of the kinase is either the channel itself or a closely associated entity. Similar experiments by Shuster *et al.* (*Nature* **313**, 392; 1985) have shown that the cAMP-dependent protein kinase can act to decrease the activity of a fourth type of potassium channel (the S channel), which is implicated in a form of learning in the sea slug *Aplysia*. Although various types of potassium channels are frequently the targets for neuromodulation, other channels are also influenced in this way. For example, DeRiemer *et al.* (*Nature* **313**, 313; 1985) recently showed that the action of a channel that controls calcium permeation is augmented when the enzyme protein kinase C is activated.

Why does the nervous system go to so much trouble to modify channel properties? One of the brain's chief powers is that it is self-modifying — one set of computing elements can, under neuronal control, be substituted for another while the basic nerve cells and their connections are maintained. Channel properties determine the information handling capabilities of neurones; altering them changes the way information is processed.

Even though we know that neuromodulatory phenomena are both common and important, we are only now starting to define the range of effects and the underlying mechanisms. In no case has the entire regulatory cascade been fully elucidated and much more must be done to work out enough specific examples before we can begin to perceive the underlying logic. Discovering how the brain modifies its own machinery is one of the most important challenges in modern neurobiology and is becoming one of the most active areas of research. □

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High-energy astrophysics

Is Cygnus X-3 a source of gamma rays or of new particles?

from Alan Watson

IF NEW results from the Soudan proton-decay project[1] are being interpreted correctly, cosmic, rather than man-made, accelerators are providing evidence either for a new elementary particle or for unexpected interactions of existing ones. The cosmic accelerator in question is the well-known X-ray and radio source, Cygnus X-3. By observing gamma rays above 10^{15} eV, groups at Kiel[2] and Leeds[3] universities have identified Cygnus X-3 as one of the most powerful emitters of charged cosmic-ray particles in the Galaxy[4,5]. These groups have shown that a few high-energy air-shower events from the direction of Cygnus X-3 display the distinctive 4.8-h modulation seen at X-ray and TeV gamma-ray energies. Because of the continuity of the spectrum from 10^{11} to 10^{15} eV (refs 2,3), and in the absence of other straightforward explanations, it has been generally assumed that these signals are gamma rays resulting from π^0 decay in the environs of the source. The new results from proton-decay experiments in the Soudan mine[1] and in the Mt Blanc tunnel (NUSEX)[6] challenge this simple picture and may demand a change in our views about particle physics.

Cygnus X-3, one of the most luminous X-ray sources in the Galaxy, is a stellar-binary system some 12 kpc from the Sun[7] in which a neutron star orbits a more massive companion star. X-rays are emitted when gas flowing from the massive star falls into the deep gravitational potential well surrounding the neutron star. The X-rays are modulated with a 4.8-h period which slowly lengthens and which is believed to be the orbital period of the system

— the minimum of the X-ray signal defines zero phase, $\phi = 0$, and is thought to occur when the neutron star is eclipsed by its companion. Signals, interpreted as gamma rays, have been seen regularly in many independent experiments from 10^{11} to 10^{16} eV, as discussed previously in these columns[8]. Here, the pattern of emission is variable but relatively sharp spikes of emission (of width $\Delta\phi \lesssim 0.1$) have usually been seen near $\phi = 0.2$ and/or $\phi = 0.65$. Nuclei accelerated in the electromagnetic fields of the neutron star may generate π^0s, and hence gamma rays, through interactions in the circumstellar material[5]. Above 10^{11} eV, cosmic-ray primaries are detected through the cascades that they produce in the atmosphere. It is an inference to say that gamma rays have produced the showers, but neither charged particles nor neutrons could be the primaries — the interstellar magnetic field is much too turbulent for the former to retain their original direction or phase coherence, whereas neutrons of 10^{15} eV have a mean path length before decay of only 30 pc. It has long been expected that gamma-ray initiated air showers should be deficient in muons by a factor of about 10 compared with proton showers, because the photopion production cross-sections are 100 times smaller than proton-proton cross-sections. In an attempt to secure the gamma-ray identification, the Kiel group[9] searched for signals from muons above 2 GeV in a shielded 65 m^2 detector within their array. It came as a shock that for the 14 'on-source' events for which they had data, the muon content was about 80 per cent rather than about 10 per cent of that in 'off-source' events: were the primaries really gamma rays? This difficulty was recognized but tended to be dismissed because the Kiel detector could not follow muon trajectories.

In this context the recent work by the Soudan group[1] demands attention. It is the first published report of a remarkable effect, apparently duplicated in the NUSEX experiment[6], which has hitherto been known only to readers of the *samizdat* literature of particle physics. The Soudan and Mt Blanc groups operate large volume proton-decay detectors at depths of 1,800 and 5,000 metres water equivalent, respectively. Both find that those straight tracks which pass through their detectors (interpreted as single muons) and which project back close to the Cygnus X-3 direction, exhibit its distinctive 4.8-h periodicity. In the Soudan experiment the enhancement of 600 GeV muons is in the phase range $0.65 < \phi < 0.90$ and consists of 84 ± 20 events, whereas for NUSEX the excess of 1 TeV muons comprises 19 ± 5.7 events in the interval $0.7 < \phi < 0.8$. The possibility of chance giving the observed distribution in each experiment is estimated separately to be between 10^{-3} and 10^{-4}.

If Cygnus X-3 is a gamma-ray source, with the rays originating from π^0 decay, then it must also be a neutrino source; both the Soudan and Mt Blanc groups have considered the possibility of neutrinos as muon parents. The Soudan group find that the local zenith angle distribution of events in the phase peak is similar to that of ordinary muons and claim that they can reject completely the hypothesis of an isotropic zenith angle distribution expected for neutrino primaries. In the NUSEX experiment, the predicted neutrino-induced muon flux is too low by a factor of about 800 to explain the observations. To explain the Soudan results, Marshak *et al.*[1] propose either a new interaction or a new neutral particle. To maintain phase coherence, such a particle must have travelled from Cygnus X-3 with a Lorentz factor above 2×10^4.

But some worries about the new experiments remain. The Soudan effect is maximized in a direction displaced by about 3° from the known radio position of Cygnus X-3, which is rather more than can be accounted for by uncertainties in the horizontal orientation of the detector. It is not clear that the background calculation correctly accounts for the possibility of variable exposure at different phases throughout the experiment. In particular the background summed over all phase bins is not equal to the source count as it should be for the background calculation described (J. Lloyd-Evans, personal communication). In the NUSEX analysis a $10° \times 10°$ area centred on the source has been required to maximize the signal; this is much larger than the $1° \times 0.5°$ error in celestial coordinates estimated for each particle track. The absence of any signal above 7 TeV also needs explaining. In neither experiment is it clear whether any statistical degrees of freedom remain unaccounted for in the probability estimates.

As a final twist to this complex story the Akeno air-shower group has just announced a result[10] agreeing with conventional views of the muon content of gamma-ray showers. In air showers with less than a thirtieth of the normal number of muons they find a 4.8-h modulated signal from Cygnus X-3 above 10^{15} eV and at phase 0.5-0.6. Both the phase and intensity agree with recent work from Haverah Park (A. Lambert *et al.*, manuscript in preparation), where confirmatory evidence will be sought with the University of Nottingham's new 40 m^2 muon detector. At the moment the gamma-ray interpretation of Cygnus X-3 is the simplest hypothesis and it may be premature to reject it. □

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Plant metabolism

Coping with drought by the sea

from Peter D. Moore

CRASSULACEAN acid metabolism (CAM) is a photosynthetic strategy characteristically associated with plants subjected to periodic or constant water stress. It is therefore, more usually described in plants of arid lands and with continental distributions than in those found in oceanic areas. It is particularly surprising that a recent study by P.P. Daniel *et al.*[1] of *Umbilicus rupestris* (wall pennywort) — a distinctly oceanic member of the Crassulaceae — should have revealed that this plant exhibits a physiological response to drought that is reminiscent of CAM.

During the past century it was observed that some succulent species of plants accumulate acid in their tissues during the night, but it was not until the 1940s that the full significance of this behaviour was understood. Leaf acidification is accompanied by the nocturnal uptake of carbon dioxide and it has become clear that there is a temporary fixation of carbon by the enzyme phosphoenolpyruvate carboxylase into a 4-carbon compound, oxaloacetate, which is subsequently converted to malate. In the daytime, the malate is decarboxylated and the released CO_2 is refixed by the Calvin cycle enzymes. The main advantage of the pathway is the opportunity it provides for water conservation, because the stomata in the leaf need only open for gaseous exchange at night when transpiration losses can be kept to a minimum.

The efficiency of water conservation in CAM plants becomes clear when their transpiration/photosynthesis ratios (grams of water transpired per gram CO_2 fixed) are compared with those from plants that have other photosynthetic strategies[2]. For CAM plants the ratio is usually 50-100, for C_4 plants it is 250-300 and for C_3 plants 400-500. The strategy is also efficient at conserving carbon, as the CO_2 generated by respiration can be trapped and retained in the same manner.

One of the costs of efficient water conservation in CAM plants is a fairly slow rate of carbon accumulation in the growth of tissues. Therefore, there are clear energetic advantages in the adoption of a flexible system in which it is possible to switch to CAM only during periods when it is of advantage to the survival of the plant, such as in times of water stress; several species of plant have now been described that can do this[3]. One is the succulent *Mesembryanthemum crystallinum*[4], found on

sandy shores and sand dunes around the Mediterranean. Other species switch to an incomplete form of CAM when they experience drought. This has been termed 'CAM idling'. In this mode, there is no fixation of atmospheric carbon during the night, but there is a nocturnal accumulation of acid resulting from the fixation of respiratory CO_2. Here, carbon economy is closely linked to water conservation.

It is this type of CAM idling that has now been observed in *Umbilicus rupestris* when it is exposed to drought. Under normal well-watered conditions, *Umbilicus* behaves like a conventional C_3 plant as far as its carbon exchange is concerned, taking up atmospheric CO_2 during the day and showing a net output of CO_2 at night. After six days of drought, the daytime uptake becomes confined to a short period in the morning, after which stomatal exchange is curtailed. The plant makes up the reduced carbon intake by lowering the respiratory losses at night when phosphoenolpyruvate carboxylase traps the CO_2 generated in respiration and converts it to oxaloacetate and then malate, resulting in tissue acidification.

Evidently the falling water potentials in the plant activate a metabolic switch, but the nature of the operation of this switch is still unknown. One of the consequences of switching is the enhancement of carboxylase enzyme activity; Daniel *et al.* have shown that the properties of the activated enzyme are somewhat modified — for example, it is less sensitive to low *p*H and to malate inhibition[5]. But they do not suggest the generation of a new separable form of the enzyme.

There remains the intriguing ecological question of why an oceanic species should possess this unusual capacity for dealing with drought. The answer must lie in its preferred habitat, for *Umbilicus rupestris* is a plant of cliff crevices and stone walls,

Wall pennywort growing epiphytically on a date palm in Seville, southern Spain.



and sometimes even grows as an epiphyte on larger plants. It can be found at the southern extremities of its range in southern Spain growing on the coarse trunks of the date palm, *Phoenix dactylifera* (see figure). In such exposed locations, often with little or no true soil around its roots, the risk of drought must be very real for *Umbilicus*, even in the wetter regions of north-west Europe. Clearly, the capacity to switch to CAM idling enables the plant to make the most of any given set of conditions. Discovery of CAM in an oceanic temperate plant forms an interesting equivalent to the tropical epiphytic ferns in which CAM has previously been described[6]. □

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Particle physics

Look before you LEP

from David J Miller

THE physics agenda for the large electron-positron machine (LEP), now being constructed at the European Organization for Nuclear Research (CERN), was first circulated in the late 1970s. At that time the Z^0 and $W^{\pm}$ were entirely theoretical particles, but they were needed by the electroweak theory of Glashow, Salam and Weinberg to carry the weak part of the force. Now that these particles have both been discovered by experimenters using the proton-antiproton ($p\bar{p}$) collider at CERN, and after the other developments of the past six years, what should the goals of LEP be? John Ellis convened a Physics Jamboree at CERN on 19 March 1985 to examine this question. Similar answers came from the five working-groups that reported: we must test the validity of the standard model theories at energies where they might begin to break down, and we must probe for the explanation of their many arbitrary parameters.

The important feature of an electron-positron (e^+e^-) machine like LEP, compared with the existing $p\bar{p}$ collider, is that there are no spectator particles to complicate the main event. Although Z^0 and $W^{\pm}$ particles can be found in $p\bar{p}$ collisions, thorough investigations of their properties will only be possible with an e^+e^- machine. Guido Altarelli (Rome) reviewed the precision studies that can be made when the e^+e^- energy is around 94 GeV, equal to or just above the Z^0 mass. In this energy region nearly all reactions will go via a resonance centred at the Z^0 particle and LEP becomes a 'Z^0 factory'. Every possible pair of particle and antiparticle into which the Z^0 can decay will be produced at roughly comparable rates — for example, the heavy tau leptons, the rare and heavy beauty quarks or the everyday down quarks. Although the rarer quarks and leptons are much heavier than the common ones, their partner neutrinos all seem to be very light, so the Z^0 could decay to particle-antiparticle pairs of new neutrinos that are the partners of still undiscovered quarks and leptons. This should allow us to prove the number of generations of heavier and heavier sets of quarks and leptons that still remain to be found after the 'beauty-top-tau' generation. Altarelli reported a technique which the LEP experimental teams believe they can use to make a definitive measurement of the number of neutrino types, and hence the number of generations, since there seems to be one neutrino per generation. This would require only ten days of experimental running when LEP has achieved its planned beam intensity.

Although the Stanford Linear Collider (SLC) in California, now also under construction, may be the first e^+e^- machine to produce the Z^0, its initial detector will probably not be good enough to make this particular measurement. Some of the other physics around the Z^0 mass will probably be investigated first by SLC groups, but it will require very patient and precise work to check all the predictions of the electroweak model — particularly to measure the higher-order corrections to its fundamental constant, the Weinberg angle. Here, the cross-checks between SLC and the four LEP experiments will be important.

Wilfred Buchmuller (CERN) described the feasts in store when we finally pass the toponium threshold — the energy at which toponium, a bound state of a top quark with an anti-top quark, can be created. Last year the UA1 experiment at the $p\bar{p}$ collider gave an estimated mass for the top quark of between 30 and 50 GeV, but no independent confirmation has yet appeared for this rather imprecise result. Toponium may therefore be found anywhere from the present limit of 43 GeV, set by the experiments at the PETRA e^+e^- collider in Hamburg, to the highest possible LEP energy. It should have many of the properties of the two well-established positronium-like quark-antiquark states, the J/psi (charm-anticharm) and the upsilon (beauty-antibeauty). Although toponium should have more excited states than the J/psi and the upsilon, all but the lowest of them will be impossible to resolve, even if they lie well below the Z^0 mass. If they lie close to the Z^0 mass, the physics could be extremely complicated since the Z^0 and toponium states would behave like nearby resonances coupled to the same input channel. They would have opposite phases, so toponium would appear not as localized bumps in the cross section but as narrow dips on the

shoulder of the larger Z^0 bump. Because of the greater mass of the top quark, toponium will be a smaller system than either J/psi or upsilon, and its detailed level-structure will give evidence for the behaviour of the quantum-chromodynamic potential at very short range. Other ways of investigating quantum chromodynamics in e^+e^- annihilation and the scattering of virtual photons from virtual photons, were reviewed by Ahmed Ali (DESY, Hamburg).

The totally new effects that might appear once the natural energy scale of the weak interactions is reached were reviewed by John Ellis (CERN). The cause of the large masses of the $W^{\pm}$ and Z^0, together with the generations of heavy quarks, is one of the great open questions in current theory. These masses may be generated by the Higgs-Kibble mechanism, which introduces a new heavy scalar particle that pervades space, modifying the vacuum and coupling most strongly to the heavy particles. No one has yet identified corresponding free Higgs bosons, although many strategies to look for them have been suggested, especially in Z^0 or toponium decay. But other more complicated theoretical schemes exist which would not necessarily need simple Higgs particles. If their thresholds are reached at LEP, the effects may be seen in anomalous events like the UA1 monojets, which some have tried to explain in terms of supersymmetric theories (see J. Ellis, *Nature* **313**, 626; 1985).

Herwig Schopper, the director-general of CERN, announced that he plans an early upgrading of LEP from the initial (50 + 50) GeV LEP 1 machine, which starts work in 1989, to the (100 + 100) GeV LEP 2. The only change needed would be to replace the accelerating radiofrequency cavities. Great progress has been made in developing the required superconducting cavities and some of them will even be included in LEP 1.

Schopper's announcement was particularly interesting after the discussion of the physics to be done at LEP 2 energies (Roberto Peccei, DESY). The bankable study, which cannot be done on any other machine, is to produce W^+W^- pairs directly via the fundamental couplings of electroweak theory. This gives yet another probe of higher order corrections. If the Higgs particle has not yet been seen, it will only be through such higher order electroweak studies that we will be able to differentiate between the different candidate models that generate masses. There is also more speculative work to be done at the higher LEP 2 energies, continuing the search for the effects of supersymmetry or for some new heavier scale of composite substructure that may underlie the present particles. The Jamboree showed that LEP has an even longer list than before of important specific questions to answer. If there are surprises as well, it will be a bonus. □

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Ocean Drilling Program

Shakedown and sea trials

*from the Leg 100 shipboard scientific party**

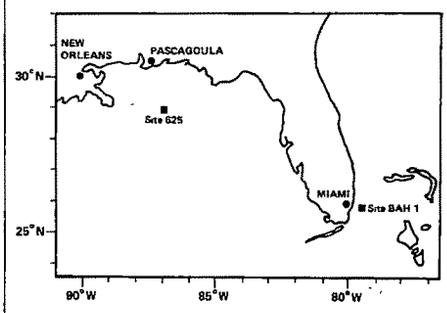
THE Ocean Drilling Program (ODP), successor to the Deep Sea Drilling Project (DSDP), commenced its field operations on 11 January 1985 with an 18-day shakedown and sea-trials cruise (Leg 100), departing from Pascagoula, Mississippi and arriving at Miami, Florida. The cruise came after an extensive conversion and overhaul of the newly chartered drilling vessel *Joides Resolution*. This drillship is 470 feet long and 70 feet wide with a displacement of 16,596 tons, a derrick towering 217 feet above the waterline, and the ability to employ a drillstring of 30,000 feet (9,146 m) in water depths up to 27,000 feet (8,632 m).

Prior to the shakedown cruise, a seven-storey main laboratory structure, geophysics laboratory and a separate library and study area were constructed aboard the vessel. Additional scientific, office and storage space has been provided; major modifications to the ship's dynamic positioning system, which enables the vessel to maintain a relatively fixed position over the hole being drilled in the sea bottom, now enable long-based and short-based line systems, as well as the original ultra-short-based line system, to be used. The system is supported by 12 powerful 750 horsepower thrusters, as well as by two main shafts each with six 750 horsepower motors. In addition, a 400-ton heave compensator, which keeps the drillstring stable relative to the seafloor, has been installed. These are only a few of the numerous modifications made to the original rig.

The science laboratories contain a complete array of seagoing research equipment that will allow shipboard scientists to carry out a wide range of physical and chemical analyses on the core samples recovered by drilling. There are laboratories to study sedimentology, physical properties, palaeomagnetics, chemistry, gas analysis, palaeontology, petrology, thin section, X-ray diffraction and fluorescence microscopy, scanning electron microscopy, and downhole measurements. In addition, facilities for photography, electronics, and refrigerated core storage are available. While the ship is in transit between drill sites, digital single-channel seismics are recorded and processed in the on-board geophysics laboratory. There is also a research-oriented computer system which is designed to perform as many routine clerical and arithmetical tasks as possible. A pair of VAX computers serve as a central processor and data library for 50 microcomputers distributed throughout the laboratories.

During the inaugural cruise, three holes were drilled at site 625, near De Soto Canyon, west Florida Shelf (see figure). The scientific objectives were to document the sedimentological, palaeontological, geochemical, geotechnical and geomagnetic characteristics of the sedimentary sequences, and to correlate alternating sedimentary and erosive sequences to worldwide changes in sea level over the past several million years. The principal operational objectives were to test the rotary advanced piston coring (APC), and extended core barrel (XCB) drilling and coring systems, and to familiarize the drillship's crew with core handling and sampling procedures.

The deepest of the three holes drilled was 625B, which penetrated to 235.2 m subbottom. This hole was continuously cored with the APC system through 197.1 m of Plio-Pleistocene section. The XCB sytem continued to termination depth in the Lower Pliocene. At the third hole (625C), an attempt was made to obtain a complete section of the uppermost Quaternary by overlapping the HPC cores taken in the previous hole between 5 and 44.5 m sub-



Location map for site 625.

bottom. A visual comparison between the overlapping piston cores indicates that the sequences in each are closely matched. On the basis of physical properties and magnetic susceptibility data, we believe that no more than about 10 cm of material remained unsampled after the double HPC coring. Since this operation was accomplished in hostile sea conditions, it clearly demonstrates the usefulness and importance of the heave compensator.

During Leg 100, the onboard scientific, drilling and operational equipment was tested under varying sea conditions and generally performed to, or even exceeded, expectations. A fairly large number of adjustments or rearrangements of equipment were required but the minor nature of these is illustrated by the fact that the ship was able to depart on 31 January, after only two days in port, for the first fully operational, internationally staffed cruise of the Ocean Drilling Program, Leg 101. □

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Split-gene evolution

Exon shuffling and intron insertion in serine protease genes

from John Rogers

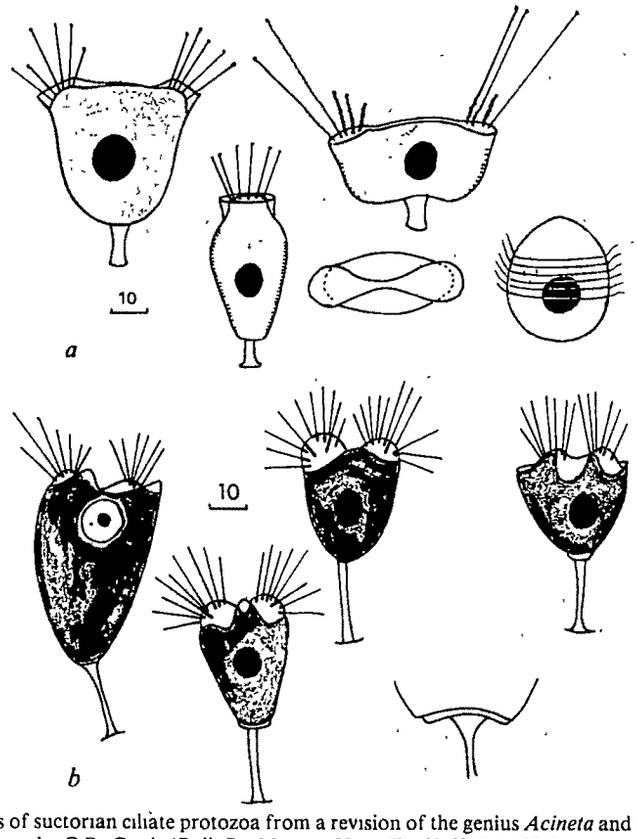
EVER since the discovery of split genes, there has been a debate about why they are split. This can be resolved into three separate problems: the origin of the introns that split the genes (separating exons from each other), the role of introns in evolution, and their present function, if any (for example, refs 1–3). Ideas on these matters can be tested against the serine proteases (proteins that have particularly clear patterns of domains[4,5]) now that more than a dozen of their genes have been sequenced. In agreement with one hypothesis, the different domains are encoded in separate exons, some of them apparently transferred from unrelated genes. But it is also clear that new introns have been inserted at various times in evolution.

Discussions of the role of introns in evolution have centred round ideas of 'exon shuffling', in which exons are supposed to be units in the evolutionary rearrangements of genes[1-3]. One should bear in mind that exon shuffling, however advantageous in the long run of evolution, cannot explain the origin of introns, nor does it amount to a present-day function for them. Nevertheless, there has been considerable interest in the idea that some exons encode protein domains, or at least coherent structural motifs, which would be good material for evolution by exon shuffling. Thus, they could be subject to duplications within a gene, as has happened in several genes such as that for fibronectin[6,7] (and the serine proteases — see below). In addition, exons could be transferred between unrelated genes to create new proteins out of old domains. Attempts to validate this more interesting possibility have not been successful. For example, the nucleotide-binding fold of metabolic enzymes is not encoded separately[2,8], and the amino-terminal signal peptide of secreted proteins, which is often more or less contained in a single exon, is too variable in sequence for its ancestry to be reliably traced. But good evidence of exon shuffling is now available from the exon/intron organization of the serine protease genes.

The serine protease superfamily[4,5] includes digestive enzymes (such as trypsin), enzymes of the blood-clotting and complement cascades and various other polypeptide-processing enzymes such as the kallikreins. (To escape from the semantic jungle of 'pre', 'pro' and 'gen' terms, I use the names of the active forms of these molecules.) New serine proteases have been arising by gene duplications for most of biological history. Those just mentioned probably diverged more than 500 million years ago, and there are even more divergent homologues in bacteria[5,9]. The protease domain itself is activated by cleavage from a larger precursor. The non-enzymatic portion often includes various structurally distinct domains with particular physiological functions.

Exon shuffling is best illustrated in the 5′ halves of the dozen sequenced genes, as shown in the figure. In each case, the coding region outside the protease domain is split by at least one intron in phase I of the reading frame (see figure) and the various extra domains within the region are encoded by separate exons or groups of exons, also separated by introns in phase I. It is presumably this coincidence of phasing that has permitted the assembly of diverse exons and the tandem duplications of some of them. An example is the kringle domain (so named because of its topological resemblance to a traditional type of Scandinavian cake). The region of the gene that encodes the kringle, flanked by phase I introns, has been duplicated independently in the genes for thrombin[10], tissue plasminogen activator[11] and plasmin[12] (not shown). This has happened even though the domain has different internal splicing patterns in these three genes[10-12] (intron insertion?), and even though the donor splice site following the kringle seems to have been replaced by a splice site that is nine codons further downstream in the gene for uPA, another serine protease[13] (intron sliding).

The tPA gene contains another two exons which, as reported by Ny *et al.*[11], provide the first example of exon transfer between otherwise unrelated genes. The third coding exon, unique to tPA, encodes a disulphide-linked 'finger' that is homologous to the tandemly repeated 'fingers' of the fibronectin molecule[14]. In fibronectin, each 'finger' is encoded in a separate exon[6], and although their boundaries have been mapped only approximately, the one example sequenced[6] does have a potential splice site in a position precisely equivalent to that in tPA. Thus, the tPA exon seems to have come from a fibronectin gene. The fourth coding exon of tPA encodes a region of homology to epidermal growth factor (EGF)[11,14]; a homologous exon is duplicated in the gene for clotting factor IX[15]. This exon may have come from a gene like the EGF gene, which contains several EGF-like segments in separate exons (G. Bell, personal communication); but any firm conclusion must await the sequencing of the EGF gene. Even further back, the fibronectin-related and EGF-related exons of all these genes may have had a common origin, as all three exons encode sequences approximating to Cys-



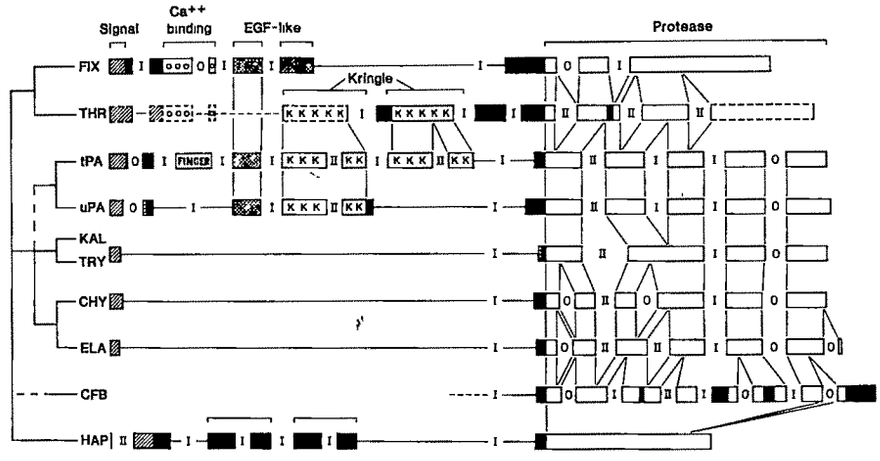
Two species of suctorian ciliate protozoa from a revision of the genius *Acineta* and its morphological relatives by C.R. Curds (*Bull. Br. Mus nat Hist. (Zool.)* **48**, 75; 1985). *a Acineta gammari*, lateral, end and top views and ciliated larva (far right); *b Acineta dentata*. Bars are 10 μm.

X-Cys-X-X-$^{Gly}_{Asn}$-X-X-Gly-X-X-Cys near their 3′ ends[14].

The domain that encodes the protease activity itself sheds light on the origin of introns. As in several other superfamilies, the positions of introns are different in different genes, and the question is whether this results from insertions or excisions. Clearly introns can be excised, at least in mammals[1,16,17]; that they can also be inserted, at least in invertebrates, is suggested by the apparently random positioning of introns in invertebrate gene families such as actin[16] and myosin[18], but this idea has never been proved. The different serine proteases, which are also pre-vertebrate in origin, show an unambiguous history of intron insertions (see figure).

This is clearest within the trypsin family (middle five lines in the figure), the family tree of which, as deduced from protein sequences, is shown at the left. These genes all have three introns in common. As one follows the branches of the family tree, new unique introns appear in the different genes — one extra in tPA and uPA, two extra in chymotrypsin and three extra in elastase. It seems that the shared introns were also inserted, as the introns in the trypsin family are entirely different from those in thrombin, and almost all are different from those in complement factor B. Such unrelated patterns could hardly have arisen by random deletions of introns. Therefore, most or all of the introns in the protease domain have been inserted. (The only apparent exceptions are the two introns of the factor IX protease domain, which match introns in elastase and factor B, respectively. But according to the amino-acid homologies, the first of these is in a segment that may have come from a chymotrypsin- or elastase-like gene by recombination, while the second is in a region of weak and uncertain relationships.)

Given that these introns have been inserted, do their positions show us anything about how or why this happened? Some regularities can be discerned. For one thing, the figure suggests that new insertions tend to occur near the middle of pre-existing exons. Thus a gene evolves towards a uniformity of exon size. This has been noted independently for the elastase gene[19] and for genes in general[8,20]. Another regularity[3,21] is that many of the introns map to surface loops, and particularly to variable-length loops, in the protein structure. Craik *et al.* [3,21,22] suggested ways in which this length variation could be caused by the presence of the introns, for example by substitution of alternative splice sites (intron sliding). Another possibility now worth considering is that introns generate length variation because they are inserted inaccurately. As introns do not resemble any known transposable elements, one can speculate that a new intron might be included as part of a longer insertion, or might be induced when an insertion activates a cryptic splice site that is nearby.



Structures of the coding parts of serine protease genes. The family tree as deduced from protein sequencing[5,9,26] is summarized schematically at left, the dashed branches being tentative. FIX is human clotting factor IX[15]; THR, human thrombin[10] (gene structure not reported for regions shown dashed); tPA, human tissue plasminogen activator[11]; uPA, pig urokinase[13]; KAL, mouse kallikrein; TRY, rat trypsin (gene structures are identical for two trypsin genes[22] and four kallikrein genes, including nerve growth factor subunits[27,28]); CHY, rat chymotrypsin b[29]; ELA, rat elastase[19] (two similar genes); CFB, human complement factor B[30] (the exons shown are preceded by other, non-homologous exons); HAP, human haptoglobin[23]; Exons are shown as boxes shaded according to their homologies. Sequences with no discernible homology to each other are in black. Introns are not to scale, but their phase relative to the reading frame is indicated, according to whether they follow the first (I), second (II), or third (0) nucleotide of a codon.

On the other hand, some introns are in conserved coding regions, and these include some of the most striking cases of independent insertions into the same region. Thus, five genes in the figure contain an intron that is 14–21 codons into the protease domain, but at least three of these are in different positions relative to codons for conserved amino acids. Even more remarkable, the penultimate introns in complement factor B and elastase fall in the same conserved glycine codon, but one nuceotide apart. These coincidences cannot reasonably be explained by intron sliding; this would require vanishingly improbable coincidences of mutations in order to shift an intron within a conserved coding region, or to shift the phase of an intron. The non-random positions of these introns seem to imply either sequence specificity at the insertion, or selection following it, for reasons that are still mysterious. The methodological lesson, however, is clear. Correlation of introns with protein structure[1,8] does not, *per se*, tell us whether the introns are original or recent additions.

A final puzzle is presented by the gene for haptoglobin[23], a member of the superfamily but not known to have protease activity[9]. Although this gene has plenty of introns in its 5′ half, dividing duplicated phase I exons as in the other genes, there are no introns in the 3′ half, which encodes the protease-like domain. This could represent the condition of the ancestral serine protease gene, intronless-like genes of bacteria. Alternatively, it could represent a much more recent processed gene, derived from the messenger RNA of a split serine protease gene via reverse transcription, which became attached to a different split gene. A similar history has been postulated for part of the (unrelated) clotting factor VIII gene[24]; this mechanism has also been proposed as a general way of evolving new proteins[25]. Processed genes are common only in mammals[17], so if one is incorporated either in haptoglobin or in factor VIII, the gene from which it was derived may still be present and detectable by nucleic acid hybridization. □

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Igneous petrology

Discordance in layered intrusions

from R.S.J. Sparks

LAYERED intrusions are the frozen remains of magma chambers. Their distinguishing characteristic is a vertical variation in mineral abundance, giving rise to a horizontal layering superficially resembling that seen in sedimentary rocks. The study of their geology and petrology has played a central role in the development of ideas on the origin of magmas. Intrusions such as the Skaergaard in Greenland provide almost perfect natural examples of crystallization under closed-system conditions. Other intrusions, such as Rhum in Scotland and Stillwater in Montana, record the history of long-lived open-system magma chambers. The classic method of investigating a layered intrusion is to collect samples through the stratigraphy and to document variations in the composition and abundance of minerals. Usually one vertical section has been taken to represent the entire intrusion. In a recent paper, J. R. Wilson and S. B. Larsen[1] describe the first comprehensive two-dimensional study of a layered intrusion — the Fongen-Hyllingen intrusion in Norway. The relationships they report provide important new facts which any theory on the origin of layered intrusions must seek to explain.

Detailed geological mapping and systematic petrology provide the key to understanding large layered intrusions, which typically cover areas of hundreds of square kilometres. This kind of study is thus a substantial and time-consuming enterprise. Indeed a layered intrusion often becomes associated with one particular scientist who may have devoted considerable time to its study; perhaps the most notable link is between L.R. Wager and the Skaergaard intrusion. Wilson and colleagues at Aarhus University in Denmark have devoted over fifteen years to investigating the Fongen-Hyllingen intrusion and they have now produced one of the most significant studies of a layered intrusion for many years.

The variation in mineral composition observed in these intrusions is termed cryptic layering, whereas the presence or absence of minerals defines the phase layering. On the scale of outcrops, the layering is made visible by small changes in the relative abundances of minerals, here termed modal layering. Wilson and Larsen document dramatic lateral variations in mineral composition and show that the modal layering observed in the field is strongly discordant to crytpic and phase layering.

The Fongen-Hyllingen intrusion was emplaced as a sill-like body at 12-20 km depth during the Caledonian orogeny, roughly 425 Myr ago. The intrusion and its metamorphosed envelope of basic and pelitic country rocks are now part of a nappe complex and prolonged erosion has exposed a longtitudinal cross-section 33 km in length. The northern part (the Fongen and Ruten Series) has the greatest stratigraphical thickness (6.2 km) and is complicated by folding. Primitive ultramafic rocks occur at the base of the sequence and there are many reversals in the mineral compositions, a certain sign of an open-system magma chamber. The southern part (the Hyllingen Series) consists of 3.6 km of modally layered rocks, and is documented in detail by Wilson and Larsen.

Both the floor (the western margin) and the roof (the eastern margin) are exposed in the Hyllingen area. Geological mapping reveals intimate interfingering of country rock with layered rocks in the southern region. Swarms of huge country-rock inclusions, with individual metabasic xenoliths up to 1 km long and a few hundred metres wide, are found throughout the sequence. The layering is completely undisturbed by these xenoliths and they seem to be in their original positions rather than having fallen in from the roof or walls. The igneous rocks are highly evolved ferrogabbros and ferrodiorites, with quartz-bearing syenites occurring next to the roof. Minerals include olivine, Ca-rich and Ca-poor pyroxenes, amphibole, plagioclase, Fe-Ti oxides, zircon, apatite, biotite and allanite. The very high Fe/Mg ratio of ferromagnesian minerals shows that all the rocks have crystallized from highly differentiated magmas. In any one vertical profile four stages of magma evolution can be recognized. Stage I is an unlayered gabbro. Stages II and IV record initial open-system behaviour with relatively constant mineral compositions followed by progressive differentiation. Stage III in the middle of the sequence records a major reversal with minerals gradually becoming less fractionated towards the top of the section.

The most important results of the study are the recognition of lateral variations in mineral compositions, which are almost as great as those recorded in vertical profiles and provide evidence by changes of mineral abundances on millimetre or centimetre scales. These layers strike north-south, parallel to the floor and roof with no deviation at the southern contact or locally around enormous xenoliths. However, mineral compositions (defining cryptic layering) change dramatically along the strike with minerals becoming more evolved towards the southern margin. For example, at the boundary between stages III and IV the olivine and plagioclase vary from Fo_{75}: An_{63} to Fo_{13}: An_{42} over a 10 km strike length. The stage boundaries are parallel to field layering, showing that changes in the differentiation trend occurred simultaneously at the same stratigraphical level. With eleven separate vertical profiles Wilson and Larsen have been able to contour lines of constant mineral composition. The contours are discordant to the modal layering by angles which are typically 20° but are as much as 90° at the southern margin. As described in their preliminary account[2], boundaries recording the appearance of new cumulus minerals (zircon and apatite) are also highly discordant to the rhythmical layering.

Conventionally, the visible modal layering is thought to represent the trace of the ancient magma-chamber floor and therefore each layer is a time-plane. If this is so then the implication of the discordance is that the magma chamber must have been compositionally stratified throughout its evolution. Wilson and Larsen argue that the dramatic lateral gradients and the lack of disturbance of layering by xenoliths eliminate crystal settling. They believe that the crystals grew *in situ* along the floor. They propose that the floor was gently tilted so that increasingly differentiated magma in a zoned chamber was in contact with the floor in the (southerly) upslope direction. Thus, the minerals systematically change composition and new phases appear up the slope. Addition of magma from the north complicated the picture by elevating the zoned magma column and causing gradual reversals as lower and more primitive magma migrates up the slope. A more elaborate model, based on the work of Irvine, Keith and Todd[3], envisages lateral accretion of layers, in which the field layering, mineral-composition layering and the accretion front (the time-plane) are all discordant.

Recent petrological literature contains several new ideas on the processes that form layered intrusions. Several workers have argued that double-diffusive convection plays an important role[3-6]. There is also field and theoretical evidence that compaction of cumulate piles and post-cumulus migration of melt[7,8] can lead to changes in mineral compositions, which further complicates interpretations of their petrogenesis. Irrespective of the merits of recent thinking and speculation, the discoveries of major lateral variations and discordances in layered intrusions must be central facts on which different hypotheses will succeed or founder. Wilson and Larsen's work should herald a new phase of research in layered intrusions, in which documentation of lateral changes will be as important as vertical variations. □

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Has inductive probability been proved impossible?

SIR—The interesting letter by Popper and Miller[1] and the subsequent correspondence[2-4] leaves in some doubt the question whether a proof of the impossibility of inductive probability been given. As this would be a remarkable achievement, it is no criticism of these authors that we raise this question again. In our view the answer is a clear "No". We shall follow your other correspondents in not burdening the discussion with references to extensive earlier literature.

The key elements in the Popper-Miller (PM) argument are: first the evidence e, when found, counter-supports the implication $h \leftarrow e$ of a hypothesis h by the evidence; and second, this is due to the negative nature of that part of the support for $h \leftarrow e$ arising from e which is not purely deductive. From this type of consideration, PM infer the impossibility of inductive probability. In both the original PM and the following discussion, the part played by the negation $\bar{e}$ was not considered explicitly. Yet it is helpful to do so in showing what is going on in these arguments, as will now be explained. Two basic results, to be formulated as "theorems", are useful preludes to this explanation.

Theorem A

$$\begin{aligned} p(h \leftarrow e|e) &\leq p(h \leftarrow e|e) + \underline{p(\bar{h}|e)p(\bar{e})} \\ &= p(h \leftarrow e) \leq p(h \leftarrow e|\bar{e}) = 1 \\ p(h \vee e|\bar{e}) &\leq p(h \vee e|\bar{e}) + \underline{p(\bar{h}|\bar{e})p(e)} \\ &= p(h \vee e) \leq p(h \vee e|e) = 1 \end{aligned} \quad (1)$$

Theorem B The following have the same sign:

$$\begin{aligned} p(h|e) - p(h) &= \underline{p(h|e)p(\bar{e})} - p(h|\bar{e})p(\bar{e}) \\ &= p(\bar{h}|\bar{e})p(\bar{e}) - \underline{p(\bar{h}|e)p(\bar{e})} \\ &= p(\bar{h}) - p(\bar{h}|e) \end{aligned} \quad (2)$$

and

$$\begin{aligned} p(\bar{h}|\bar{e}) - p(\bar{h}) &= \underline{p(\bar{h}|\bar{e})p(e)} - p(\bar{h}|e)p(e) \\ &= p(h|e)p(e) - \underline{p(h|\bar{e})p(e)} \\ &= p(h) - p(h|\bar{e}). \end{aligned} \quad (3)$$

Theorem A shows the origin of theorem 1 of PM that

$$p(h \leftarrow e) > p(h \leftarrow e|e) \qquad (\text{if } p(e) \neq 1) \quad (4)$$

as follows. The underlined terms are positive, so that (4) could be incorporated in (1). Note that $p(h \leftarrow e) = p(h \vee \bar{e})$ allows for the possibility of $\bar{e}$, which possibility is ruled out by the assumption that e has occurred. This makes the inequality (4) intuitive as a logical necessity (which is incidentally independent of any inferential relation between e and h).

Since $h \leftarrow e \equiv h \vee \bar{e}$, $\bar{e}$ must "support" $h \leftarrow e$ maximally, or $p(h \leftarrow e|\bar{e}) = 1$. It is intuitively to be expected, therefore, that e "counter supports" $h \leftarrow e$. These statements interpret Theorem A (an extension of theorems 1 and 2 of PM).

Theorem B shows relations between four "support" functions whose definition

$$s(h, e) = p(h/e) - p(h) \quad (5)$$

was introduced into the correspondence by Jeffrey[3]. They are shown as differences in different ways. Adopting the nomenclature of your correspondents (which is possibly misleading), we call the underlined terms "non-deductive". The introduction of negations is again helpful here, for they show, using (5), that the "non-deductive" part in (2) appears with a positive sign (support) in $s(h, e)$, but with a negative sign (counter support) in $s(\bar{h}, e)$. Similarly for (3). Thus the remark by PM that all probabilistic support is purely deductive requires some different definition of these terms or some restriction on their interpretation.

Theorem B also tells us something very reasonable about the function (5):

$$\begin{aligned} &s(h, e) \geq 0 \text{ implies } s(h, \bar{e}) \leq 0, \\ &s(\bar{h}, e) \leq 0,\ s(\bar{h}, \bar{e}) \geq 0 \end{aligned} \quad (6)$$

while $s(h, e) \leq 0$ implies $s(h, \bar{e}) \geq 0$,

$$s(\bar{h}, e) \geq 0,\ s(\bar{h}, \bar{e}) \leq 0 \quad (7)$$

In words, if e supports h then it counter-supports $\bar{h}$, and also $\bar{e}$ counter-supports h but supports $\bar{h}$. The converse is given by (7).

The above considerations explain, we hope, some of the results obtained by PM, by your correspondents and by us. They do not really go beyond basic facts such as: $h \vee e$ follows deductively from e, $h \vee \bar{e}$ follows deductively from $\bar{e}$, together with probability algebra. In that sense, they are obtained by strict deduction and have nothing to do with induction. It is for these reasons that we have to give the answer "No" to the question in the title.

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POPPER AND MILLER REPLY—In 1878, Peirce drew a sharp distinction between "*explicative, analytic,* or *deductive*" and "*amplifiative, synthetic,* or (loosely speaking) *inductive*" reasoning. He characterized the latter as reasoning in which "the facts summed up in the conclusion are not among those stated in the premisses". This was the background to our brief letters (refs 1, 2 in Wise and Landsberg's letter). We identified the amplifiative part, relative to any evidence e, of any hypothesis h, with the conditional proposition $h \leftarrow e$; and we proved quite generally that if $p(h, e) \neq 1 \neq p(e)$, then

$$s(h \leftarrow e, e) = -ct(h, e)ct(e) < 0 \quad (1)$$

where

$$s(x, y) = p(x, y) - p(x)$$

and

$$ct(x, y) = 1 - p(x, y).$$

Accordingly, the ("amplifiative") part of the hypothesis h that goes beyond the evidence e is always counter-supported by the evidence. This was our thesis. (The other part, which we wrote $h \vee e$, is of course a deductive consequence of e, and is deductively supported by e.) Both the measures of support and of content are additive:

$$s(h, z) = s(h \vee e, z) + s(h \leftarrow e, z) \quad (2)$$

$$ct(h, z) = ct(h \vee e, z) + ct(h \leftarrow e, z) \quad (3)$$

(Equations (2) and (3) are valid for all h, e and z, and thus for $z = e$.) Wise & Landsberg note that there are other ways of writing $s(h, e)$ in the form $\ulcorner \pm s(x, e) + \pm s(y, e) \urcorner$; for example

$$s(h, e) = -s(\bar{h} \vee e, e) + -s(\bar{h} \leftarrow e, e) \quad (4)$$

This equation (4) is a simplification of their formula (2). It can be obtained by substituting $\bar{h}$ for h in our (2) above, and multiplying by -1. Obviously, neither $\bar{h} \vee e$ nor $\bar{h} \leftarrow e$ is part of the content of h. Thus we fail to see how our (4)—that is to say, their (2)—can possibly challenge our thesis, or have any bearing whatsoever on it.

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Gastric cancer and salivary nitrate and nitrite

SIR — Forman *et al.*[1] reported lower salivary and nitrite levels in healthy subjects from areas of Great Britain with high gastric cancer incidences, compared with subjects from areas of that country with low incidences of this cancer. These unexpected findings were used to argue against the theory[2,3] that the intra-gastric formation of carcinogenic *N*-nitroso compounds (NOC) from nitrite is involved in the aetiology of gastric cancer. Although this theory remains controversial, I have three objections to the suggestion that the results presented by Forman *et al.* disprove the NOC theory.

(1) A lower nitrate intake was reported[1] in the diet of the high-risk subjects, which accounted for their lower levels of salivary nitrate and nitrite. Since most dietary nitrate (76-89% in this study) arises from vegetables, which supply much of our dietary vitamin C, the high-risk subjects

probably ingested less vitamin C than did those from the low-risk areas. Hence the high-risk subjects could have produced more intra-gastric NOC, despite their lower salivary nitrite levels. The relevant property of each vegetable may be the ratio of vitamin C to nitrate content, and not nitrate content alone[4]. The intake of fresh fruits and vegetables is negatively correlated with gastric cancer incidence[2], suggesting that the critical components here are vitamin C and other nitrosation inhibitors.

(2) The same argument applies to socio-economic class, since the "lower" classes have relatively high incidences of gastric cancer[2] and showed lower salivary nitrate and nitrite levels in the reported study[1], but would be expected to consume less vitamin C in fresh fruits and vegetables.

(3) Forman *et al.* reported lower salivary nitrate and nitrite levels in cigarette smokers than in nonsmokers, as did Ladd *et al.*[5]. Both groups attributed this finding to the high level of salivary thiocyanate in smokers. Smokers have raised incidences of various types of cancer, possibly including gastric cancer[1,2]. Some of these cancers are likely to be induced by NOC in tobacco smoke or produced *in vivo* from smoke constituents[6]. These findings in smokers do not, as suggested[1], discredit the NOC theory because thiocyanate is a powerful catalyst of nitrosation[7] and we might therefore expect smokers to show an increased NOC formation, despite their lower levels of salivary nitrite.

In 1981 Ohshima and Bartsch[8] developed a method of measuring the potential for *in vivo* (probably intragastric) nitrosation. They fed nitrate and the amino acid L-proline to volunteers, and detected microgram amounts of the noncarcinogenic nitrosamine, *N*-nitrosoproline, in the 24-hour urines. Using this test, Ladd *et al.*[5] reported that smokers given nitrate and proline indeed had a greater urinary excretion of nitrosoproline than did non-smokers, despite the smokers' lower salivary nitrate and nitrite levels. Similar nitrosoproline data were reported by Hoffmann and Brunnemann[9]. These findings prove that salivary nitrite levels cannot be used to predict the potential for intragastric NOC formation.

In conclusion, it is interesting that salivary nitrate and nitrite levels follow the opposite trend to that expected from the NOC theory. However, *in vivo* nitrosoproline formation[5,8,9] offers a more valid test for potential *in vivo* NOC formation and the exposure to nitrosation inhibitors, such as vitamin C, and catalysts, such as thiocyanate, must be taken into account. (The role of vitamin C was addressed in ref. 1, but with a different emphasis.)

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FORMAN *ET AL.* REPLY — In our study, we did not set out to disprove the theory "that the intragastric formulation of carcinogenic *N*-nitroso compounds (NOC) from nitrite is involved in the aetiology of gastric cancer", but rather to test the hypothesis "that an increase in nitrate exposure may represent an increased cancer risk". We agree with Mirvish that the negative relationship we observed between salivary nitrates and nitrites and gastric cancer could be due to an association between the consumption of vitamin C (or other protective factors in vegetables) and the consumption of nitrates and suggested this in our paper. We agree, too, that the increased secretion of thiocyanates by smokers might account for the increased risk of gastric cancer in cigarette smokers that has been reported in several series. We clearly stated in the paper that our results do no more than "weigh against the idea that environmental nitrates and nitrites play a major role in determining the risk of gastric cancer in Britain" and we emphasised that this should not "be taken to imply that nitrate-related *N*-nitroso compound carcinogenesis has no role in the development of gastric tumours". Our conclusion is supported by the recently published results of Beresford showing that in Britain the risk of gastric cancer is negatively correlated with the nitrate content of the water supply in over 200 urban areas (*Int. J. Epidemiol.* **14**, 57-63; 1985) and by our observations on men producing nitrate fertilizers, who are certainly exposed to unusually large amounts of nitrates, and whose mortality from gastric cancer is almost identical with that experienced by other men in the same part of Britain (our work, in preparation).

It is clear that whatever the role of NOC in human carcinogenesis may be, direct exposure to nitrate *per se*, does not appear to be a critical risk factor in the United Kingdom. We agree that *in vivo* nitrosoproline formation has the potential for a useful test of *in vivo* NOC formation and are ourselves conducting more research in this area.

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Spreading rate of an Arctic ice shelf

SIR — In connection with problems of creep and *à propos* the work of Doake and Wolff[1] on the flow law for polar ice sheets, Weertman[2] calls for measurements of the spreading rate of the Ward Hunt Ice Shelf, Ellesmere Island, Arctic Canada. This was an oversight on Weertman's part because, when I was in charge of field operations in the area for the Canadian Defence Research Board, I arranged for such measurements to be made under the supervision of Dr G. Konecny, then of the Univeristy of New Brunswick.

The work was performed with a geodimeter during the field seasons 1964, 1965 and 1968, and the data were analysed in detail by Dorrer[3], who derived a value of 1.17×10^{-4} yr^{-1} for the spreading rate where the mean thickness of the ice shelf was 38 m. Weertman now agrees that both Doake and Wolff's ice shelf data and Dorrer's ice shelf data appear to go against a linear creep law.

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Powers of ten *correctly* expressed

SIR — Claude Liébecq (*Nature* **314**, 586; 1985) thinks that an ordinate with figures ranging from 0 to 10 and labelled "Radioactivity (c.p.m. $\times 10^{-3}$)" is labelled incorrectly if, for example, one of the values included is 5,000 c.p.m. It is indeed labelled incorrectly if one thinks of the label as an equation in the form "Radioactivity = 5,000 × c.p.m.". But if, with more logic, one thinks of the words written vertically simply as a label describing what the figures are, then "c.p.m. $\times 10^{-3}$" is correct.

In other words, instead of substituting "5" for the "c.p.m." in the expression "c.p.m. $\times 10^{-3}$" (giving the incorrect "Radioactivity = 5×10^{-3}"), one may understand the ordinate to mean "c.p.m. $\times 10^{-3} = 5$", or "c.p.m. $= 5 \times 10^{3}$".

Clearly, the meaning of the ordinate can be read in different ways, and there ought to be agreement on how to read it. I suggest that the words on the ordinate be viewed, as I believe they already are by a majority of my colleagues, simply as a label describing the figures, and not as part of an equation.

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Prospects for an ageing population

from Jacob A. Brody

The ageing populations of industrialized societies are more likely to be encumbered by increased morbidity than some optimistic arguments would suggest.

OLIVER Wendall Holms, American physician-poet, likened human ageing to the obsolescence of a "one-hoss shay".

Two metaphors of ageing:

Have you heard of the wonderful one-hoss shay,
That was built in such a logical way
It ran a hundred years to a day,
And then, of a sudden, it.

You see, of course, if you're not a dunce,
How it went to pieces all at once,
All at once, and nothing first,
Just as bubbles do when they burst

End of the wonderful one-hoss shay.
Logic is logic. That's all I say

In stark contrast are W.B. Yeats' lines in the poem "Sailing to Byzantium":

Consume my heart away; sick with desire
And fastened to a dying animal
It knows not what it is

HUMAN lifespan, or survival potential[1], cannot be less than 110 to 115 years; some humans have survived that long. It is even possible that lifespan will be found to exceed this estimate, perhaps by a considerable amount. Life expectancy, on the other hand, the average of the years of life from birth or other stated age, is a projection based on accumulated data[1]. In the United States, life expectancy is now 71 years for males and 78.3 years for females, an average of about 74 years for both sexes[2]. This discrepancy between life expectancy and lifespan, about 40 years, is a matter of legitimate concern.

What are the prospects for the years ahead? In an influential article published in 1980, J.F. Fries[3] postulated that the mean age of death, or the average potential life expectancy, would eventually become 85 years, with 95 per cent of deaths occurring between ages 77 and 93. This consideration is not easily reconciled with current life expectancy in the United States, which at age 85 is 6.3 years and at age 91 remains 4 years[4]. Most other authors[5-7] and the Social Security Administration[8] have therefore concluded that average life expectancy will increase to more than 85 years within a century. Yet, Fries describes his conclusions as: "a set of predictions that contradict the conventional anticipation of an ever older, ever more feeble, and ever more expensive-to-care-for populace These predictions suggest that the number of very old persons will not increase, that the average period of diminished physical vigour will decrease, that chronic disease will occupy a smaller proportion of the typical lifespan, and that the need for medical care in later life will decrease." This conclusion offers a comfortable solution as a daunting problem to health policymakers and fiscal planners, but may engender a dangerous optimism. Here I present a less simplified account of the balance between mortality and morbidity in this century and the next.

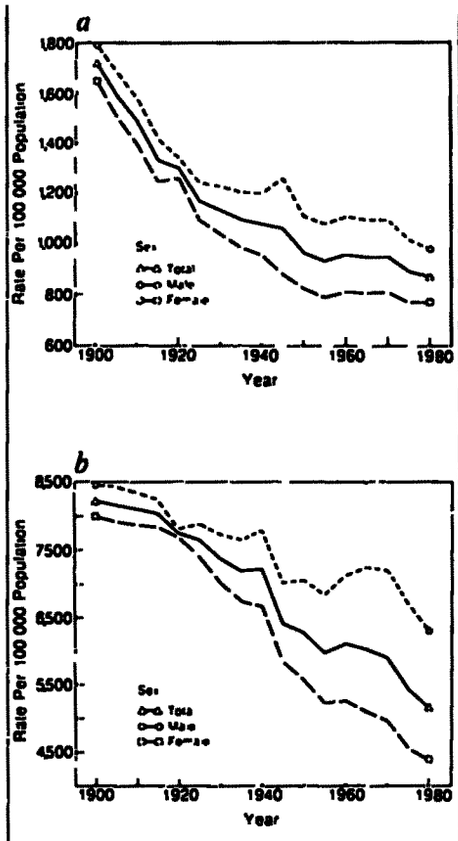


Fig. 1 *a*, Mortality rate for years 1900-79, by sex (all ages) *b*, Mortality rates for years 1900-79, by sex (ages 65 and over). Source National Center for Health Statistics, various volumes of *Vital Statistics of the United States*.

Mortality reduction

In 1900, only a quarter of the US population survived to the age of 65. Now almost 70 per cent achieve this age, and the proportion of those aged 80 and over is 30 per cent and increasing[9]. Within a very few years, about half the population will live more than 80 years. But the pattern of reduction in mortality has not been smooth over time, or easily explicable. Figure 1*a* shows that the mortality rate per 100,000 for all ages fell from 1,720 to 868 between 1900 and 1980. Half of the remarkable decrease observed this century had been complete by about 1920. Neither physicians nor social planners were prime movers in the period during which the greatest mortality reduction occurred — improved living environments and better sanitation were the major factors in the decline.

Among those aged 65 and over, mortality had declined by barely 5 per cent by 1920 (Fig. 1*b*), an indication that the older population was resistant to whatever factors caused the conservation of life in the general population. By about 1945, however, half of the decline in mortality among those 65 and over in this century had been completed. The era between 1920 and 1945 was an historic one in the United States — including the Second World War, the Depression, the beginning of Social Security and Prohibition. While any number of influences may have caused the decline of mortality by 1945, specific medical or social interventions are unlikely to have been major contributors.

During the period from 1945 to about 1968, when it may be thought that medical and social factors should have been reducing mortality rates for the entire population, the overall rate was generally unchanged, with an actual increase in mortality among those aged 65 and over. This seems to have reassured policymakers and planners and, in a sense, lulled them into the false assumption that the maximum potential for human life expectancy had been achieved[10]. Yet since about 1968, mortality rates have persistently declined with only minor perturbations (Fig. 1). Declining mortality among those 65 and over has been remarkable, causing a disproportionate accumulation of the oldest and most vulnerable sector of the population.

Can this recent trend be ascribed to medical and social interventions? Undoubtedly they have had some impact, particularly by the application of medical means for the reduction of blood pressure and, in the United States, by the improvement of medical care through Medicare and other health-insurance programmes. There has also been reduction in smoking and some improvements in diet and exercise; while the scientific demonstration of the benefits of diet and exercise is fragile, presumably some good comes from an intelligent and moderate approach to eating and activity. But careful analysis of the data about the powerful determinants of human health and longevity, such as the avoidance of smoking and the use of antihypertensive agents, reveals that neither of these influences can explain either the extent or the timing of the decline of mortality in the elderly since 1968[11]. The major impact of blood-pressure control and

reduced smoking has yet to be fully realized, so there are likely to be even greater gains in life expectancy in future years.

Yet, actuarial arguments such as those used by Fries, as well as the projections of the Social Security Administration, serve as the cornerstone of major policy decisions. My own discomfort with this approach stems from my conviction that we do not have satisfactory answers to the questions of what has brought about this dramatic mortality decline of the last few decades, and of what still sustains it.

In the past 150 years, there have been major shifts in the causes of mortality and morbidity which have profoundly influenced attitudes towards public health and policy. Important diseases have disappeared or diminished, but for the most part we do not know why. Many of the greatest medical triumphs are not easily attributable to our own constructive action[12].

Tuberculosis was the chief cause of death in the United States and northern Europe at the turn of the century, but even by then there had been a very rapid decline in mortality from tuberculosis since 1856 (Fig. 2). The tuberculosis bacillus was first identified in 1882, and specific therapies for tuberculosis were introduced only in the late 1940s These developments were, therefore, of small importance in the decline of the disease. Similarly, for reasons not understood, deaths from scarlet fever declined precipitously from 1870 to 1900, although the sulpha-drugs and penicillin were not in common use until the Second World War. Moreover, surprising shifts in causes of death are not confined to diseases of infectious aetiology The rate of mortality from accidents among the elderly has declined rapidly since 1950[13] as have deaths from stroke and stomach cancer[14], while conversion hysteria, one of Freud's most frequent diagnoses, seems to have vanished.

One phenomenon that has, however, had a great impact on health trends and thus on current attitudes is the remarkable decline since the late 1960s in mortality from ischaemic heart disease[15] which, in my opinion, created a false sense of optimism about the extent to which we are in control of our survival[11,16]. Heart attacks appear to be a twentieth-century medical chimaera. There is no real mention of the classical heart attack or myocardial infarction before 1900: the generally accepted first reference is Herrick's in 1912[17]. It is possible but unlikely that the medical profession missed the diagnosis of myocardial infarction or systematically misclassified it; the extraordinarily dramatic nature of a heart attack, with its crushing chest pain and pain radiating down the left arm make it a notable event Surely our authors and playwrights would have described an event so frequent and dramatic as we now witness, but there are no good descriptions of heart attacks in a literature rich with accounts of gout, epilepsy, apoplexy and

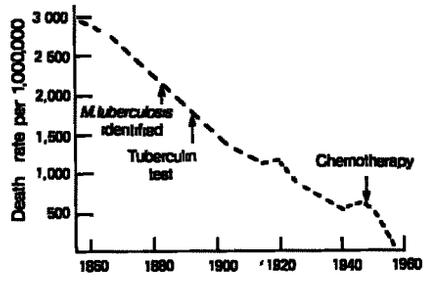


Fig. 2 Respiratory tuberculosis in the total population Source. Mean death rates from various diseases, England and Wales, 1860-1960. From ref 45

tuberculosis. It is probable that acute myocardial infarction was projected into prominence by circumstances arising early in this century, probably associated with the introduction of cigarette smoking and different types and amounts of fat in the diet. Mortality from heart attacks has declined rapidly in the United States since 1968, but this decline has not occurred throughout the world. In Sweden[18] and Japan[19] there has been no comparable decline, although life expectancy is greater than in the United States.

Mortality from heart attacks increased during a period when general mortality was declining. Cause-specific mortality shifted early this century from infectious diseases to chronic diseases and now half of all deaths are caused by heart disease, a large fraction of which result from acute myocardial infarction. After the Second World War, the decline in mortality ended, and was followed by a plateau and then a rise (Fig. 1). This may have resulted from an abatement of the forces that had reduced mortality early in the century, and an increase of mortality from heart attacks. Acute myocardial infarction, however, appears to have been a chance phenomenon which, while causing serious damage, depended for its maintenance and increase on tenuous and complex factors, certainly including smoking and diet The rapid decline in deaths from acute myocardial infarction began in the late 1960s and it is likely that United States age-specific mortality rates since 1900 would have shown a smooth steady decline over the entire years had it not been for the shifting pattern of mortality from this cause.

So this underlying momentum for life extension seems not to be determined by a decline in cause-specific mortality, but rather to be part of a pattern in which the potential lifespan is ever more closely attained But concomitantly with increased longevity, there has also been a steady increase in average height of successive generations for more than a century Gains in growth probably result from the reduction in infectious diseases and from better nutrition in the formative years. There is evidence, in some populations, that growth may now have reached a plateau[20].

It is hard not to think that there must be a relationhsip between these phenomena. Each generation is more closely approaching the human growth potential and this process is apparently reflected in successively-improved trajectories for survival. Therefore, studying the past history of disease and mortality data can yield only fragile guesses about human survival.

The problem is further compounded because 17 per cent of current deaths in the United States occur in people born before 1900, before the era of complete and accurate vital statistics, so we do not have complete data from even a single cohort upon which to base projections. But it is a fact that older people are living longer[9]. Moreover, no other species has ever had our potential for controlling our environment, so that the limits of life expectancy or maximum attainable longevity are not known. There are no empirical data of great value to instruct us on the limits of these unprecedented happenings.

Disability

Life expectancy is increasing at a remarkable rate among the elderly, but are their lives more satisfactory? Scientific documentation of quality of life and life satisfaction is elusive. Present data indicate a modest gain in healthy years lived and a far greater increase in years of compromised physical, mental and social function[2,21-24]. The net increase in survival translates into more years of ill health.

Logically, greater longevity should be associated with increased years of good health, but critical studies are insufficient. Limited evidence is accumulating in current studies of successive aged cohorts. But there are no data suggesting an age-specific decline in deafness, blindness, hip fracture, osteoarthritis and Alzheimer's disease and related disorders[9].

We lack methods to measure the prevalence of good or compromised health. A promising measurement of health in terms other than of disease and death[27] is "active life expectancy", the endpoint being the loss of independence in daily life. This study documented a substantial decline in active life expectancy between age 65 and 84. Men were shown to die shortly after losing independence, while women lived on with diminishing functional abilities suggesting that, in the future, as male patterns more closely resemble those of females, the number of person-years lived after active life expectancy will further increase. Currently, limitations of activity due to chronic health conditions, in the age groups 65 to 74 years, 75 to 84 years, and 85 years and older are 41 per cent, 51 per cent, and 60 per cent, respectively[28] Annual rates of admission to short-stay hospitals per 1,000 population for these age groups are 299, 451, and 507, and the rates of admission to nursing homes per 1,000, for the same age groups, are 15, 68, and 216 If populations were appropriately studied over time, a pattern of increased active life expectancy might emerge.

Time/trend analysis of health status,

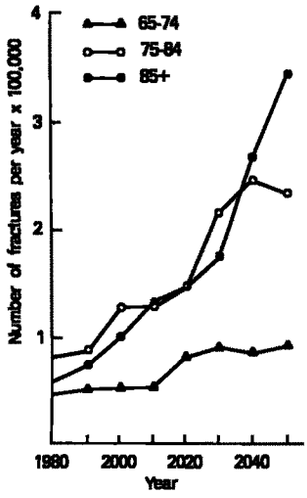


Fig. 3 Projected number of hip fractures annually in the United States by age 1980-2050 Source. NCHS and US Bureau of Census projections.

however, is unreliable. Colvez and Blanchet[29] found a disturbing rise in morbidity and disability since 1957, as did a recent Veterans Administration study[30]. Wilson[31], of the National Center for Health Statistics, commenting on the Colvez and Blanchet report, questions the ability "of health statistics to reflect what might really be happening to health status." He offers plausible explanations for the observed increases in morbidity and disability including artificially high results caused by increased sensitivity of the screening instrument, to a change in attitude towards self-reporting as a result of more liberal retirement benefits for health reasons and improved survival of the chronically ill (implying that the increase of morbidity is real). All this merely emphasizes that there are not sufficient data to reach conclusions about whether people are indeed enjoying better health. Increased public health awareness further confounds interpretation of trend analysis. For instance, as education about hypertension increased in the United States, more people had blood pressure determinations which are a key item in a health-status questionnaire, so that the rate of reported hypertension has increased[29,30].

Longitudinal studies, in which subjects are regularly examined over time, avoid some of the obvious weaknesses of the sequential and cross-sectional surveys but are few in number and, because of the cost and logistical problems, follow only relatively small cohorts. Yet intelligent health policy rests on such research data. Nobody seriously doubts that the disease burden increases with age. It is essential, however, to monitor morbidity, disability and even the quality of life to determine the trends of health as life expectancy increases

Population

The dangers of misinterpreting data are matched by those of presuming knowledge that does not exist. The combination of increasing life expectancy and maturation of the "baby-boom" population will produce extraordinary increases in the numbers of older people in the United States. Within about 40 years (by 2025), the population 65 and over will rise from 11.3 to 20 per cent of the total and will comprise about 60 million individuals. By 2050, the population 85 and over will have risen from about 2 million to more than 16 million[3]. In 10 years, more than half of the people now aged 65 and over will be aged 75 and over. When we now lament that our medical and social systems cannot at present provide for those 65 and over, we forget that it is after age 75 that health problems increase sharply[32]. A concerned government should focus on the realities of the future, not be seduced by fragile notions that the numbers of very old people will not increase, that impairment of physical vigour will decrease and that the result will be the need for less medical care in later life[3].

Three specific examples illustrate physical, mental, and social realities that will occur in the United States unless miraculous advances in science and health management develop in the very near future. In 1980 there were approximately 200,000 people with hip fractures whose median age was 79. Census projections indicate that by the year 2000, before the ageing of the "baby-boom" population, the number will have risen to 330,600 and, by 2050, to more than 650,000, including 340,000 over age 85 (Fig. 3).

In 1980 there were more than 2,000,000 people with Alzheimer's disease and related disorders whose mean age was about 80 years. By the year 2000 the number will rise to 3,800,000 and by 2050 there will be more than 8,500,000, of whom almost 5,000,000 will be age 85 and over (Fig. 4).

There are now about 1.1 million people age 65 and over in nursing homes whose median age is about 80. By the year 2000, the number will rise to 2,200,000 and in 2050 a conservative estimate projects a total of 5,400,000.

Prospects

Unlike the "one-hoss shay", the human body outlives its joints, its cognitive and memory capacities, its vision, its hearing and the many other substrates of disability While concern is limitless, there is at present no accepted scientific prospect that the net increase in healthy years will exceed the increase of years of dysfunction We are, however, far short of reaching the potential of better health and quality of life in later years. Two vehicles offer promise: health promotion through the improvement of personal health practices throughout life, and major research efforts to understand and postpone the ageing processes that inexorably move the elderly into an increasingly morbid and disabled state.

The value of health promotion, involving people in self-care and in achieving wholesome lifestyles is obvious, but a crisis of credibility is emerging in the pursuit of

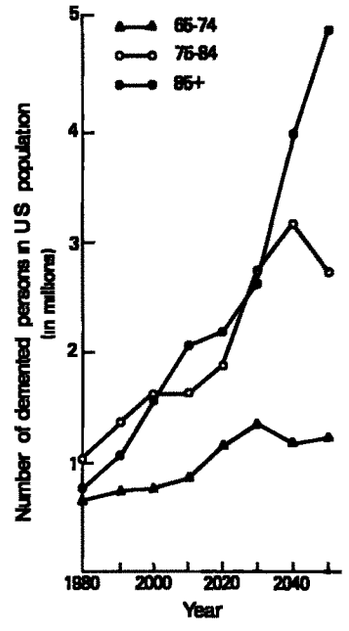


Fig. 4 Projected number of demented persons in the United States by age: 1980-2050. Source, NTA prevalence estimates and US Bureau of Census projections

this goal. One hazard is overstating knowledge when scientific evidence is weak or lacking. In the face of the growing numbers of old and very old people, we must admit that we do not know causes of, and cannot prevent, most chronic diseases[33]; only a fraction of the cancers, heart diseases, strokes, neurological diseases and arthritides are understood, and we know virtually nothing about the causes and prevention of the senile dementias that afflict 20 per cent of people 80 years of age and older[34].

Nobody doubts that smoking and excessive drinking are harmful. Data substantiating the importance of effective treatment of high blood pressure are robust, and at least one study has shown that lowering cholesterol levels is beneficial[35]. Data relating to exercise, weight control, special diets and salt restriction in the absence of hypertension are much less persuasive. Nor do we know how great would be the reduction in morbidity and disability if we produced a population of paragons. Indeed, with almost 70 per cent of the US population now surviving their 65th year, data of Branch and Jette[36] indicate almost no association between most of the commonly advocated personal health practices and mortality among the elderly. To maintain our credibility, we have an obligation to admit our ignorance of causation and our propensity to engender guilt and anxiety among the elderly in areas in which the benefits of specific preventive techniques are not scientifically documented[16].

Another area of health promotion that requires rethinking is behaviour modification through health education. Although considerable benefits may be achieved by altering lifestyles, the results so far have been uneven and equivocal[37,38]. There may

be a systematic obstacle in health education related to an individual's educational level. The classic US study by Kitagawa and Hauser shows a distinct survival advantage among the better educated[39] and is supported in the current literature[40,41]. A recent British investigation reported similar findings[42], noteworthy because medical services have been available free in Britain since 1948.

Education (and its concomitants, all of which are harder to measure) has a powerful effect on life expectancy. People with minimal education may not be reached by the available means of health education. Elimination of smoking, moderation of alcohol consumption, diet control and other attemps at modification of lifestyle will have by-passed this less well educated group, which continues to inflict needless self-harm. We need a new direction in behaviour modification through health education, with the primary target being those with fewer years of education. We may be wasting much of our resources by failing to modify clearly harmful health practices in this inaccessible high-risk subpopulation.

In research, it is important that we should place emphasis on conditions that become more prevalent with ageing, such as blindness, deafness, Alzheimer's disease, acute and chronic heart disease, diabetes, and hip fracture, and that we should develop mechanisms to postpone them. This approach has increasing value as life expectancy reaches or passes 85 years, as illustrated with hip fractures and the associated osteoporotic process. Our data[43] indicate that hip fractures start to rise in frequency in the early forties and thereafter increase exponentially, doubling every 6 years (Fig. 5). If research on bone metabolism could provide a mechanism for delaying osteoporosis so as uniformly to postpone the onset of the exponential rise in hip fracture, the incidence of this condition would be reduced by 50 per cent[44]. Research directed at the precursors of disability is the most effective tool to prevent chronic disease and attendant disabilities.

The most urgent need is of an understanding of human natural history beyond age 65, using the emerging data on elderly populations. The increase in life expectancy among the elderly is unprecedented in human history and probably relates to longer survival trajectories for successive cohorts rather than to specific disease control. The causes of the systematic alteration between 1900, when 25 per cent of the population survived their 65th year, and 1980, when almost 70 per cent did so, demands explanation.

Meanwhile, in our state of ignorance, making assumptions with major policy implications is dangerous. When we speculate in good faith, but on a basis of soft data and with projections that human history has already denied, we run the risk of ignoring Hippocrates' basic admonition that

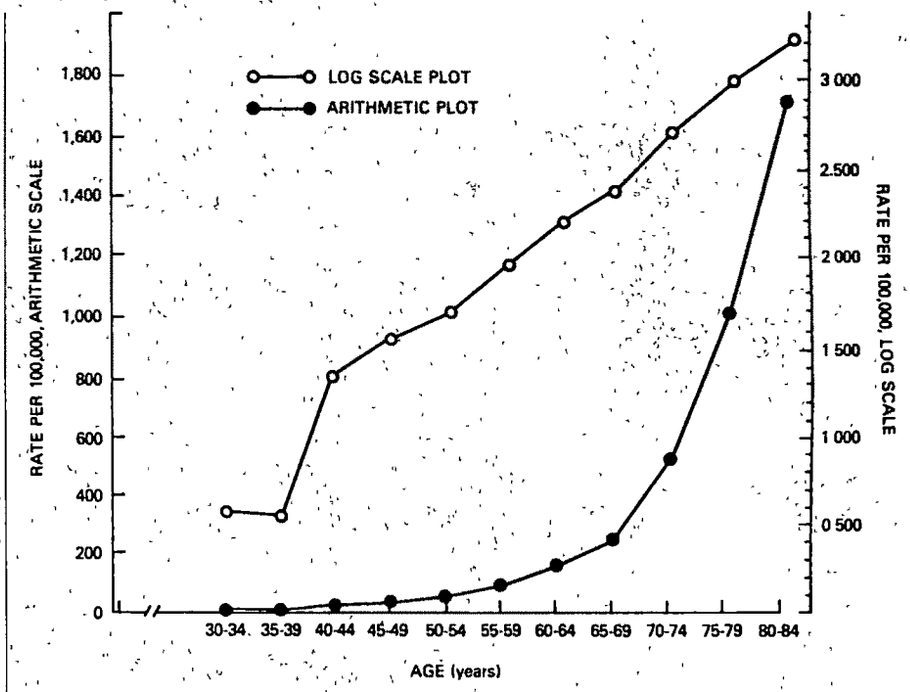


Fig. 5 Age-specific hip fracture incidence among white women. Source: National Hospital Discharge Survey 1975 to 1979.

"the physician must be able to tell the antecedents, know the present and foretell the future — must meditate these things, and have two special objects in view with regard to diseases, namely, to do good or to do no harm."

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Mass distribution in the galactic centre

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New infrared and submillimetre spectroscopic measurements of the gas dynamics in the central 10 pc of our Galaxy make a convincing case that the mass distribution at the centre of the Galaxy is more concentrated than a spherical isothermal stellar cluster. The measurements fit a point mass of $\sim 4 \times 10^6$ $M_\odot$ but are also consistent with a cluster where stellar density decreases with radius (R) at least as fast as $R^{-2.7}$, or a combination of a point mass and a stellar cluster. The dynamical information combined with previous 2-μm observations favour a large point mass, which is presumably a massive black hole.

THE possible existence of massive black holes at the nuclei of galaxies is an important long-standing question. The lack of spatial resolution has limited the work in external galaxies, and the observational difficulty in establishing a reliable answer to this question even in our own Galaxy is quite formidable. The centre of our Galaxy is obscured visibly, and material along the line-of-sight to the centre hampers radio investigations. Here we discuss new information on the gas dynamics in the galactic centre, available from spectroscopy in the infrared and submillimetre regions. This new information allows a determination of the rotational velocity of the interstellar material between ~0.5 and 10 pc from the centre and gives some discrimination between possible mass distributions. The observations entering into the analysis comprise a large body of data which adds considerable detail to our knowledge of the velocity and mass distribution in the central 10 pc of our Galaxy. These data will be presented and discussed in detail elsewhere[1-4]. Here, we provide a brief summary of our present understanding of the galactic centre based on these new results. Distance to the galactic centre is assumed to be 10 kpc.

Previous observations of inner 10 pc

Figure 1 indicates the distribution of interstellar gas and dust in the inner 10 pc of our Galaxy[2,5]. High-resolution Very Large Array (VLA) maps[5-7] show that much of the free-free radio continuum emission from Sgr A West, the radio source in the galactic centre, is distributed in large-scale filamentary structures which may be spiralling into or out of the galactic centre, or orbiting around it[5-7]. There is also an unusual radio point source (Fig. 1, refs 8, 9), whose position almost exactly coincides with the infrared source IRS16 which is very close to if not at the dynamical centre of the Galaxy[10,11]. Spectroscopic measurements of the ionized gas show hydrogen and helium with a velocity range of 1,500 km s^{-1} within 3 arc s of IRS16 (refs 12, 13). At 10–20 arc s from IRS16, measurements of singly ionized neon[14] and of hydrogen recombination lines[15] indicate substantially lower velocities. A statistical analysis based on the velocity dispersion of Ne II-emitting clouds as a function of distance from IRS16 implies that a point mass of $\sim 3 \times 10^6 M_\odot$ combined with an isothermal stellar cluster (density $\propto R^{-2}$, where R is the radius) is a more probable mass distribution for the centre than a R^{-2} stellar cluster, but the level of significance of this result is not high[14].

Far-infrared continuum observations indicate that a ring or disk of neutral dust and gas surrounds the centre from a radius of ~2 pc outward and that inside this region dust and gas is of substantially lower average density[16]. These data (Fig. 1) imply that the neutral dust disk is oriented approximately in the plane of the Galaxy such that two lobes representing the inner edge of this disk appear on either side of the ionized region, apparently encompassing it. Far-infrared measurements of the O I fine structure line at 63 μm show that this disk rotates around the galactic centre, with an axis approximately that of general galactic rotation[17]. Emission from H_2 molecules at 2 μm indicates that the disk contains hot (~2,000 K) gas, which is probably shock-excited[18]). Radio observations of the 21-cm H line and the 1-0 rotational line of CO show that cooler material in the disk extends to ≥6 pc from the centre[19].

New data on galactic centre

New spectroscopic results provide a much more detailed dynamic picture of the ionized gas features by a more complete examination of Doppler shifts of the 12.8-μm Ne II fine-structure line. A much clearer picture of the velocity distribution in the neutral disk out to ~10 pc from the galactic centre comes through mapping of various far-infrared lines, including: (1) fine-structure lines of O° in the ground state (at 63 and 146 μm); (2) the fine-structure line of C^+ at 158 μm; and (3) the $J = 7 \rightarrow 6$ rotational transition of CO at 372 μm. The O I and C II data were taken on the Kuiper Airborne Observatory with a Fabry-Perot spectrometer[2,3], the Ne II line with a Fabry-Perot-grating spectrometer using the IRTF telescope on Mauna Kea, Hawaii[1], and the 7→6 transition of CO with a newly developed heterodyne spectrometer, also at the IRTF[4].

Ionized streamers. New spectra of the Ne II line were taken at 110 different locations with 1.5-6-arc s resolution, principally along the structures in the VLA radio continuum map. The questions of interest were whether the velocities of the filamentary structures are continuous and characteristic of material in systematic motion, and which arms of the various streamers are parts of the same dynamic structures. The results and their interpretation are discussed in detail in ref. 1 (see also ref. 15) and are summarized here.

There are at least two long, coherent streamers of ionized gas. Their velocities and spatial patterns suggest that the gas follows simple orbits around the galactic centre, although there are some deviations from a smooth velocity pattern. As proposed by Lo and Claussen[7], the arc of ionized gas extending from ~30 arc s south of IRS16 to ~15 arc s west of the centre continues further north to ~30 arc s north of the centre (the 'western arc' in Fig. 1) Velocities of the 16 spectral-line centres at approximately equally spaced positions along this ~180° arc vary linearly with declination from −100 to +100 km s^{-1}, with more-or-less random deviations of ~7 km s^{-1} r.m.s. This corresponds to what one expects from the projection of a rotating circle of radius 1.7 pc and rotational velocity of 110 km s^{-1} made up, however, of individual clouds that are not following precisely the same

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orbit. This rotational velocity allows for the observed ellipticity of the arc, which makes the axis of this circle tilted with respect to our line of sight by ~70°. Its extremes lie at the inner edge of the neutral dust and gas disk detected in far-infrared continuum and line radiation[16,17]. As the rotational velocity and projected linear velocity gradient agree with those of the neutral gas at the inner edge of the neutral disk, the western arc is probably the ionized inner surface of the disk. The rather clear-cut circular orbit of the western arc allows a determination of the enclosed mass. If the mass distribution within 1.7 pc is spherical, it contains $4.7 \pm 1 \times 10^6\ M_\odot$. That the circular orbit is ionized and hence visible in Ne II over only about half of its circumference may result from shadowing of a central ionizing source. The shadowing cloud may be, for example, the 'northern arm', which could interrupt ultraviolet radiation west of the centre.

The northern arm (Fig. 1) is another long structure which appears to be a continuous kinematic feature, with velocities varying from 137 km s^{-1} on the northern end to about zero at the declination of the centre. The average change of velocity with distance along this feature is three to four times greater than along the western arc. Hence, this gas feature is closer to the dynamical centre. The velocities in the northern arm reach a maximum of 137 km s^{-1} at a projected distance of 15 arc s (0.75 pc) from IRS16, suggesting that $\sim 4 \times 10^6\ M_\odot$ would have to be enclosed within this radius if this feature were a circular orbit. However, the significant curvature of the velocity curve of the arm indicates that the gas feature is not at constant radius, so that a simple circular orbit analysis is not appropriate. A quantitative examination of the velocities and positions shows that the ionized gas in the northern arm is well described by an eccentric orbit about a point mass, such as a parabola, hyperbola or elongated ellipse. Deviations from such a fit are ~2 km s^{-1} r.m.s., and velocities seem to vary continuously, suggesting an elongation of a single cloud. The inferred orbit passes within 0.5 pc of the central mass of $3.5 \times 10^6\ M_\odot$ located within a few arc s of IRS16. The other model which is of particular interest here is an orbit in an isothermal cluster; such an orbit fitting the measurements of the northern arm still requires a mass of $\sim 3.4 \times 10^6\ M_\odot$ within a radius of 0.5 pc; hence a mass $\sim 11.5 \times 10^6\ M_\odot$ would lie within 1.7 pc, substantially exceeding the mass observed within that radius. It follows that most of the mass contained inside the outer orbit at $R = 1.7$ pc (the western arc) is also within ~0.5 pc of the centre. This mass distribution can result from either a central point mass or a stellar cluster more concentrated than an isothermal cluster, with density falling off steeper than R^{-2}[7].

Neutral material. We have examined the velocities of the neutral material extending from ~30 arc s to several minutes of arc, or ~10 pc from the galactic centre in the far-infrared and submillimetre regions with 30–60-arc s angular resolution. The most recent and improved O I measurements are described in detail elsewhere by R.G. *et al.*[2], the C II results by J.B.L. *et al.*[3] and the CO $J = 7 \rightarrow 6$ results by A.H. *et al.*[4].

The relative intensities of the two O I fine-structure lines and the C II line, as well as the relative intensities of various CO rotational lines[2,4], show that most of the neutral material is in clumps with a hydrogen density near 10^5 cm^{-3} and a gas temperature near 300 K. The total mass of the gas in the neutral disk within ~5 pc is a few times $10^4\ M_\odot$, and <10% of the volume is filled by high-density gas. Maps of intensities of these lines show that the galactic centre gas disk is tilted by ~20° with respect to the galactic plane and may be quite thin (thickness ≤1 pc).

The velocities of all far-infrared and submillimetre emission lines are blueshifted at negative galactic longitudes and redshifted at positive longitudes, with a sharp transition at the centre position. The distributions of integrated line intensities and absolute values of the velocities as a function of longitude offset are very similar on either side of the centre. Rotation is the simplest gas motion fitting this symmetrical distribution and the other observed characteristics of the data. From the orientation of the disk of gas, noted above, and from the velocities measured, the rotation axis must be near that of the general galactic rotation. The velocity at $R = 1.7$ pc determined from the O I and CO measurements is in excellent agreement with the orbital velocity of the western arc. The spectra indicate that velocities decrease with increasing distance from the centre. The velocities at the peaks of the profiles are ±70 km s^{-1} at $R \simeq 2$ pc on respective sides of the centre, and decrease over the next several parsecs to ±50 km s^{-1} at 6 pc and ±27 km s^{-1} at 10 pc. Beyond ~10 pc, the velocity patterns become less clear because the C II lines associated with the dense and warm material close to the centre become weaker and may be contaminated by radiation from other sources along the line of sight. A spectrum of the 1-O CO line at $R = 4.5$ pc (ref. 19) and two Ne II profiles taken at $R = 2.7$ and 5 pc (ref. 20) also support the fall-off of rotational velocity between 2 and 10 pc. The rotation curve is consistent with a 'keplerian ($R^{-1/2}$)' fall-off resulting from orbits around a point mass, and excludes an isothermal cluster.

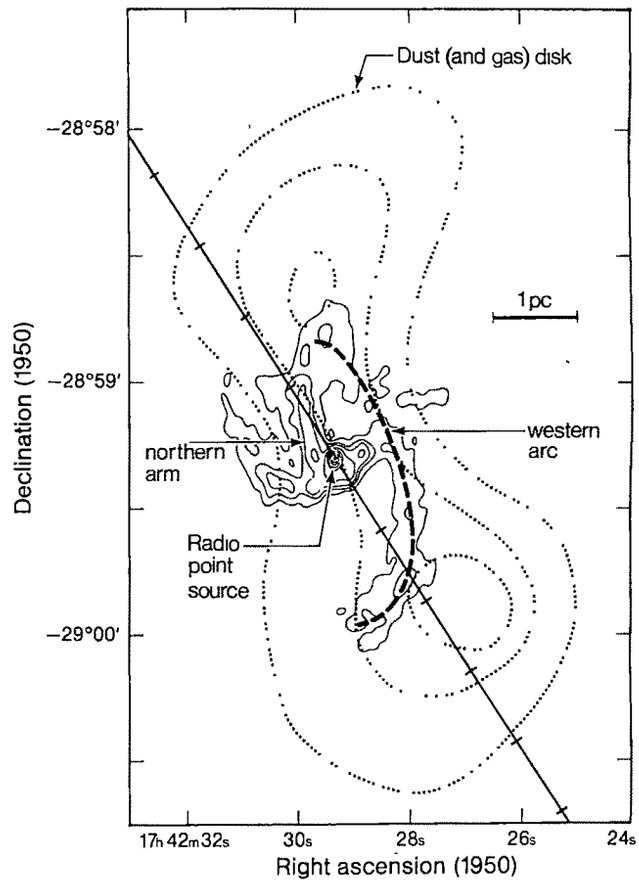


Fig. 1 Sketch of gas in the central 10 pc of the galactic centre. A VLA map of the 2-cm thermal radio continuum emission at a resolution of 2.5 arc s [5] is indicated by the solid contours. Dotted contours give the far-infrared continuum emission from warm dust[2,16]. The galactic plane is marked by diagonal line with ticks every pc. In addition to the filaments marked, the bar is the structure stretching from north-west to south-east ~10 arc s on either side of the radio point source.

In addition to rotation, the neutral disk exhibits some non-systematic motion. For example, lines in many locations are as broad as 100 km s^{-1}, and there is a separate blueshifted cloud ($v = -20$ to -80 km s^{-1} with respect to the local standard of rest) away from the galactic plane north-west of the centre. Nevertheless, for distances greater than 1.7 pc, the measurements give clear evidence of an average rotation. At least some of the irregular motions result from a turbulent velocity dispersion of the gas clumps. In the most central region, O I and CO lines show still larger widths, corresponding to velocities detec-

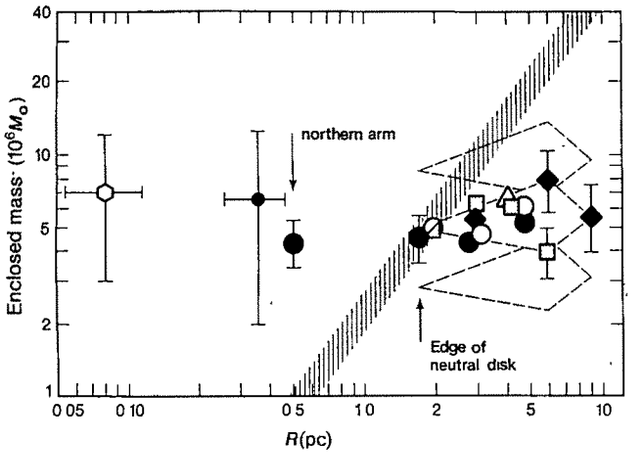


Fig. 2 Enclosed mass as a function of radius from IRS16 The mass distribution is derived from several measurements of the kinematics of neutral and ionized gas discussed in the text With the exception of the mass enclosed within the northern arm from Serabyn and Lacy's fitting of eccentric orbits, all masses were derived assuming circular orbits: $M_{\odot} = v^2 R/G$. Typical error bars are indicated. Velocities in the neutral disk were corrected for an angle between the rotation axis and line of sight (inclination) of 69°. Rotational velocities of the neutral gas in the disk were derived from the terminal velocities (velocity at half maximum), corrected for a local 'turbulent' velocity dispersion of FWHM 50 km s^{-1}. The large dashed diamonds above and below the measured points show where these data on the neutral gas would be without such a correction for non-circular motions, and where they would be if line centroids rather than edges were taken. Relative positions of the points within the dashed diamonds would be essentially unchanged. He I, 2.1 μm (○); Ne II, 12.8 μm (●); O I, 63 μm (○); C II, 158 μm (◆); CO 7→6, 370 μm (▨), CO 1→0, 0 26 cm (△). The Ne II data are from refs 1, 14, 20; O I data from ref. 2, C II from ref. 3; CO 7→6 from ref. 4; and CO 1→0 from ref. 19. The 2-μm He I data (from ref. 13) indicate that velocities of ±750 km s^{-1} occur in a region within a radius of 1-2 arc s, leading to the cross at $R \sim 0.08$ pc. The shaded diagonal bar indicates the mass distribution of a cluster with $4.7 \times 10^6 M_{\odot}$ within a radius of 1.7 pc and a radial dependence of enclosed mass $R^{1.3}$ (from the 2-μm light distribution; refs 23, 24)

ted earlier for Ne II and indicating that there is neutral material in many of the ionized clumps. Normally, the best estimate of the rotational velocities in an edge-on disk comes from the extreme ('terminal') velocities of the profiles. In the galactic centre, these edges are significantly broadened by the turbulent motions (Δv_{turb} (FWHM) ~ 30-50 km s^{-1}). We correct for this broadening in our estimates of the rotational velocities. As indicated in Fig. 2, if turbulence of this magnitude is instead added to the average rotational velocity to calculate average kinetic energy, the mass contained inside a given radius would be increased for the neutral gas measurements, but the fractional rate of change of mass with distance in this region would not be affected. This general result for the fractional rate of change of mass results from the observed decrease in velocity with distance, which makes it relatively insensitive to details of line widths and turbulence.

Discussion

Figure 2, which shows the mass inside of various radii, gives an overview of the mass distribution in the galactic centre. We have derived the masses from the measured velocities assuming a spherical mass distribution. The maximum influence on the derived rotational velocities of corrections for the noncircular motions in the neutral disk are schematically indicated by large dashed diamonds. We choose the points as placed on Fig. 2, using the corrections stated in the legend, as giving the most likely mass values. For other choices, points within the central dashed diamond would approximately maintain their relative positions but be moved with this diamond to its upper or lower positions, depending on one's choice of corrections. In addition to the estimates for the disk, 'western arc' and 'northern arm', we have also indicated a mass estimate from the velocity dispersion of eight Ne II clouds within 8 arc s of IRS16 (from the earlier work by Lacy *et al.*[14]). The line-of-sight velocity dispersion of the inner Ne II clouds, most of which are located in the 'bar' region, is $\sigma_v = 145 \pm 50$ km s^{-1} (ref. 14). We can estimate a mass from the data in Fig. 2 using the virial theorem (enclosed mass $= 3\sigma_v^2 R/G$; see ref. 14), which assumes the gas velocities represent a steady-state distribution. (G is the gravitational constant.) This estimate is about the same as the mass determined from the highest velocity of these clouds (±260 km s^{-1}) assuming circular motion. Finally, a mass estimate within 1-2 arc s of IRS16 originates from the total width of He I and H I lines measured by Geballe *et al.*[13], with the assumption of circular motion. In contrast to the other measurements discussed above, it is not certain that the innermost Ne II and He I/H I velocities represent circulation or any simple orbits. The Ne II clouds in the bar region may be falling into the centre[7], and the He I/H I motions may result from an expanding wind from IRS16 (ref. 13). Hence, these data should be given less weight in the derivation of the mass distribution.

Taken together, the large body of data shown in Fig. 2, measured with different methods and subject to different uncertainties and systematic effects, make a convincing case that at small radii the apparent mass approaches a constant.

The measurements are consistent with a point mass of $4 \pm 1 \times 10^6 M_{\odot}$, or a combination of a $3\text{-}4 \times 10^6 M_{\odot}$ point mass and a small stellar cluster ($1\text{-}2 \times 10^6 M_{\odot}$ within 2 pc and $5\text{-}10 \times 10^6 M_{\odot}$ within 10 pc). They are also consistent with a stellar cluster and no point source if the stellar density decreases as fast or faster than $R^{-2.7}$, and thus exclude an isothermal (R^{-2}) distribution. Some globular clusters and elliptical galaxies have radial density profiles as steep as $R^{-2.7}$ or steeper[21]. Concentrated globular clusters such as M15, for example, have radial density profiles outside their core radii (~0.01-1 pc) which approximately follow a power law $R^{-\alpha}$ with $2.4 \leq \alpha \leq 2.8$ (ref. 22) Such a gradient is consistent with the present data and the main difference between the present case and globular clusters would then be the higher central density and smaller core radius (~0.01-0.05 pc) of the galactic centre mass distribution.

However, additional information on the stellar distribution in the galactic centre, available from observations of the 2.2-μm stellar surface brightness distribution[23,24], makes it unlikely that such a concentrated stellar cluster dominates the mass in the central 2 pc of the Galaxy. The distribution of 2.2-μm surface brightness drops off with $R^{-0.7}$ between 0.05 and 1.5 pc radius from IRS16 (ref. 24), and with $R^{-0.8}$ between 1 and 30 pc (ref. 23) implying a density approximately proportional to $R^{-1.75}$. There is no sign of a flattening in the inward increase of surface brightness, even at the smallest scales observable (≤0.05 pc; ref. 24). The slope of the 2.2-μm emission line is less steep than that of a cluster in isothermal equilibrium, and certainly not steep enough to explain the spectroscopic velocity measurements. The shape of the 2.2-μm light distribution combined with the dynamical information thus suggests strongly that the stars distributed similar to the 2.2-μm light do not contain the major fraction of the mass within 3 pc. Therefore, if the 2.2-μm light distribution in the centre is a measure of the total mass distribution of stars, the mass concentration at the galactic centre probably results from a central point mass of $\sim 4 \times 10^6 M_{\odot}$, most likely in the form of a single massive black hole. Note that the particular radial distribution observed for the stellar cluster would be a natural consequence of a black hole that is more massive than the stellar cluster out to a given radius. Theoretical calculations show that a steady-state balancing between infall of stars towards such a hole and tidal disruption by the hole gives a stellar density distribution proportional to $R^{-1.75}$, similar to what is observed in the galactic centre[25].

There are several reasons why the evidence for a highly concentrated mass at the galactic centre surrounded by a less

massive stellar cluster could be misleading. For example, the 2-μm light distribution may not reflect the mass distribution of the galactic centre stellar cluster. The stellar mass distribution may be more concentrated towards the centre than indicated by the 2-μm light if the stars emitting at 2 μm do not represent a major fraction of the stellar mass. However, the 2-μm light distribution is believed to measure the distribution of K and M giants and supergiants; these would normally be the more massive members of the cluster and should be more concentrated than the cluster as a whole.

A spheroidal or triaxial cluster would have velocities that decrease with distance from the centre rather than the simpler behaviour associated with spherical clusters. In the central plane, however, where velocity measurements are considered in the present study, although the ionized clouds are asymmetrically arranged, they contain little of the total mass and the distribution of gas around the galactic centre is rather symmetrical in both density and velocity. Hence, any extreme ellipticity in the stellar cluster that would give the observed velocity distribution seems improbable. A mild ellipticity of the mass distribution would not change the results qualitatively, but would only decrease the total amount of calculated mass. In the case of the northern arm, one might wonder whether some broadly distributed gas, providing friction to the orbit of any streamer of gas, can produce the curvature and accelerations observed. Quinn and Sussman[26] have proposed such a distribution of hot gas as an explanation for the morphology of the ionized streamers in the galactic centre. The Ne II observations of the western arc and the northern arm, however, exclude the specific model proposed[1], because the measured velocities are substantially different from those predicted by the model. Any more general friction-induced infall model is only possible if it can explain both the almost undisturbed circular orbit of the western arc and the presence of clouds moving as fast as 260 km s^{-1} (ref. 14) very near the galactic centre.

If a black hole accounts for $4\times10^6\ M_\odot$, the measurements in Fig. 2 allow a stellar cluster mass of only $1\text{–}2\times10^6\ M_\odot$ within $R=2$ pc. The total luminosity of the cluster within this radius, which is deduced by assuming the 2-μm radiation represents a distribution of 4,000 K giant stars, is $\sim4\times10^6\ L_\odot$ (refs 23, 24). Hence, the mass to luminosity ratio of the cluster is $\leq0.5\ M_\odot/L_\odot$, which is much lower than typical for old clusters ($M_\odot/L_\odot\sim$ 1.5:5; ref. 21). It may indicate relatively recent star formation activity or a lack of low mass stars. The total luminosity of the region inside $R=1$ pc deduced from the ionizing flux or the longer-wavelength infrared continuum radiation is $\sim2\times10^7\ L_\odot$ (ref. 16), and hence if all the mass were assumed to be stars, the ratio $M_\odot/L_\odot$ would be very low.

Related observations

The turbulent and non-circular motions of the ionized and neutral gas in the galactic centre are significantly larger than in other dense clouds in the Galaxy or than one would expect in equilibrium. It is likely that this gas has been strongly perturbed within a time as recent as a few orbital periods ($\sim10^5$ yr). This disturbance was possibly produced by material which fell into the galactic centre from an appreciable distance. Estimates from the amount of material present and its clumpy distribution indicate that material should fall into the centre at an average rate of $\sim10^{-4}\text{–}10^{-3}\ M_\odot\ yr^{-1}$ (ref. 14). This average rate should generate substantially more energy flux than is presently seen in the region. However, if there were episodic infall, the present perturbations and possibly also the low density of material within the inner radius of the disk could be the result of a past larger infall and very high resultant energy flux. This would also imply that we are now in a time of relative quiescence.

It has previously been argued that the ultraviolet radiation required to ionize gas in the galactic centre could not come from a single point-source, because a black-body source or one with the characteristics of a stellar atmosphere would not produce enough ultraviolet without being a stronger source of infrared than has been observed[27,28]. Now we find that these arguments are not conclusive. Henry *et al.*[10] have already suggested that much of the radiation could originate from the source IRS16. It does seem possible to fit the amount of ultraviolet radiation required to ionize the Sgr A West H II region and the observed infrared intensity of IRS16 to what would be expected from a radiating atmosphere similar to that of an O-type star of surface temperature ~35,000 K and surface gravity of $\log g=5$ (ref. 1), although a large number of O-type stars would be required. The surface gravity tends to decrease radiation energetic enough to ionize Ar^+ or S^{2+}, fulfilling the observed conditions of very few Ar^{2+} and S^{3+} ions, while at the same time providing enough photons to produce the observed Ne^+. Thus ultraviolet and infrared observations seem to be consistent with production of the ultraviolet excitation in the central few parsecs by a single localized source, such as a black hole.

Conclusions

New infrared and submillimetre measurements of the dynamics and distribution of material around the galactic centre have increased knowledge of the structure and mass distribution of this region. A large amount of data seem to fit a model, involving: (1) an unusual concentration of mass not in the form of ordinary stars, most probably a central black hole of mass $4\pm1\times10^6\ M_\odot$; (2) an ionized cavity of relatively low gas density surrounding the centre and of radius 1.7 pc. This cavity also contains an additional $1\text{–}2\times10^6\ M_\odot$ mass in a stellar cluster; (3) a circulating disk of dense ($\sim10^5$ cm^{-3}), warm (300 K) and clumpy neutral gas between 1.7 and ~10 pc which is ionized along much of its inner edge. Irregular gas velocities indicate strong perturbations of the motions in this region over the past few 10^5 yr.

The observed velocities also fit a stellar cluster with no central point mass if the stellar density distribution decreases at least as fast as R^{-2}[7]. However, the case without a central black hole is excluded if the observed 2-μm light distribution is a fair representation of the mass distribution of the stellar cluster.

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Terrestrial xenon isotope constraints on the early history of the Earth

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Comparison between ^{129}I-radiogenic ^{129}Xe and ^{244}Pu-fissiogenic ^{136}Xe components in terrestrial xenon suggests that the Earth's inner region accreted a few tens of millions of years earlier than the outer region from which the atmosphere evolved. The results also indicate that there has been no substantial mixing of the two regions since the Earth's accretion.

SINCE the discovery of excess ^{129}Xe in meteorites[1], Xe isotopes produced from ^{129}I (half-life $t_{1/2}=17$ Myr) and from ^{244}Pu ($t_{1/2}=82$ Myr) have been a prime source of information on the early history of meteorites. Although there has been considerable work on Xe in meteorites, there is still relatively little Xe isotope data for terrestrial samples: nevertheless, some very interesting results have emerged, notably the confirmation of large excess ^{129}Xe and $^{132-136}Xe$, relative to air Xe, in gas from a CO_2 well in New Mexico[2-5]. There have also been reports of excess ^{129}Xe in some mantle-derived materials, such as mid-ocean ridge basalts (MORB)[6-8], mantle xenoliths[9-11] and diamonds[12]. The existence of excess ^{129}Xe indicates that the source region for the excess ^{129}Xe must have been isolated for almost the whole history of the Earth from the atmosphere and/or the rest of the mantle from which the atmosphere was generated. It has been argued[6,7,13-15] that the isolation of this source must correspond to degassing of the mantle and, therefore, that this degassing, and the major generation of the atmosphere, occurred very early, within a few half-lives of ^{129}I after the formation of the Earth.

We now examine published data on Xe isotope composition in terrestrial samples and discuss their implications for the early history of the Earth. We show that the terrestrial Xe isotope data need not lead to the conclusion that the excess ^{129}Xe requires very early mantle degassing, as generally assumed.

Terrestrial Xe isotope data

From published Xe data, we have constructed three isotope plots for granites, MORBs and upper-mantle derived rocks (mostly mantle xenoliths) (Figs 1-3). The data for New Mexico CO_2-well gas Xe is shown in the plots for granites (Fig. 1). In plotting these correlation diagrams our only selection criterion was to discard those with abnormally large error assignments. For step-heating data, we discarded the lowest temperature fraction, which would probably include large air contamination, and we chose as a representative value, the fraction with the largest amount of Xe. For the New Mexico CO_2-well Xe, we used the latest experimental data[5] (Fig. 1).

Granites (Fig. 1) show a very clear linear correlation which must be due to the addition of ^{238}U-fissiogenic Xe isotopes[16] as granite typically has relatively high concentrations of U. The slopes of the correlation lines (solid lines) in the $^{134}Xe/^{130}Xe$-$^{136}Xe/^{130}Xe$ and $^{132}Xe/^{130}Xe$-$^{136}Xe/^{130}Xe$ diagrams give the ratios of the fission yields $^{134}Y/^{136}Y$ and $^{132}Y/^{136}Y$, respectively, which agree well with the laboratory values of 0.832 ± 0.02 and 0.59 ± 0.017 for spontaneous fission of ^{238}U. Figure 1 also shows the correlation lines (dotted lines) corresponding to ^{244}Pu-fissiogenic Xe, which do not fit the data well. The isotope composition for New Mexico CO_2-well Xe falls on the correlation lines, indicating that the excess ^{132}Xe-^{136}Xe is also due to ^{238}U-fission, as was concluded by Phinney *et al.*[4]. However, the CO_2-well gas Xe has significant excess ^{129}Xe, which is not seen in granites. This excess ^{129}Xe has been confirmed independently[3-5] and its existence seems to be established.

Excess ^{129}Xe may also exist in MORB (Fig. 2) and mantle-derived xenoliths (Fig. 3). In addition, ^{136}Xe and ^{134}Xe appear to show slight excesses relative to air Xe. However, correlations between $^{136}Xe/^{130}Xe$ and $^{134}Xe/^{130}Xe$, $^{132}Xe/^{130}Xe$ are vague and it is not possible to resolve whether the excess $^{132-136}Xe$, if real, is due to ^{238}U-fission or to ^{244}Pu-fission. The only known source for excess ^{129}Xe is the extinct nuclide ^{129}I. The existence of the excess ^{129}Xe indicates that some part of the Earth had incorporated a non-trivial amount of short-lived ^{129}I and has not undergone extensive exchange with the atmosphere, or the source of the atmosphere, since before the decay of ^{129}I was substantially complete. For excess ^{131}Xe-^{136}Xe, both ^{238}U-fission and ^{244}Pu-fission could be responsible. Except for crustal rocks, which are more enriched in U, ^{244}Pu-fissiogenic Xe is likely to be more important than ^{238}U-fissiogenic Xe in the Earth as a whole[17].

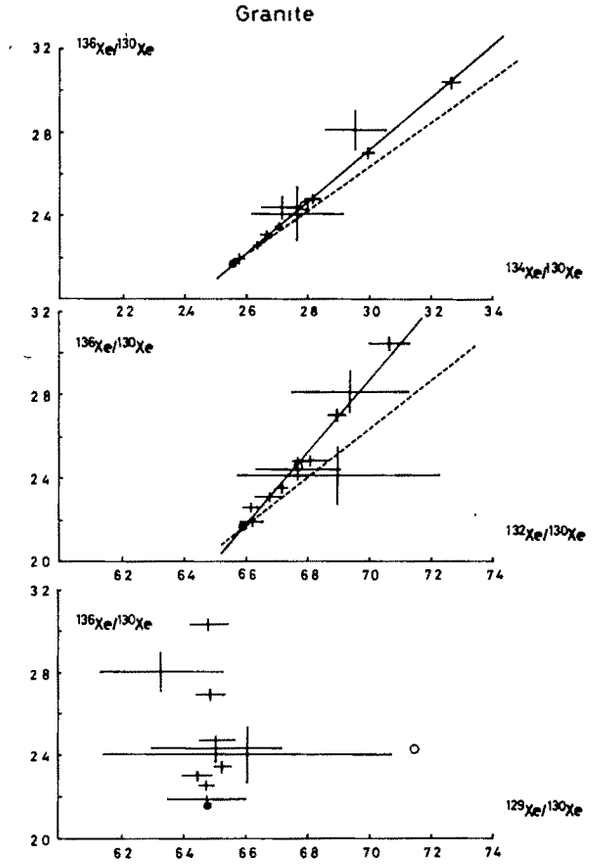


Fig. 1 Three isotope plots for granite samples[16]. Also shown are New Mexico CO_2-well gas Xe (O, data from ref. 5) and air Xe (●). Note that the CO_2-well gas Xe data lie on the correlation lines, which characterize ^{238}U-fissiogenic Xe (solid lines). For comparison, a correlation line for ^{244}Pu-fissiogenic Xe is also shown by a dotted line.

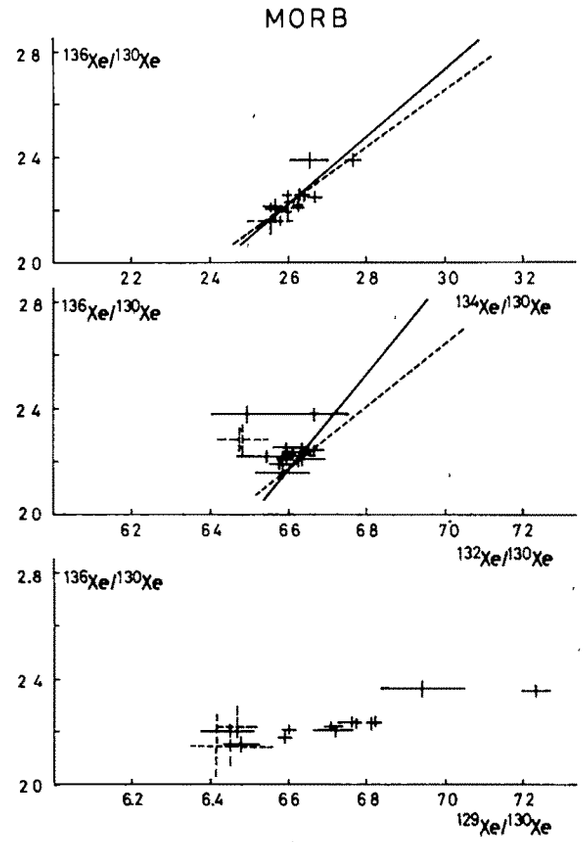


Fig. 2 Three isotope plots for MORB. Solid cross, data from refs 6, 7 Dotted cross, data from ref. 27. Note that neither the ^{244}Pu-fissiogenic nor the ^{238}U-fissiogenic lines fit the data

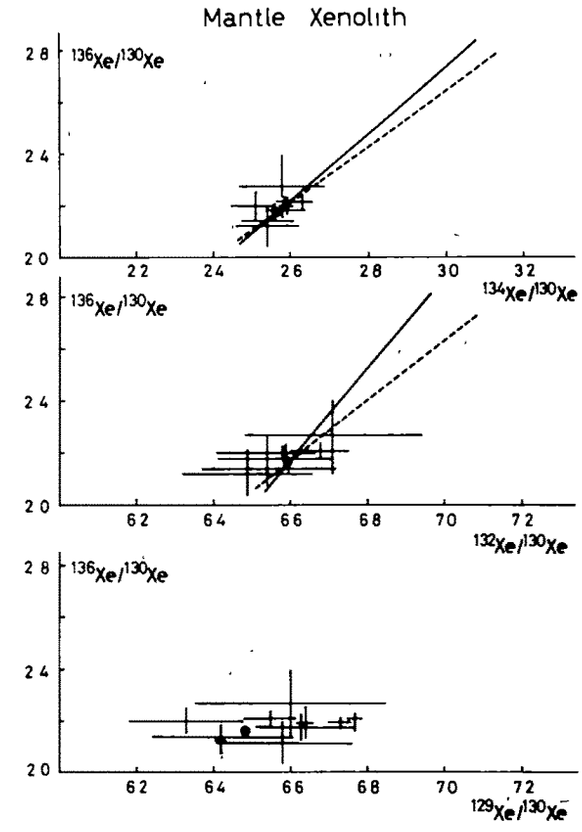


Fig. 3 Three isotope plots for mantle-derived xenoliths (data from refs 9, 10, 28) ●, Air Xe A slight excess may be seen in ^{129}Xe.

Xe closure time

In using radiogenic isotopes as chronological tracers, a fundamental problem is to identify when the parent–daughter element system closed. The nucleogenic Xe isotopes that are important in terrestrial Xe are spontaneous fissiogenic Xe (^{131}Xe, ^{132}Xe, ^{134}Xe, ^{136}Xe) from ^{238}U and ^{244}Pu and radiogenic ^{129}Xe. Once the system is closed for ^{129}I/Xe and ^{244}Pu/Xe ratios, Xe isotope evolution obeys a simple radioactive decay law and is predictable. Because our main concern here is the early history of the Earth and thus with I–Xe and Pu–Xe closure; we can disregard the ^{238}U–Xe system in much of the following discussion because of the long half-life of ^{238}U.

In gas of a solar composition, Xe isotope evolution would be negligible because of very low I/Xe and Pu/Xe ratios; only in condensed materials greatly depleted in noble gases would Xe evolution be significant. Theoretical treatments of early Solar System history typically conclude that condensation and fragmentation into planetesimals (bodies a few kilometres in diameter) would occur quite rapidly, on a 10^4-yr timescale at the Earth's orbit[18,19], but that accretion of planetesimals into the major terrestrial planets might take significantly longer, of the order of 10^7–10^8 yr (ref. 20). This is a significant time for short-lived ^{129}I and ^{244}Pu. While condensation and accretion result in closure of solid elements such as Pu and I, Xe closure might be established only after collisions among planetesimals, because impact shock could result in the effective degassing of volatiles[21]. In this way, it would be possible for early and late-accreting material to have significantly different Xe closure times and contribute significantly different amounts of radiogenic Xe, expecially ^{129}Xe, even if it all had the same basic chemistry and the same initial I/Pu ratio.

Interpretation

According to Pepin and Phinney[22,23], about 6.7% of atmospheric ^{129}Xe originates from the decay of ^{129}I, and 4.7% of atmospheric ^{136}Xe originates from the decay of ^{244}Pu. Subtracting these nucleogenic components from the air Xe composition, we can define a 'primary' air Xe (equivalent to "corrected atmosphere Xe"[23]); this is presumably the Xe composition at the time of isotopic closure. It is a straightforward matter to develop equations for the compositional evolution after closure and to include a single episodic degassing event. Using subscripts '0' for closure and 'd' for degassing, and setting $t_0 = 0$, we have for $0 \leq t \leq t_d$ (from Xe closure to degassing):

$$\frac{^{129}\text{Xe}}{^{130}\text{Xe}} = \left(\frac{^{129}\text{Xe}}{^{130}\text{Xe}}\right)_0 + \left(\frac{^{129}\text{I}}{^{130}\text{Xe}}\right)_0 (1-\exp(-\lambda_{129}t))$$
$$\frac{^{136}\text{Xe}}{^{130}\text{Xe}} = \left(\frac{^{136}\text{Xe}}{^{130}\text{Xe}}\right)_0 + \left(\frac{^{244}\text{Pu}}{^{130}\text{Xe}}\right)_0 {}^{136}\text{Y}_{\text{Pu}}(1-\exp(-\lambda_{244}t)) \quad (1)$$

where λ_{129} and λ_{244} are the decay constants for ^{129}I and ^{244}Pu and $^{136}Y_{Pu}$ is the yield of ^{136}Xe per decay of ^{244}Pu.

If the degassing occurred before ^{129}I and ^{244}Pu had decayed completely, Xe isotopic composition in the residue continued to evolve. If we assume that a fraction $(1-f)$ of the Xe was degassed at $t = t_d$, the evolution for $t > t_d$ becomes

$$\frac{^{129}\text{Xe}}{^{130}\text{Xe}} = \left(\frac{^{129}\text{Xe}}{^{130}\text{Xe}}\right)_0 + \left(\frac{^{129}\text{I}}{^{130}\text{Xe}}\right)_0 (1-\exp(-\lambda_{129}t_d)) + \frac{1}{f}\left(\frac{^{129}\text{I}}{^{130}\text{Xe}}\right)_0 \exp(-\lambda_{129}t_d) \times (1-\exp(-\lambda_{129}(t-t_d)))$$
$$\frac{^{136}\text{Xe}}{^{130}\text{Xe}} = \left(\frac{^{136}\text{Xe}}{^{130}\text{Xe}}\right)_0 + \left(\frac{^{244}\text{Pu}}{^{130}\text{Xe}}\right)_0 {}^{136}\text{Y}_{\text{Pu}}(1-\exp(-\lambda_{244}t_d)) + \frac{1}{f}\left(\frac{^{244}\text{Pu}}{^{130}\text{Xe}}\right)_0 {}^{136}\text{Y}_{\text{Pu}} \exp(-\lambda_{244}t_d) \times (1-\exp(-\lambda_{244}(t-t_d))). \quad (2)$$

Figure 4 shows a single-stage Xe-isotope evolution curve, for

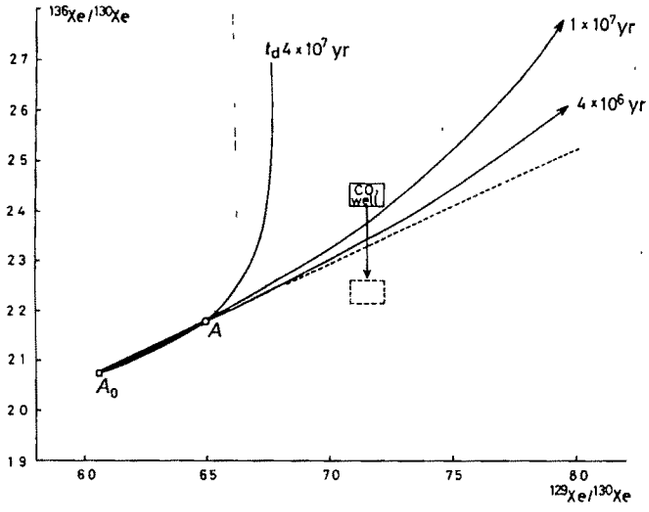


Fig. 4 Xe isotope evolution model in the Earth. *A*, Air Xe, A_0, primary air Xe (see text). A_0 is the Xe-closure time in the Earth Owing to ^{129}I and ^{244}Pu, Xe isotopes in the Earth had evolved along the paths A_0–A At t_d Xe was degassed into the atmosphere, where the Xe isotopic ratio was quenched to give air Xe (A). After t_d, Xe in the Earth has continued to evolve. The evolution curve is determined by specifying t_d. We also show the CO_2-well gas Xe (refs 4, 5). Solid rectangle, the range for the measured isotope composition; dotted rectangle, isotopic composition (maximum value) corrected for the ^{238}U-fissiogenic ^{136}Xe

which atmospheric Xe is assumed to be degassed at $t = t_d$. Although drawn for $f = 0.5$, the choice of f ($0 < f < 1$) does not significantly affect the general character of the evolution. Equation (1) clearly shows that the specification of t_d determines $(^{244}Pu/^{130}Xe)_0$ and $(^{129}I/^{130}Xe)_0$, as both $(^{136}Xe/^{130}Xe)_{air}$ and $(^{129}Xe/^{130}Xe)_{air}$ are assumed to be known. This situation can also be seen in Fig. 4: an evolution curve passing through A_0 (primary air Xe) and A (present air Xe) can be uniquely determined by specifying t_d. The shorter the t_d, the further the curves grow after the degassing, since shorter t_d requires greater I/Xe and Pu/Xe and much of the decay occurs after the degassing. Note that Xe isotope composition in any degassed region ($t_d > 0$) must be located in the region above the dotted line, that is, the extension of the line connecting A_0 and A in Fig. 4; qualitatively, the ratio of fission ^{136}Xe to radiogenic ^{129}Xe must increase with time because of the greater half-life of ^{244}Pu.

Let us now consider the CO_2-well gas Xe. Figure 4 plots the CO_2-well gas Xe both as measured and as corrected for ^{238}U fissiogenic ^{136}Xe. Phinney *et al.*[4] estimated that at least 80% (and perhaps all) of the excess ^{136}Xe is due to ^{238}U-fission (rather than ^{244}Pu fission). We have re-estimated the fraction of the ^{238}U-contribution to the excess ^{136}Xe using a least-squares method, with the result that it is at least 80%. Figure 4 shows that the CO_2-well gas Xe corrected for ^{238}U-fission lies below the dotted line, where the correction involves subtracting 80% of the excess $^{136}Xe/^{130}Xe$, relative to the air ratio, from the observed ratio (that is $(^{136}Xe/^{130}Xe)_{corr} = 2.448 - (2.448 - 2.1747) \times 0.8 = 2.2294$). The corrected value should be an upper limit, as the ^{238}U-fissiogenic contribution is likely to be larger than 80%. By the above argument, this observation indicates that the CO_2-well gas Xe (corrected for ^{238}U fission) was not derived from the residue from which atmospheric Xe was degassed, nor entirely as a mixture of air with Xe from this residue, because such Xe must lie above the dotted line.

The fundamental difference between the source region of the well-gas Xe and that of air Xe is that the former must have had a smaller $(^{244}Pu/^{129}I)_0$ value. It is easy to show that the Xe isotope evolution curve which evolved from A_0 to terminate at the nominally corrected well-gas composition is uniquely specified by a $(^{244}Pu/^{129}I)_0$ value of 2,105, which is much smaller than the minimum permissible value for the source of atmospheric Xe ($(^{244}Pu/^{129}I)_{min} = 3{,}306$, corresponding for $t_d \gg 1/\lambda_{244}$). This difference may have resulted from chemical differentiation in the Earth or from chemical heterogeneity among the planetesimals which accreted to form the Earth. In the former case, the chemical differentiation must have occurred before ^{129}I or ^{244}Pu had decayed, that is, within a few tens of millions of years after the formation of the Earth. In the latter case, the difference in $(^{224}Pu/^{129}I)_0$ among planetesimals may reflect a variation in the Xe-closure time of different planetesimals. If the time lag is responsible for the difference in the $(^{244}Pu/^{129}I)_0$ ratio, the time interval required can be estimated from

$$\left(\frac{^{244}Pu}{^{129}I}\right)_{0,outer} \Big/ \left(\frac{^{244}Pu}{^{129}I}\right)_{0,inner} = \exp\{(\lambda_{129} - \lambda_{244})\Delta t\} \quad (3)$$

Setting $(^{244}Pu/^{129}I)_{outer} > 3{,}306$ and $(^{244}Pu/^{129}I)_{inner} = 2{,}105$ in the left-hand side of equation (3). We obtain $\Delta t > 1.4 \times 10^7$ yr.

Earth's early history

The excess ^{129}Xe in some terrestrial materials indicates that the region from which the excess ^{129}Xe was derived must have been isolated from the atmosphere for nearly the whole history of the Earth. We argued above against the commonly held view[6,7,13–15] that the source region for the excess ^{129}Xe in the CO_2-well gas represents the degassed mantle from which air Xe was derived.

We propose the following scenario for interpreting coherently the available Xe isotope data. The Earth formed by accretion of impacting planetisimals. Planetesimals were originally formed by the fragmentation process[24], underwent mutual collision to grow further and finally impacted onto the Earth. The early planetesimals constituted the inner part of the Earth. As the accretion time of the Earth is plausibly at least a few tens of millions of years[18,19] and the time of the final impact of the planetsimals onto the Earth must be an upper limit to their Xe-closure time, there could well have been a few tens of millions of years time gap in Xe-closure time between the inner and outer regions of the Earth. Hence, we expect that the inner part of the Earth had smaller $(^{244}Pu/^{129}I)_0$ as well as larger $(^{244}Pu/^{130}Xe)_0$ and $(^{129}I/^{130}Xe)_0$ ratios. If we assume that the CO_2-well gas Xe (except for the ^{238}U fission component) was derived from the inner part of the Earth, while air Xe was degassed from the outer region, we could explain why the CO_2-well gas Xe is characterized by excess ^{129}Xe but not a corresponding excess of ^{244}Pu-derived $^{131-136}$Xe. The comparison between air Xe and CO_2-well gas Xe then gives about 1.4×10^7 yr for the minimum time interval between the formation of the two regions. If the timescale for atmospheric degassing is of the order of 10^8 yr, as inferred from very high $^{40}Ar/^{36}Ar$ ratios in the upper mantle[25], then the upper-mantle Xe composition should be nearly the same as the atmospheric Xe composition.

Excess ^{129}Xe has been reported in some mantle-derived samples. The largest excess ^{129}Xe reported for terrestrial materials is for MORB by Allègre *et al.*[7] who argued that the excess ^{129}Xe in MORB is indicative of very early degassing of the MORB source region. The argument can be examined by means of Fig. 4. However, the difficulty in identifying ^{244}Pu-fissiogenic Xe does not allow such a test. Hence, until the origin of the excess $^{131-136}$Xe is resolved, the conclusion by Allègre *et al.* should be treated with caution.

The high $^{40}Ar/^{36}Ar$ ratios in MORBs relative to air Ar are not inconsistent with the rapid timescale for degassing (within a few tens of millions years after Earth's formation) argued by several authors on the basis of the excess ^{129}Xe in the mantle-derived materials[6,7,13–15]. However, note that while the high $^{40}Ar/^{36}Ar$ ratio has been established throughout the Earth's history owing to the much slower decay of ^{40}K, the excess ^{129}Xe was established soon after the formation of the Earth. In the upper mantle, where material movement such as mantle convection and subduction are likely to have operated throughout geological time, it is difficult to suppose that the early established excess ^{129}Xe could have survived these geological disturbances. The mixing of subducted materials into the mantle is especially difficult to reconcile with the persistent existence of excess Xe

in MORB because, unlike lighter noble gases, Xe is notoriously 'sticky' and is likely to be carried down into the mantle with the subducting ocean sediments, which have 2-4 orders of magnitude enrichment of Xe relative to common volcanic rocks[26]. Hence, it may be more reasonable to suppose that the excess ^{129}Xe observed in MORBs (and also in other mantle-derived materials) did not originate in the degassed upper mantle, but was transported there from some other region (possibly from the deeper mantle). This source had been isolated from the mantle-atmosphere system for almost the whole history of the Earth as shown by the presence of excess ^{129}Xe in the CO_2-well gas Xe.

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Quaternary structure of the acetylcholine receptor

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The five membrane-spanning subunits of the acetylcholine receptor have been resolved in electron microscope images and are shown to lie at pentagonally symmetrical positions around the channel over a large fraction of their length. The channel consists of a wide synaptic portion and a narrow portion extending through the membrane into the interior of the cell.

SIGNALLING between excitable cells is commonly mediated by the release of neurotransmitters, which bind subsequently to receptors in the target membranes and induce in them a change of permeability. The nicotinic acetylcholine receptor, the best understood such receptor, effects the change by opening a channel in the membrane through which cations, principally sodium and potassium, diffuse. This action is initiated by the cooperative binding of neurotransmitter to the α-subunits, the two smallest of five (stoichiometry $\alpha_2\beta\gamma\delta$) assembled in a ring around the channel (see refs 1-3 for review).

The complete amino-acid sequences of the four types of polypeptide comprising this receptor have recently been deduced[4-7] and indicate extensive homology between the subunits (see ref. 8 for review). Predictions based on these data suggest a common motif of secondary structure for the portions of the subunits inside the lipid bilayer[9,10], with features having important implications concerning the movement of ions through the channel and the mechanism of the gating event[11,12]. An understanding of the molecular details now requires direct structural information on the different transitional states.

Here we report on a quaternary configuration of the acetylcholine receptor, determined by three-dimensional electron image analysis of tubular crystals grown from native membrane vesicles. Previously, a pentagonal molecular outline had been resolved in individual images of stained tubes, suggesting that the subunits are disposed regularly around the channel[13]. The three-dimensional maps obtained by combining information from many images now reveal details of the subunits and of the channel at different levels throughout the structure. We demonstrate that there is indeed an approximate 5-fold axis of symmetry along the channel axis over a large fraction of the molecule extending from within the lipid bilayer into the synaptic cleft.

Table 1 Three-dimensional data from images of tubes

	Frozen	Stained
No. of images*	35	52
Range of tilt angles	0-60°	0-65°
No. of independent lattice lines†	21	24
No. of Fourier terms used in synthesis‡	208	271
Average phase error/image§	13.5°	11.9°

* Values for the axial ratio b/a and included angle γ were 1.787 ± 0.01, $120.17 \pm 0.19°$ (frozen) and 1.797 ± 0.01, $117.33 \pm 0.48°$ (stained), from 20 measurements, with a, b and γ as defined previously[13]. Thus, the ratio of lateral to longitudinal dimensions is ~5% smaller for the stained than for the frozen tubes.

† Data include all significant lattice lines (except for the (0, 0)) to a resolution of 25 Å; these are the (0, 2), (0, 3), (0, 4), (0, 5), (1, −5), (1, −4), (1, −3), (1, −2), (1, −1), (1, 0), (1, 1), (1, 2), (1, 3), (2, −5), (2, −4), (2, −3), (2, −2), (2, −1), (2, 0), (3, −3), (3, −1) lattice lines in both cases, and in addition the (0, 1), (3, −5), (3, −2) for the tubes in stain.

‡ Obtained by sampling the continuous amplitude and phase curves at regular 0.0025-Å^{-1} intervals.

§ Based on cumulative comparisons of individual peaks over a range of 0.003 Å^{-1} along the lattice lines.

Data collection and analysis

The tubes, which were prepared from the electric organ of the ray *Torpedo marmorata*, are composed of receptors arranged in a p2 surface lattice and oriented with the synaptic side outwards[13]. They are flattened against the support film during preparation (see Fig. 2 legend), forming two almost planar superposed layers (Fig. 1). Images of the tubes in frozen solution and in negative stain were obtained to provide information on the complete protein and the hydrophilic surfaces, respectively.

For the analysis, all the selected tubes had surface lattices in orientations close to the example in Fig. 1*b*; therefore, variations in their unit cell parameters were less than described previously[13]. The tubes were tilted at angles of up to 65° to the incident electron beam and the amplitudes and phases of the Fourier components were determined to a resolution of 25 Å from digitized areas of these images. Data were then combined cumulatively by standard methods[14] to map the continuous variations of amplitude and phase along each reciprocal lattice line (Fig. 2). The three-dimensional Fourier syntheses were from the terms obtained by sampling these curves at regular intervals

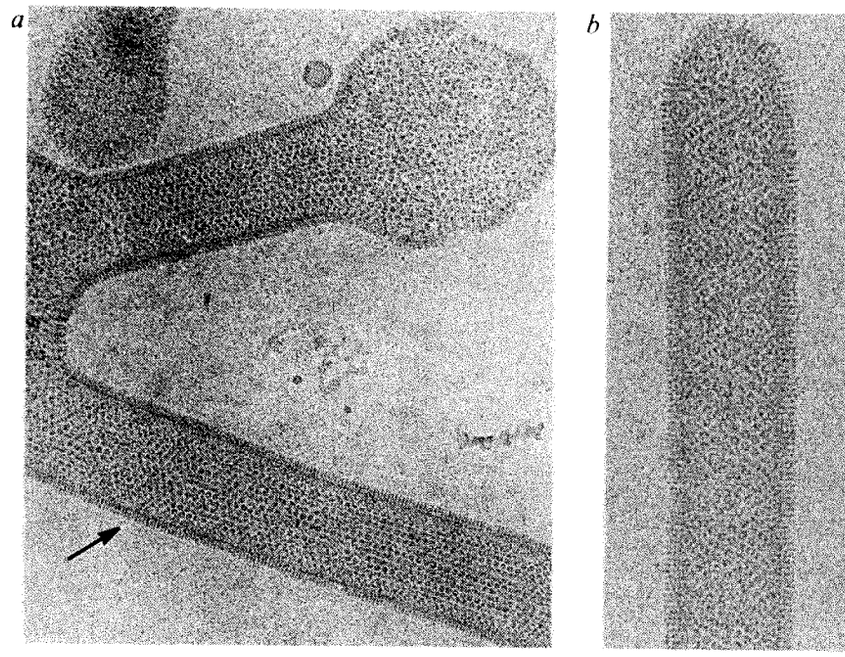


Fig. 1 Electron microscope images of tubular crystals of acetylcholine receptor embedded in thin films of frozen buffer (100 mM Tris-HCl, *p*H 6.8). The complex patterns arise from superposition of *en face* views of the receptors located on the top and bottom surfaces. Striated zones at the tube borders are a result of the receptors being viewed more nearly from the side; when aligned parallel to the tube axis, as in *a*, the receptors in these zones form distinct 120–140-Å wide bands (arrow) and seem to be viewed exactly from the side. Magnifications: *a*, ×112,000; *b*, ×182,400.

and assuming the unit cell dimensions in the plane of the membrane to be $a = 90.0$ Å, $b = 160.8$ Å, $\gamma = 120.2°$, determined from the frozen specimens. The densities synthesized from the stained specimens were multiplied by −1 to correct for their opposite contrast. Further details are given in Table 1.

The similarity between the two three-dimensional maps was evaluated by comparing sections at various levels throughout the two structures. The sections were parallel to the plane of the membrane and the comparisons included all the densities in a single unit cell. The best overall correlation was achieved with a relative displacement of 11 Å perpendicular to the membrane between the origins of the two maps, reflecting differences between the centres of scattering density for the two cases. Corresponding values for the correlation coefficients were >0.8 over most of the ~140 Å length in which features were clearly contrasted (Fig. 3), except for a narrow zone identified as the hydrophobic core of the bilayer (see below). Hence, with the exception of this zone, the two maps resemble each other closely.

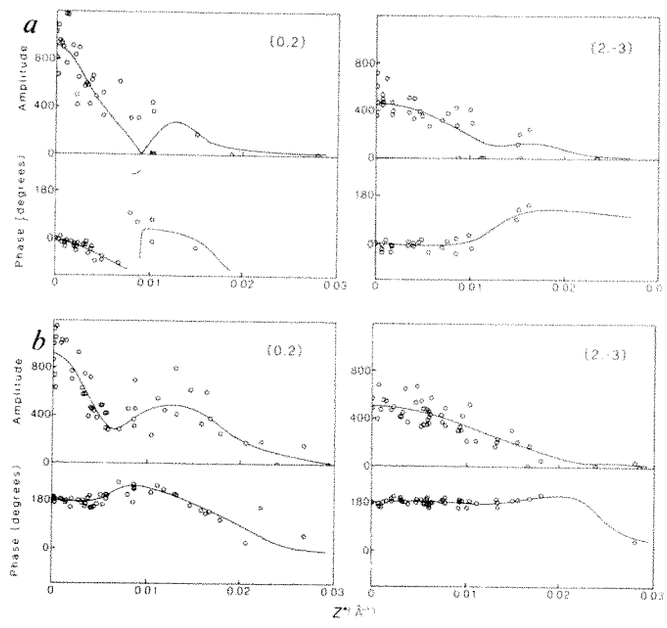


Fig. 2 Comparison of amplitude and phase variations along typical lattice lines after combining images from tubes in: *a*, ice; *b*, stain, according to the 2-sided plane group p21. The continuous curves through the data points were computed by a least-squares method with amplitudes weighted according to the reliability of the corresponding phases[22]. Z^* is the distance from the midpoint of the lattice line.

Methods. Tubes were prepared as described previously[13] and applied to carbon support grids rendered highly charged by glow discharge in amylamine vapour[23]. For freezing, grids were blotted with filter paper for 4 s, then plunged rapidly into liquid ethane cooled to below −130 °C; for staining, they were washed in 2% sodium phosphotungstate *p*H 7.2 and then dried. Frozen specimens were stored under liquid nitrogen and later examined in a Philips EM400 electron microscope using a cold holder maintained at −174 °C. The electron optical magnifications were ×40,800 (ice) and ×28,500 (stain), and images were recorded at 100 kV on Kodak SO163 film developed for 8 min in concentrated D19 developer. A Philips low-dose kit was used to minimize radiation damage and to ensure that all images incorporated in the three-dimensional datasets were of tubes which had received total doses of no more than ~15 electrons per Å^2. Optical adjustments were performed at a nominal magnification on the screen of ×90,000 to minimize variability in focus level from one image to the next. Tube images were evaluated by optical diffraction (see ref. 13) for the extent of structural preservation and degree of flattening, and then evaluated further by computer. For the computer analysis, densitometry and Fourier transformation of the central portion of the tubes were as described previously[13]; criteria for selection of images for incorporation into the three-dimensional datasets included the strength of the diffraction maxima above background and the accuracy with which they fit a regular two-dimensional net. Also, areas of interest were considered unsuitable if the corresponding under-focus values (see below) were outside the range 8–16,000 Å. Variability in lattice parameters between tubes necessitated the use of special methods to derive accurate values of tilt angle and axis. All images were recorded in pairs. With the frozen specimens (*a*) the second image of each pair was of the carbon support film only, after intensifying the electron beam to sublimate the ice. Diffraction patterns from these images displayed a series of concentric rings[24] extending to spacings <10 Å. Defocus values could be measured locally with an accuracy of ±250 Å over a wide range, based on matching such patterns against a calibrated set. Tilt parameters were computed from the defocus values measured at the four corners of each film. Typical errors, estimated by comparing values from the four alternative planes passing through the different sets of three points, were 2° in tilt angle and 4° in direct of tilt axis, slightly larger errors applying to smaller tilts (<10°). With the stained specimens (*b*), the second image was recorded (with negligible in-between irradiation) at another angle of tilt, differing from the first by a pre-selected amount (typically 15°). Tilt parameters were computed from the changes in reciprocal lattice geometry between the two images. In this case the independent values obtained from the two sides of each tube could be compared, and the errors indicated were found to be of similar magnitude as in the first method.

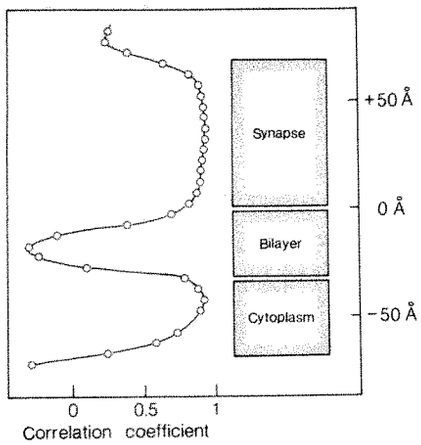


Fig. 3 Plot of correlation coefficients obtained by comparing sections from the three-dimensional maps of the frozen and stained tubes. The ordinate indicates the various levels through the structure, perpendicular to the membrane, at which the values were calculated. The hydrophobic core of the bilayer is identified by a zone showing poor correlation. The synaptic and cytoplasmic domains show good correlation over most of their extent; the poorer correlation near the extremities of the structure may be due to slight flattening of the stained receptors in the direction normal to the membrane plane.

Exact correspondence, particularly at the extremities of the structure, is not expected because of minor alterations resulting from, for example, drying of the stain[15].

Structure of the receptor

The three-dimensional map calculated from the frozen tubes (Fig. 4) shows details of the entire receptor molecule, both in the lipid bilayer and in the aqueous surroundings on either side. The molecule is resolved into five rod-shaped subunits (Fig. 4*a*) of about equal cross-section, which lie predominantly perpendicular to the membrane plane. The five subunits are contained within a ~140-Å-long by ~80-Å-diameter cylindrical shell, and delineate a water-filled opening, presumed to be the channel, along the axis of this cylinder.

This map does not distinguish between the portion of the oligomer embedded in the bilayer and those protruding outside, because lipid and water contribute about equally to the image contrast at low resolution. The portions, however, can be identified by correlation with the map from the stained tubes. A ~ 30-Å zone in that map appears almost featureless—neither the channel nor the surrounding protein is visible—and must correspond to the hydrophobic core of the bilayer, which is inaccessible to the stain. Consequently, there is a length of ~30 Å, the correlation coefficients of which have particularly low values (Fig. 3), and the matching of structural detail is poor (Fig. 5*b*) compared with that on either side (Fig. 5*a*, *c*). Thus the correlation places the hydrophobic core nearest to the cytoplasmic end of the structure, leaving a length of 35–45 Å protruding on this side, compared with ~70 Å on the other.

The channel is nearly 30 Å wide at the synaptic end of the oligomer (Fig. 4*b*, uppermost), but becomes too narrow at the cytoplasmic end to be seen. The way in which the channel narrows cannot be discerned accurately at the resolution achieved in the present study, but the abrupt exclusion of the anionic stain at the level of the bilayer surface, on the synaptic side, suggests that a large reduction in its diameter occurs in this region.

The cross-sectional area of the membrane-spanning portion of the subunits is of interest because of the different models that have been proposed for the internal structure of this portion. To estimate this value, we assumed the molecular boundary to be at the contour level for which the total enclosed volume of glycosylated protein is 350,400 Å^3, calculated from the composition[8]. The corresponding cross-sectional area per subunit was 540 Å^2, averaged over the extent of the bilayer, whereas for the

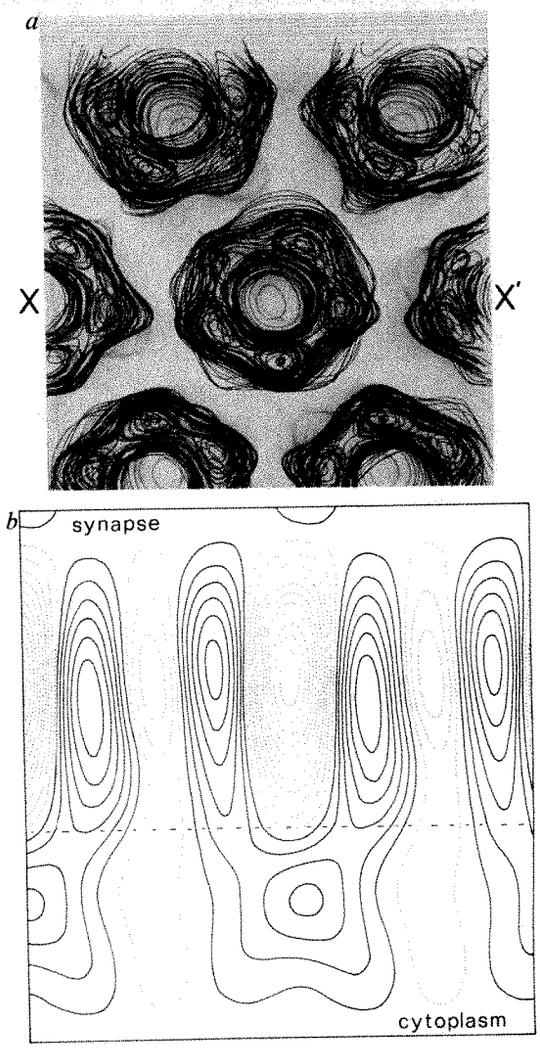


Fig. 4 *a*, Three-dimensional map of receptor molecules in the crystal lattice as they would appear viewed from the synaptic cleft; successive sheets are of sections parallel to the membrane plane separated by spaces corresponding to 5 Å. The centre-to-centre separation between receptors in the horizontal direction corresponds to 90 Å. *b*, Section along the line XX′ in *a*, through the channel axes and perpendicular to the plane of the sheets; the central plane of the bilayer is indicated by the broken line. The phosphotungstate stain is excluded from the channel at a level ~15 Å above this line. Only contours corresponding to regions in which protein is concentrated are shown in *a*; all contours are shown in *b*. The outermost, full-line contours in *a* and *b* are close to the zero contour in Fig. 5 and represent the molecular boundary consistent with the volume calculated from protein composition.

synaptic and cytoplasmic portions it was 550 and 375 Å^2, respectively. As an approximate check on the value for the cross-section in the bilayer, a three-dimensional map was also calculated with approximate terms included for the central (0, 0) lattice line. These terms were derived from transforms of the striated border regions of tubes, as in Fig. 1*a*, using the (1, 2) lattice line (associated with the striation periodicity) to set the scale for the amplitudes and the origin of the phases. From the molecular boundary now on an absolute scale, a mean value of 500 Å^2 was obtained for the subunit cross-section in the bilayer, in reasonable agreement with the estimate based on protein composition.

Pentagonal symmetry

The five subunits are arranged regularly around the central axis of the receptor for much of its length, so that the structure has approximate pentagonal symmetry about this axis. The r.m.s. departures from exact symmetry are, respectively, 1.2 and 1.4 Å in radius and azimuth averaged over the portion of the protein

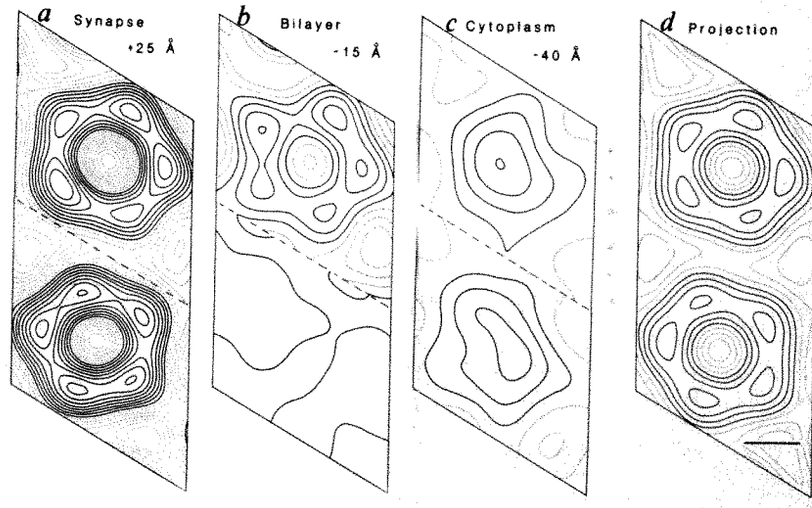


Fig. 5 *a–c*, Sections at different levels through a unit cell from the three-dimensional maps; *d*, the projected structure in frozen solution. The pair of receptors shown is thought to correspond to the δ–δ subunit linked physiological dimer[13]. In *a–c* the regions and levels indicated relate to Fig. 3; contours in the upper and lower halves refer to the frozen and the stained tubes, respectively; the two halves are related by symmetry as in *d*. The positive and zero contours are indicated by continuous lines, the negative contours by dotted lines; data from the (0, 0) lattice line, not included in the Fourier syntheses, have only a minor effect on the position of the zero contour. The scale bar (bottom right) corresponds to 25 Å.

in the hydrophobic core of the bilayer, and 0.8 and 1.9 Å averaged over the contiguous ~30-Å portion on the synaptic side. The total extent of this symmetrical region comprises about half of the length of the molecule, sufficient to dominate its appearance in projection (Fig. 5*d*). Nearer to the extremities, particularly on the cytoplasmic side, the pentagonal symmetry is not as marked, suggesting more profound structural differences between individual subunits in these regions? On the cytoplasmic side the distribution of mass about the axis of the oligomer is asymmetrical (Fig. 5*c*) and the cross-section is smaller, as if the parts of the subunits here have different lengths.

Discussion

The tubular crystals have led to a quantitative description of the quaternary structure of the acetylcholine receptor in a natural lipid-containing environment. The receptor is a cylindrical molecule having almost constant lateral dimensions (mean diameter ~65 Å) except for the part on the cytoplasmic side of the bilayer, which is narrower. It is composed of five rod-shaped subunits, each up to ~140 Å long. They lie approximately perpendicular to the membrane plane and are asymmetrically placed in relation to the bilayer, having two to three times more of their combined mass on the synaptic than on the cytoplasmic side. Subunits are similar in cross-section and are arranged symmetrically around a central opening, the presumed channel through which cations diffuse. The channel is partitioned equally into a wide (25–30-Å diameter) portion at the synaptic end of the structure and an unresolved narrow portion extending through the membrane into the interior of the cell.

In previous studies, electron images of stained molecules, viewed *en face* and from the side, have been used to deduce the overall shape of the protein and channel[16,17,25–27]. Morphological observations have also been combined with X-ray or neutron diffraction data to obtain models for the cylindrically averaged distribution of mass[18,19]. Seen most directly in frozen solution, the molecule appears more elongated and more angular in shape than has generally been supposed.

Identification of the membrane-spanning portion of the structure (Fig. 3) partitions the protein mass on either side in a way that is consistent with models inferred from the amino-acid sequences. However, the probable correctness of different models cannot be evaluated on this basis because of the possibility that the extrinsic cytoplasmic protein of apparent molecular mass 43,000 (ref. 20), thought to be present in the tubes, is bound regularly to the crystallized receptor and contributes some extraneous mass.

A striking aspect of the entire oligomer, previously suspected[1] but not seen until the present study, is that the subunits are arranged in almost equivalent positions around the channel, conferring on the structure an apparent high degree of pentagonal symmetry. This is most exact in the lipid bilayer and over a contiguous ~30 Å region on the synaptic side, and may be even more extensive than observed because minor errors in our data would make it less pronounced. Such symmetry, combined with the observed amino-acid sequence homology, makes it probable that the individual subunits share common features of tertiary organization over the lengths that they are in contact. These properties could be important in facilitating a coordinated movement of all subunits around the channel, such as may occur in switching between different transitional states[8].

The resolution of the analysis described here is insufficient to elucidate the internal structure of the subunits, but the observed details are consistent with models speculating that their membrane-spanning portions are composed of equal numbers of closely packed α-helical rods, or other configurations of the polypeptide chain[28]. The value suggested for the subunit cross-section in this region, 500–540 Å^2, favours models with five α-helices extending through the membrane[10,12] (assuming, for example, a packing similar to that in bacteriorhodopsin[21]).

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Direct evidence using tritium data for throughflow from the Pacific into the Indian Ocean

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Basin-wide exchange between the Pacific and Indian oceans through the Indonesian Archipelago has received attention, both in relation to the oceans' role in the Southern Oscillation[1] and in efforts to balance the salt and mass fluxes of the individual basins[2–5]. Wyrtki[6] made the first indirect estimate using hydrographic data of a mean annual transport from the Pacific to the Indian Ocean of 2×10^6 m^3 s^{-1} in the upper 200 m. Recent estimates have been considerably higher: 14×10^6 m^3 s^{-1} by Piola and Gordon[2], 10×10^6 m^3 s^{-1} by Godfrey and Golding[5], and 5×10^6 m^3 s^{-1} by Godfrey and Ridgeway[3]. However, Wunsch *et al.*[4] concluded that, as a result of inverse calculations on sections across the South Pacific, there was a negligible throughflow transport. A meridional maximum in bomb-produced tritium (half-life 12.4 yr) observed in the South Equatorial Current (SEC) of the Indian Ocean is used here to show direct evidence that in the mean there is a net transport (in the upper 300 m) of 5×10^6 m^3 s^{-1} from the Pacific into the Indian Ocean.

The use of bomb tritium, which is found in the oceans in the form of HTO, has become increasingly popular[7–9] as a conservative tracer for oceanic circulation processes with decadal time scales. Decay-corrected tritium data collected during the Geochemical Ocean Sections (GEOSECS) Expeditions[10] are anomalously higher in the South Indian than either the South Atlantic or South Pacific oceans. The presence of the Indian subcontinent at 25° N rules out a North Indian Ocean source, the asymmetric fallout pattern[11,12] rules out a Southern Hemisphere source. Toggweiler and Trumbore[13] found anomalously high bomb-produced ^{90}Sr in Indian Ocean corals which they attribute to throughflow of North Pacific water.

Figure 1 is a map showing tritium at the surface of the Indian Ocean[10]. Pre-bomb surface values may be considered to be <1 TU (1 TU = $10^{18}\times$mole fraction of (^{3}H/^{1}H)). Experimental error is <0.1 TU. Tritium values are highest (>6 TU) in the Bay of Bengal as result of runoff; yet the high values are confined to a shallow surface layer. Contrast the Arabian Sea where the highest evaporation in the Indian Ocean occurs, surface waters there contain 2.5 TU. In the Southern Hemisphere, the highest tritium values are found in the westward-flowing SEC located between 10 and 20° S. Tritium >3.5 TU is found from about 5° N to 30° S, and it coincides fairly well with an intrusion of low salinity observed throughout the year[14]. The tritium contours in Fig. 1 generally follow an east-west pattern because the first order transport of water is advection by the zonal wind-driven currents.

Figure 2 is a map showing tritium at 200 m in the thermocline of the Indian Ocean[10]. Tritium values have decreased to <1 TU in the Bay of Bengal showing that high tritium in the runoff there is confined to a shallow surface layer. Its effect is only local because of dilution during subsequent mixing with low ambient levels of tritium. The zonal patterns at 200 m are similar to those at the surface; highest values are shifted slightly polewards. This is probably due to poleward contraction of the gyre with depth[15], and perhaps also downwelling south of 15° S. Thus, at the time of sampling, bomb tritium had already mixed well into the thermocline of the subtropical gyre resulting in a maximum in the region of the SEC as a prominent feature down to 300 m. Most of the transport in the SEC occurs above 300 m (ref. 14).

The intrusion of low salinity/high tritium water into the South Indian Ocean is due to a combination of throughflow from the North and South Pacific, and precipitation[6] which is very high near Indonesia. In general, twice as much tritium makes its way into the oceans through molecular exchange as through rain[12,16]. In regions where precipitation and runoff are high, unusually low salinities and high tritium will be found, for example, the Bay of Bengal. However, the high tritium in the Bay of Bengal is confined to a shallow surface layer and has only a local effect. In addition, the tritium measured in precipitation in the few years before the GEOSECS Indian Ocean expedition was twice as high over the Bay of Bengal as that measured in precipitation over Indonesia[17]. This would result in even lower surface ocean tritium in the component from precipitation than in the Bay of Bengal runoff water. Thus, precipitation is not the main source for the low salinity/high tritium intrusion.

Wyrtki[6] concluded from hydrographic data that, even though the surface currents in the Indonesian Archipelago are subject to monsoonal variations, in all layers (except for the oxygen minimum) movement of water from the Pacific (via the Timor Current) to the Indian predominates. The transport has decreasing intensity with depth. Although the upper waters in the western tropical South Pacific contribute to the throughflow, they are too low in tritium[8] and high in salinity[18] to be a major source. On the other hand, GEOSECS tritium in the Pacific North Equatorial Current (NEC)[8] was twice as high as the South Indian Ocean. Maps by Wyrtki[6] and Tsuchiya[14] show some of the westward-flowing NEC turning south near Mindano, both feeding into the North Equatorial Counter Current and

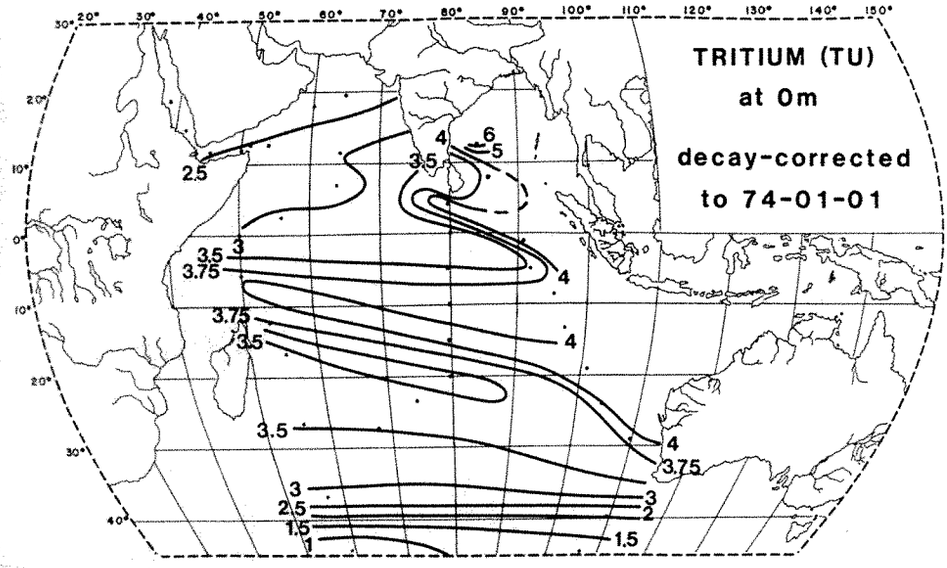


Fig. 1 Map of tritium (TU) at the surface of the Indian Ocean decay-corrected to 74-01-01. Data were collected as part of the GEOSECS program and analysed by Ostlund and Brescher[10]. •, Station locations.

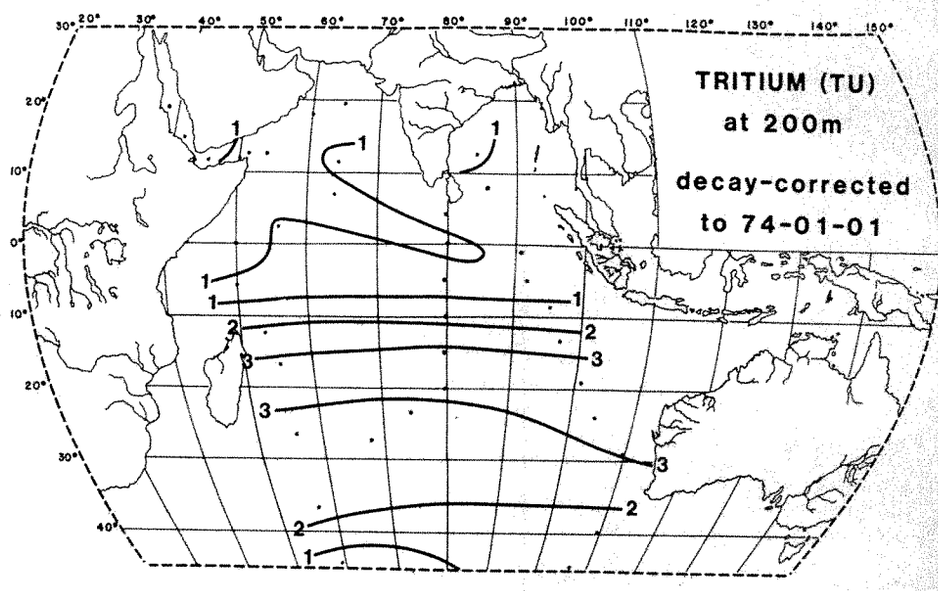


Fig. 2 Map of tritium (TU) at 200 m in the Indian Ocean decay-corrected to 74-01-01. Data were collected as part of the GEOSECS program and analysed by Ostlund and Brescher[10] (1982). •, Station locations.

entering the Indonesian Archipelago. Temperature and salinity near Mindano correlate with those in the western basins of the archipelago[5,6], in agreement with the idea of the North Pacific being a major source for the low salinity/high tritium intrusion in the Indian Ocean.

The additional information obtained when tritium is used as a tracer for oceanographic processes is particularly important for this problem because it provides an integrated picture of the decadal mean circulation. Tritium data[10] are combined with annual averages of temperature and salinity[19] into a box model to calculate the net transport from the Pacific into the Indian Ocean. The meridional extent of the box is chosen from 10 to 20° S and the depth range is 0-300 m; that region is fairly homogeneous in water mass properties and it encompasses most of the transport of the SEC. The eastern and western extent of the box are determined by geography—the Indonesian Archipelago and Madagascar.

The box model calculation[20] assumes that mass and chemical balances within the box are entirely consistent with pure advection. The first-order physics are:

$$u\frac{\partial C}{\partial x}+v\frac{\partial C}{\partial y}=\frac{\partial C}{\partial t}-\lambda(C) \qquad (1)$$

where according to convention u is the advection in the east (x)/west direction, v is the advection in the north (y)/south direction, C is the concentration, t is time, and λ is the decay term for tritium. If equation (1) is integrated over the volume (V) of the box then:

$$C_N v_N A_N + C_S v_S A_S + C_E u_E A_E = \frac{\Delta C}{\Delta t} V - \lambda C V \qquad (2)$$

where the C_is represent the average concentrations and the A_is represent the areas along the boundaries of the box. Table 1 gives the actual values for C_is which were obtained by integrating the data[10,19] observed along the boundaries of the box. Equation (2) is written for volume, salinity, temperature and tritium, giving a total of four equations and three unknowns. Using a least squares regression it may be solved for the advective velocities: v_N, v_S and u_E.

The air-sea exchange ($\Delta C/\Delta t$) may be evaluated using literature values. The net freshwater loss to the atmosphere by the Indian Ocean between 10 and 20° S calculated by Baumgartner and Reichel[21] is 0.14×10^6 m^3 s^{-1}. The heat flux between 10 and 20° S in the Indian Ocean estimated by Talley[22] was a net loss from the ocean to the atmosphere of 2 W m^{-2}.

Rates of addition of tritium by precipitation and vapour exchange to the South Indian Ocean are given in 5° latitude bands by Weiss and Roether[12]. When averaged over the time of the bomb transient, the deposition rate for the region 10-20° S is balanced by tritium decay to within the 30% error given for the deposition rate. Although subsurface observations of tritium are only available from the GEOSECS expedition for the Indian Ocean, there are observations spanning at least 10 yr in the NEC, which is the source region for the low salinity/high tritium intrusion. After 1974, decay has balanced input in the NEC[23], because we were already on the flat part of the deposition curve. Therefore, it is reasonable to consider the surface and subsurface tritium in a steady state.

The results of the regression together with the statistical errors in the coefficients are given in Table 1. The net transport into the box across the eastern boundary corresponds to the throughflow from the Pacific into the Indian Ocean; it is $5.1\pm0.4\times10^6$ m^3 s^{-1}. There is a small net northwards transport across 10° S (out of the box) of $-2.4\pm0.1\times10^6$ m^3 s^{-1} and a small net southwards transport across 20° S (out of the box) of $-2.6\pm0.4\times10^6$ m^3 s^{-1}. The north/south transports are difficult to assess physically as they encompass the surface Ekman poleward flow and part of the equatorward convergent geostrophic flow, as well as the branching of the SEC at its western terminus. A possible explanation for the integrated net northwards flow across 10° S could be related to the fact that Madagascar extends only to 10° S. Schott (personal communication) has recently found a strong northwestwards current at the northern tip of Madagascar.

Using the same data as described above, the model was run for a box 0-100 m deep encompassing the mixed layer. The

Table 1 Average concentrations and resultant net transports calculated from a box model for the region 10-20° S in the Indian Ocean

	N(10° S)	S(20° S)	E
0-300 m			
C (temperature, °C)	18.57	20.61	20.42
C (salinity, ‰)	34.85	35.39	34.43
C (tritium, TU)	2.4	2.5	3.0
Area (10^8 m^2)	20.7	19.8	2.9
Transport (10^6 m^3 s^{-1})	−2.4	−2.6	+5.1
Error (10^6 m^3 s^{-1})	±0.1	±0.4	±0.4
0-100 m			
C (temperature, °C)	25.66	24.30	25.70
C (salinity, ‰)	34.80	35.20	34.20
C (tritium, TU)	3.5	3.6	3.8
Area (10^8 m^2)	6.9	6.6	1.0
Transport (10^6 m^3 s^{-1})	±1.7	−3.6	+2.0
Error (10^6 m^3 s^{-1})	±0.7	±0.2	±0.5

Positive (negative) transports indicate a net flow into (out of) the box across that boundary.

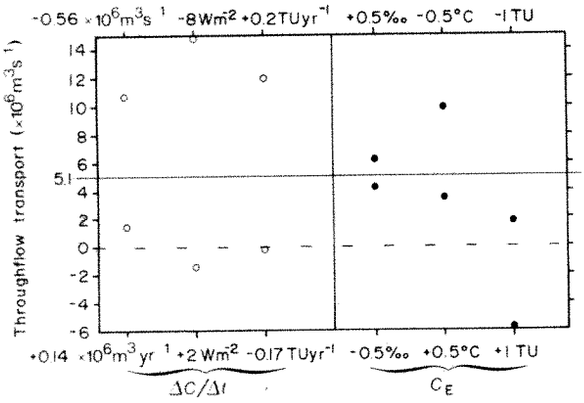


Fig. 3 Comparison of the box model result of 5.1×10^6 m^3 s^{-1} throughflow to variations in: ○, air-sea flux parameters ($\Delta C/\Delta t$) ($\Delta C/\Delta t$ is varied for: freshwater, heat, and tritium input); and ●, concentrations along the eastern boundary of the box (C_E) (C_E is varied for salinity, temperature, and tritium).

results of that regression are also included in Table 1. The net transports across both 10° S ($1.7 \pm 0.7 \times 10^6$ m^3 s^{-1} into the box) and 20° S ($-3.6 \pm 0.2 \times 10^6$ m^3 s^{-1} out of the box) are southwards; this is consistent with theories of a surface Ekman poleward flow. There is a net transport into the box across the eastern boundary of $2.0 \pm 0.5 \times 10^6$ m^3 s^{-1}. This calculation, which gives only 2×10^6 m^3 s^{-1} for the throughflow (0–100 m), shows that more than half of the transport calculated for the box 0–300 m is below the mixed layer.

Figure 3 shows the sensitivity of the throughflow transport to uncertainties which are at least several times the errors in the data. At either extreme in the balance between the tritium decay rate (-0.17 TU yr^{-1}) and deposition rate[12] (0.20 TU yr^{-1}), the effect is to decrease to nearly zero or double the throughflow transport. The uncertainties in the freshwater and heat air-sea fluxes are unknown, although it is unlikely that the directions of the fluxes are unknown. A fourfold increase in these fluxes results in more than a doubling of the throughflow transport. Varying concentrations along the eastern (Fig. 3), northern and southern boundaries of the box result in net positive throughflows, except when C_E and C_N for tritium are increased by 30% (1 TU). These sensitivity tests always result in a net northward transport across 10° S; this is consistent with observations of a northwestward current at the northern tip of Madagascar (F. Schott, personal communication). The tests also result in a positive correlation between throughflow and southwards transport across 20° S; this is consistent with the assertion by Piola and Gordon[2] that the upper layer throughflow transport must be balanced by southwards flow across 35° S in the Indian Ocean.

A throughflow transport exceeding 10×10^6 m^3 s^{-1} is unlikely. Godfrey and Golding[5] pointed out this high a transport would require large upper-layer velocities >1 m s^{-1} in some of the Indonesian passages; these have not been observed. In addition, the throughflow would then amount to 30–50% of the total volume transport for the SEC which is $30\text{–}40 \times 10^6$ m^3 s^{-1} (F. Schott, personal communication). Because of the high tritium intrusion, it is also unlikely that there is no net throughflow transport or even that it is reversed. When the regression was run for three equations and three unknowns, successive omission of salinity, heat and tritium gave throughflows respectively of 2.0, 13.4 and 10.9×10^6 m^3 s^{-1}; direction of the transports across the other two boundaries remained unchanged. Salinity has the most redundancy, that is freshwater, heat and tritium are the three most linearly independent parameters. These sensitivity analyses suggest that the box model calculation of 5×10^6 m^3 s^{-1} for the throughflow transport is probably good to within ±50%.

The tritium data show a direct link between the Pacific and Indian oceans. The interannual variability in the throughflow could have direct implications for climate because it is coincident with a warm pool of 28 °C or higher[14]. The warm pool is found to extend from the western Pacific through to the Indian Ocean. The unique relationship of the Southern Oscillation to sea-surface temperature (SST) in the region extending from the warm pool southwards to northern Australia[24,25] has been shown to have a high correlation[26]. In addition, SSTs in that region have been shown to have an influence on the monsoon[27,28], because the largest region of deep atmospheric convection occurs over the warm pool. Changes in intensity and location of this convection zone are related to variability in both the global atmospheric circulation and the monsoon, and are probably also influenced by changes in the underlying warm, low-salinity throughflow water.

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Importance of thrust faulting in the tectonic development of northern Victoria Land, Antarctica

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The present configuration of Wilson Group, Bowers Supergroup and Robertson Bay Group in northern Victoria Land (Fig. 1) has been attributed to high-angle block-faulting[1–3] or more recently[4] to major strike-slip faulting along the Lanterman and Leap Year Fault zones. In this latter interpretation[4], adjacent units have allochthonous relationships and constitute separate geological terranes. We offer additional support for the concept of allochthonous terranes in northern Victoria Land but suggest here an alternative

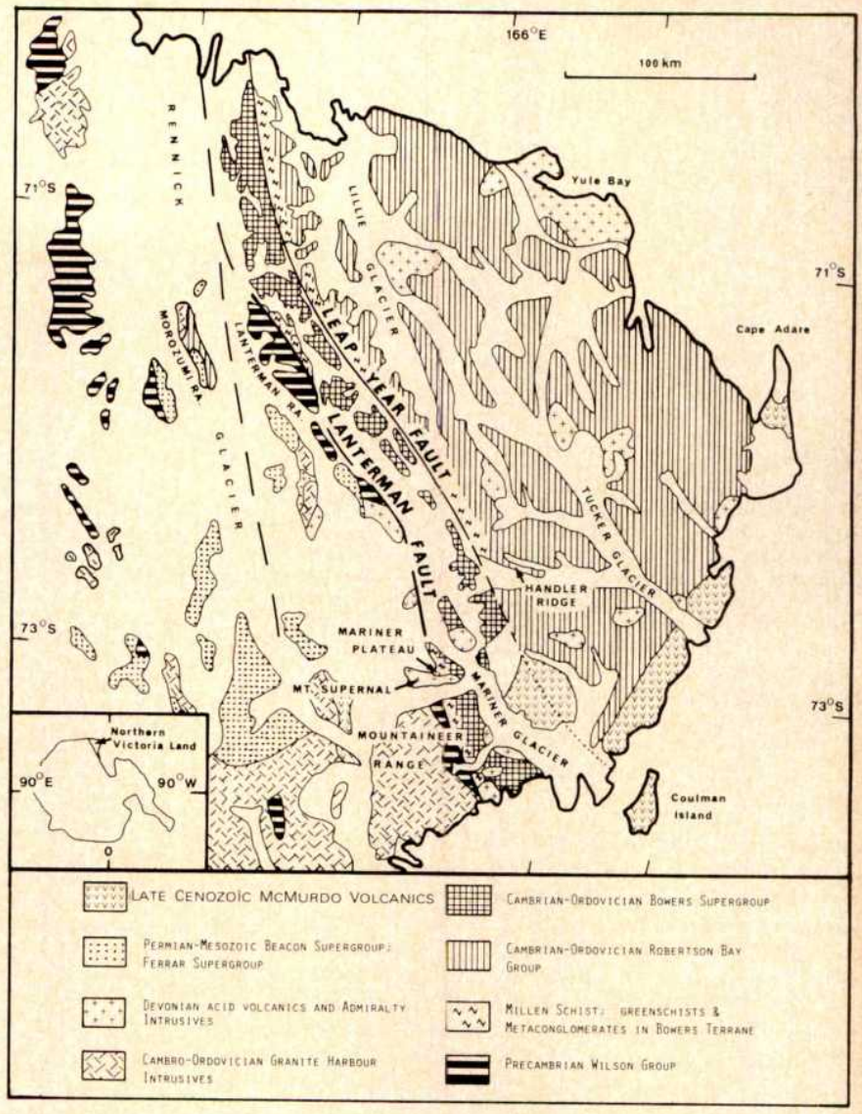


Fig. 1 Geological map of northern Victoria Land (compiled from data in refs 1, 3, 4, 12).

model to explain terrane juxtapositions. Our model emphasizes the role of thrust tectonics and implies severe structural telescoping of entire terranes with significant loss of intervening crust. We further suggest that this occurred during the Lower Palaeozoic Ross Orogeny in response to the collision of Antarctica with a westwardly directed eastern continental block. This requires that the northern Victoria Land segment of Gondwana was a zone of major plate convergence throughout the Cambrian–early Ordovician period.

The Wilson terrane contains mainly (?Precambrian) amphibolite facies gneisses intruded by Lower Palaeozoic S-type granitoids (Granite Harbour Intrusives[5,6]). These gneisses have undergone four phases of deformation[7–9], the first two of which were accompanied by intense flattening and development of boudinage. A conspicuous stretching lineation defined by elongated mineral aggregates is widespread throught the Lanterman Range and also occurs in Wilson Group gneisses immediately west of the Bowers Supergroup in the Mountaineer Range. In the Lanterman Range, this lineation originated during the second phase of deformation and everywhere plunges steeply down-dip within the north-west-trending regional foliation (Fig. 2*a*). The third and fourth phases of deformation in the Lanterman Range were associated with retrogressive metamorphism to the greenschist facies and the widespread development of crenulation structures[7,8].

The Bowers terrane contains a Cambrian–early Ordovician regressive sequence[2,10] which has at its base the Sledgers Group. Sledgers Group comprises two mutually interdigitating units[10]: the Molar Formation composed of marine volcanogenic sediments and the Glasgow Volcanics consisting of pillow lavas, basaltic and andesitic breccias, and subordinate dacite–rhyolite[4,10]. Basic rocks within the Glasgow Volcanics are interpreted [4] as primitive island arc tholeiites.

Overlying[2,10] the Sledgers Group are the non-volcanogenic, shallow or marginal marine, Mariner Group and the non-marine Leap Year Group.

Two other units are also included here in the Bowers terrane; the Lanterman and Husky Conglomerates, both of which occur only in the Lanterman Range along the western margin of the terrane. They are characterized[7] by intensely flattened pebbles and provide important clues about the nature of tectonism along the contact between the Bowers and Wilson terranes. Other conglomerates with severely flattened, or sometimes even stretched, pebbles occur in the Molar Formation immediately south of the Meander Glacier in the Mountaineer Range[3,11,12].

The Bowers Supergroup was mainly metamorphosed under prehnite–pumpellyite or pumpellyite–actinolite facies conditions[13] and generally underwent folding about gently plunging, north-west-trending axes[13,14]. Multiply-deformed greenschist facies rocks are restricted[7,11,12] to a narrow (2–4 km) belt immediately east of the contact with Wilson Group (Fig. 1). Highly strained conglomerates in this belt exhibit characteristics of both flattening and constrictional deformation[7,11]. Fold axes within this belt also show extremely variable plunge, sometimes steepening quite markedly over short distances, although they always maintain a constant north-west orientation within their axial surfaces (Figs 2*b*, *d*–*f*). This suggests non-cylindrical folding, possibly even the presence of sheath folds. Where

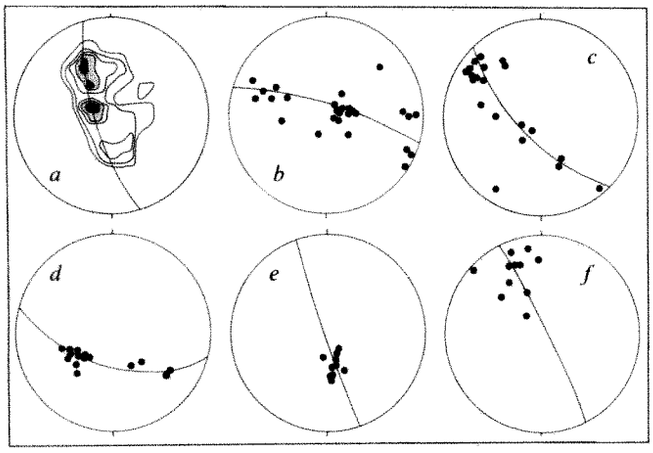


Fig. 2 Equal area stereographic projections of regional structures in the different terranes of northern Victoria Land. Solid lines, dominant schistosity or cleavage; dots, lineations and fold axes except in *a* which includes 112 stretching lineations from Wilson Group gneisses in the Lanterman Range. Contour interval is 1%, 3%, 6%, 9% and 12% per 1% area. *b*, Bowers terrane immediately east of Wilson terrane in the Lanterman Range. *c*, Robertson Bay terrane, data from ref. 14. *d–f*, Bowers terrane; *d*, belt of greenschist facies rocks immediately east of contact with Wilson terrane; *e*, greenschist facies rocks on the Mariner Plateau; *f*, virtually unmetamorphosed Bowers supergroup from area in the lower Mariner glacier. Shading accentuates contour interval.

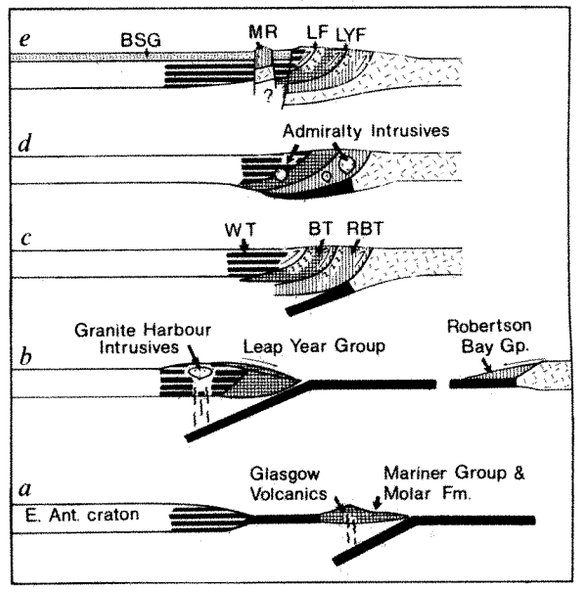


Fig. 3 Tectonic model for northern Victoria Land. *a*, Middle–late Cambrian: development of Glasgow Volcanic arc concomitant with the deposition of Molar Formation and the Mariner Group in a forearc basin. To the east, Robertson Bay Group is deposited as a clastic wedge with continental provenance. *b*, Late Cambrian–early Ordovician: following accretion of the Glasgow Volcanic arc onto the Gondwana continental margin, the centre of magmatic activity migrated westward leading to the intrusion of the Granite Harbour Intrusives and the penecontemporaneous deposition of Leap Year Group (following uplift and erosion of granites). *c*, Ordovician: Ross Orogeny with substantial crustal thickening and underthrusting of one terrane beneath the next. Deformation and regional metamorphism with the Bowers Supergroup; retrogressive metamorphism within the Wilson terrane. *d*, Devonian–Carboniferous: intrusion of Admiralty Intrusives. *e*, Post-Ferrar Supergroup Rennick block faulting[1]. Differential uplift and erosion in the Morozumi Range remove the overlying Wilson and Bowers Supergroup rocks to create a tectonic window in which Robertson Bay Group sediments are exposed. Abbreviations: BSG, Beacon Supergroup; BT, Bowers Terrane; LF, Lanterman Fault; LYF, Leap Year Fault; MR, Morozumi Range; RBT, Robertson Bay Terrane; WT, Wilson Terrane.

asymmetrical folds have been observed[9,11], they generally indicate tectonic transport to the north-east, at right angles to the structural trend of the Bowers terrane. Since the Leap Year Group contains only one-fold generation, and locally[10,11] rests unconformably upon Sledgers Group (without intervening Mariner Group), some deformation of Bowers rocks evidently occurred prior to deposition of the Leap Year Group.

The Robertson Bay terrane contains mainly marine sediments of continental provenance[1,2,15,16]. Along with Bowers Supergroup, these rocks were intruded by the Devonian–early Carboniferous Admiralty Intrusives which possess I-type characteristics[3,17,18]. Although formerly considered to be Precambrian or possibly Cambrian in age, the recent discovery[19,20] of Ordovician fossils in the Robertson Bay Group at Handler Ridge makes a Cambrian–early Ordovician age more likely.

The structural relations of the Robertson Bay Group and Bowers Supergroup are strikingly similar. To the east, weakly metamorphosed[21,22] Robertson Bay Group contains[14,21,23] a single phase of upright, variably plunging folds which trend north-west (Fig. 2*c*), whereas close to the Leap Year Fault there is a belt of multiply deformed greenschist facies rocks (Millen schists)[23,24]. These schists occupy a structural position comparable with that of the greenschist facies rocks along the western margin of the Bowers terrane. Both belts lie immediately east of major tectonic boundaries: the Leap Year and Lanterman Faults respectively. This implies a genetic as well as spatial relationship between the faults and greenschist facies rocks.

The Leap Year Fault has been traced[3,25,26] across virtually the entire length of northern Victoria Land. Recent mapping[11] suggests the Lanterman Fault (type locality[14,25] in the Lanterman Range) extends southwards into the Mountaineer Range. In both areas, Wilson Group rocks are thrust[7,11] against the Bowers terrane (Fig. 1). Moreover, on the Mariner Plateau (Fig. 1), the Lanterman Fault is cut by one of the Devonian Admirality Intrusives (Mt Supernal Granite). Mylonites in this same region partly recrystallized during the associated contact metamorphism. Because the country rocks had also undergone regional metamorphism and deformation before granite emplacement[11], we infer that both the thrust faulting and the regional deformation and metamorphism are manifestations of a pre-Devonian event: the Lower Palaeozoic Ross Orogeny.

The Lanterman and Leap Year faults both possess features compatible with major strike-slip faults. They have straight traces, dip steeply and are marked by wide crush zones, up to 500 m wide in the case of the Leap Year Fault[3]. These few features aside, there is no additional evidence for strike-slip faulting. Both faults are better interpreted as major thrusts. Along the entire length of the Lanterman Fault in both the Lanterman and Mountaineer ranges, Wilson Group amphibolite facies gneisses are consistently juxtaposed against lower grade Bowers Supergroup rocks. The faulted contact between these two units, clearly visible north of Mitten Spur in the Lanterman Range, dips steeply south-west. Slickensides along this fault indicate that movement was directed towards the north-east[7]. Parallel, and presumably related, thrust faults occur[9,12] within the Mountaineer Metamorphics west of the Lanterman Fault. They too bring higher-grade rocks over lower-grade ones. A syn-metamorphic thrust fault has also been reported[23,24] in the Millen Range just east of the Leap Year Fault.

In the western Lanterman Range, Lower Ordovician Granite Harbour Intrusives are thrust over Permian tillites and slate[8]. Late-stage reverse faults also affect the deformed conglomerates in the eastern Lanterman Range[7]. Some thrusting clearly postdates the Ross Orogeny and may represent the reactivation of pre-existing structural discontinuities. Reorientation and/or modification of the earlier thrust faults seems likely.

Based on our interpretation that the Lanterman and Leap Year faults are major thrust boundaries, we present an alternative tectonic model for northern Victoria Land (Fig. 3). It is at variance with earlier ideas[3] of a Bowers graben and precludes significant strike-slip faulting[4] along the Lanterman and Leap Year faults during the early Palaeozoic. In our model, the present disposition of rock types is primarily the result of massive telescoping along the Lanterman and Leap Year faults with one terrane underplated beneath the next. Elements of a primitive island arc (Sledgers Group) were accreted onto the Gondwana margin before a continent-continent collision with significant loss of oceanic crust. The Robertson Bay Group represents an accretionary wedge brought into juxtaposition with the Bowers Supergroup from the east following closure of the intervening ocean. Our model not only implies allochthonous relationships between adjacent terranes but substantial crustal thickening beneath the Transantarctic Mountains during the Ross Orogeny.

Other attributes of our model include:

(1) It provides different source areas for the compositionally dissimilar Sledgers and Robertson Bay Group sediments.

(2) Structural telescoping and significant underthrusting of Bowers Supergroup below the Wilson terrane brought island-arc tholeiites (Glasgow Volcanics) within 6 km of the calc-alkaline Granite Harbour Intrusives and removes earlier objections[4] that the Bowers terrane is too narrow for a typical arc

(3) Fold vergence, the common structural trend of all three terranes, and the down-dip stretching lineation in Wilson Group gneisses are consistent with thrusting directed towards the north-east.

(4) Thrusting immediately following regional metamorphism and/or cessation of subduction emplaced hot Wilson Group rocks over the Bowers terrane. This produced higher temperatures (greenschist facies metamorphism) and the intense flattening observed in rocks along the western margin of the Bowers terrane. Similarly, the Millen schists east of the Leap Year Fault resulted from the thrusting of Bowers rocks over the Robertson Bay terrane.

(5) The Robertson Bay and Bowers terranes are underlain by oceanic crust which provided possible source rocks for the Admiralty I-type granites.

A weakness in our model is the lack of an appropriate suture zone between the Bowers and Robertson Bay terranes. The Leap Year Fault is one possible choice but no fragment of a subduction complex, mélange, blueschist terrane or ophiolite belt is preserved along its trace. An additional problem is the absence of continental crust east of the Robertson Bay terrane, although rocks of suitable age have recently been reported[27] on Surgeon Island in Yule Bay.

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Diagenesis of sediments beneath the Ross Ice Shelf and their sedimentary history

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Micropalaeontological studies of sediment cores collected from beneath the Ross Ice Shelf, Antarctica, give conflicting stratigraphical interpretations. The diatom flora initially indicated an *in situ* succession of later middle Miocene age[1,2], which was supported by the occurrence of benthic foraminifera[3]. However, subsequent diatom analyses record the presence of flora with mixed ages (mostly Miocene but also Pliocene and Pleistocene) throughout both of the two lithological units present in the cores. These flora are taken to indicate repeated reworking, which most recently occurred no earlier than the late Pleistocene[4]. These contrasting views have since been re-affirmed[5,6]. Our studies of sediment geochemistry show self-consistent patterns in organic carbon, organic nitrogen and sulphur diagenesis which suggest a Recent, *in situ* origin, at least for the upper unit.

The 47 Ross Ice Shelf Project (RISP) cores collected at site J9 (82°22′ S, 168°28′ W) in the 1977-78 field season have lengths ranging from 15 to 122 cm and contain an upper light olive-grey unit (9-30 cm thick) at the base of which is a thin (<1 cm) brown-orange, iron-rich layer, providing a sharp colour contrast with the darker olive-grey lower unit (>86 cm) thick[3]. Both upper and lower units consist of pebble-granule diatomaceous mud, soft and sloppy in the upper unit but firm and compact in the lower. Quartz, feldspars, clays and diatoms generally comprise >80% of the sediments and the only discernible mineralogical difference between the two units is the presence of minor chlorite and vermiculite in the upper unit[7].

Siliceous diatoms are the most abundant microflora and occur uniformly throughout the upper and lower units Calcareous benthic foraminifera also occur in the lower unit but are absent from the upper unit[1-4]. There are two conflicting views as to the age of the microflora. One view is that the diatoms are well-preserved, except in the upper unit, and indicate a middle Miocene age with the foraminifera in the lower unit being slightly older (early to middle Miocene). This view is consistent with the presence of an *in situ* middle Miocene succession, with the upper lithological unit representing an alteration product of the underlying sediment[2,7]. The alternative view crucially depends on the recognition of minor proportions of smaller, more delicate and poorly preserved Pliocene to late Pleistocene diatoms, which have been recorded amongst the predominantly middle Miocene flora throughout both units. The presence of flora with mixed ages is taken to indicate subglacial reworking, probably in the late Pleistocene (21,000-17,000 yr BP) and possibly also earlier[6]. Thus, there are controversial questions concerning the timing of deposition and the extent of reworking and alteration in the RISP cores, which are of importance in establishing the history of the West Antarctica Ice Sheet.

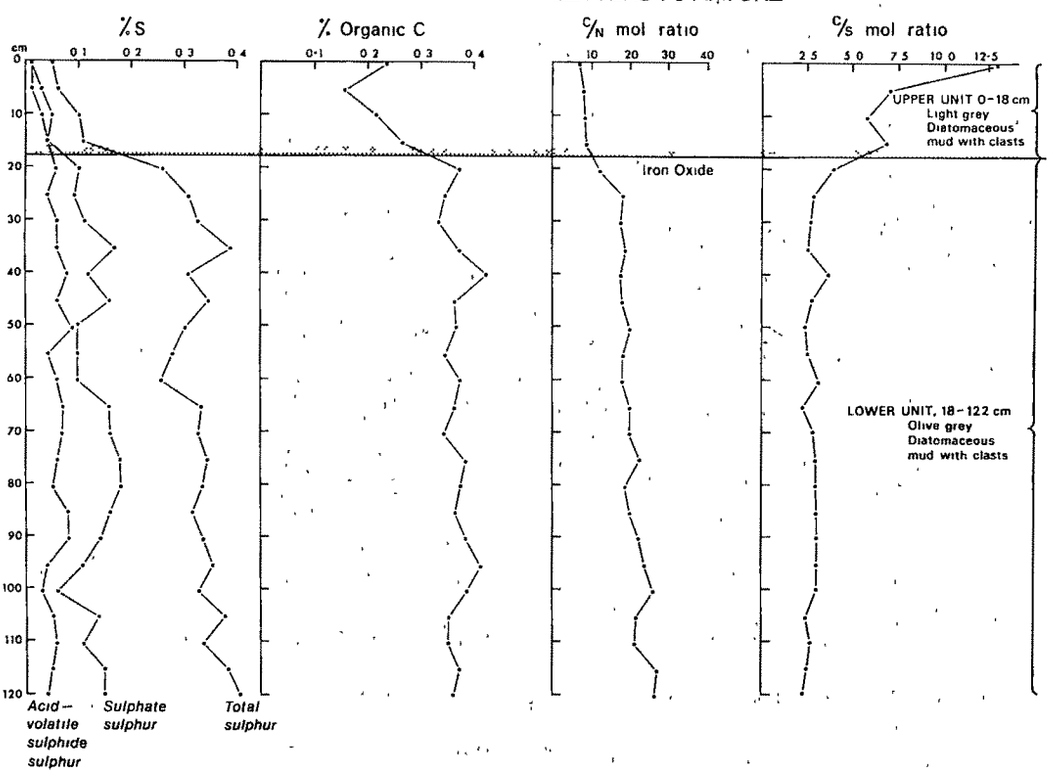


Fig. 1 Depth trends in S species, organic C and the C/S and C/N mole ratios in RISP core no. 12.

A study of chemical diagenesis was undertaken to clarify aspects of the sedimentary history. The longest RISP core, no. 12, was selected to define chemical gradients most accurately. Excess water was allowed to drain away before cores were frozen for transportation[3]. However, samples were received in a dry, unfrozen state and chemical effects may arise from sulphide oxidation and contamination by salts from dried porewater: the latter effect is small. This core provided 25 samples located at 5-cm intervals over 122 cm. These were crushed to <90 μm and analysed for organic C, organic N (by Hewlett Packard CHN analyser), acid-volatile sulphides and gravimetrically for total S and sulphate-S. Organic C and organic N determinations were made at 600 °C to minimize breakdown of carbonates[8]. Both results agreed closely with determinations at 1,200 °C following treatment with dilute HCl which removes calcareous material, exchangeable ammonium and also some fixed ammonium[9]. Organic C analyses in both cases also agreed closely with an alternative combustion method[10] and are consistent with those published elsewhere[11].

Water contents were measured before sampling (G. S. Boulton, personal communication) and decreased from 22.7% near the top of the core to 16.1% at the base. For porewaters of average seawater composition, marine sulphate could not exceed 0.01% S and most sulphate must arise from sulphide oxidation. Total S is accordingly treated as sulphide-S.

The depth profiles demonstrate the following differences in the amounts and compositions of the phases participating in diagenesis (Fig. 1). (1) Organic C (0.16–0.27%) and total S (0.05–0.11%) contents are both smaller in the upper unit than in the lower unit (organic C 0.34–0.42%, total S 0.26–0.41%). (2) The total sulphur, C/S and C/N ratios change progressively with depth through the upper unit and across the boundary, reaching near-constant values at depths >25 cm.

These features arise as a result of microbiological diagenesis of sedimentary organic matter, principally by sulphate reduction.

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

Bacterial sulphate reduction occurs only in the absence of oxygen but such conditions are common in marine sediments, which then allow anaerobic sulphate-reducing bacteria to oxidize metabolizable organic matter to dissolved carbonate species and so produce H_2S by the reduction of seawater sulphate. The H_2S reacts with detrital iron minerals to give black, acid-volatile iron sulphides which are then slowly converted to pyrite. Sulphide contents thus increase with depth (from low surface values) in Recent sediments deposited in conditions where depth and time of burial are related. Such gradients have been described in many different depositional environments[12–14]. Furthermore, in sediments deposited under an oxygenated water column, pyrite formation is apparently limited by the fraction of organic matter which can be metabolized. Thus continuing sulphate reduction is accompanied by a decreasing ratio of organic C to sulphide-S(C/S), which typically reaches a constant value at depths of <2 m, when metabolizable organic C is mostly exhausted[13–15]. Total organic C is a poor measure of metabolizable organic C in a sediment, since the latter depends on the extent of preservation in the water column and is also affected by benthic activity and terrestrial contributions[16]. However, most organic N is present in proteins which undergo rapid preferential destruction such that the C/N mole ratios of organic matter reach constant values, typically of about 15 or more, after only 1–2 m burial in Recent sediments[17,18].

Recent sediments deposited in a depth–time sequence thus show near-surface gradients of an increasing sulphide content, increasing C/N ratio and a decreasing C/S ratio. These gradients can provide a useful signature of age. The observed gradients in total S, C/N and C/S ratios in the RISP sediments are similar to those observed in Recent sediments and are also internally consistent, in that the addition of sulphide (gradients in total S and C/S) corresponds to a decrease in the metabolizable fraction of the organic matter (as indicated by the C/N gradient). A Recent origin, at least for the upper unit, is also indicated by the C/N ratios, which are close to the range in diatoms and plankton (5–6.6)[17]. Contributions from fixed ammonium may cause these values to be low, although most fixed ammonium is associated with illitic clays[19] which rarely exceed 30% of similar Ross Sea Miocene sediments[20]. The near-surface RISP samples have C/N values which clearly indicate that the sediment was mixed with fresh organic material on deposition. Such material typically survives near-surface anaerobic microbiological decay for between a few hundred to a few thousand years, as indicated by the time needed to reach uniform C/N and C/S values[13–17]. Diagenetic nitrogen loss is slower in oxic sediments (where it does not occur concomitantly with pyrite formation) but is still completed within <120,000 yr (ref. 19), except where

rates of bacterial metabolism may be limited by the diffusion of electron acceptors through a large thickness of sediment[21]. Thus, although the absence of benthic activity[22] and low metabolic rates in the water column[23] may diminish the decay of metabolizable nitrogenous material, it seems certain that the upper unit contains organic matter of Recent (or at the very oldest, late Pleistocene) origin. Radiocarbon ages support a Recent origin[4]. Recycled spores and pollen have been identified in RISP sediments[2,11], but the C/N ratios of plant material (13–26) are much larger than the C/N values in the upper unit, and recycled terrestrial material cannot be the main organic matter source.

The diagenetic gradients indicate that deposition of the upper unit has occurred postglacially, with fresh organic matter being incorporated during the reworking of older sediments. Thus the geochemical data are consistent with the occurrence of a reworking event which mixed Pliocene and Pleistocene fauna with the Miocene forms[4], but not with the existence of an *in situ* Miocene succession[1,2]. No reworking of the upper unit has occurred following deposition at the present site, otherwise no depositional or diagenetic variations would occur. In this respect, we agree with the conclusions based on ^{10}Be measurements, although these imply a pre-Quaternary origin at the latest for the upper unit[24]. Such a prolonged period of non-deposition is incompatible with the existence of reduced S species, and nitrogenous organic matter, at the sediment–water interface. Comparatively large ^{10}Be ages may result from diminished rates of ^{10}Be deposition at high latitudes and in the presence of an ice cover[25].

As the two units are so similar in detrital mineralogy, their main chemical differences arise as a result of alteration by *in situ* diagenesis which differs only in degree and not in direction. The extent of alteration by diagenetic sulphate reduction is mainly controlled by metabolizable organic matter concentrations and a fundamental difference between the two units is the lower organic C content of the upper unit, even though the diagenetic loss of organic C is not yet completed here. Hence, the lighter colour of the upper unit is probably due to a smaller content of black, iron monosulphides arising from both a lower initial organic C content and unfinished sulphate reduction. Complete diagenetic alteration of the upper unit would result in a sediment with less organic C and S than the lower unit, although the progressive C/N and C/S trends would suggest that the two units would ultimately be very similar in these respects.

The total S, C/S and C/N trends appear to pass through the iron oxide layer (at 18-cm depth) without major perturbation, but by 25 cm have closely approached the values at greater depths. The depth at which diagenesis is mostly complete thus lies only a little below the iron oxide layer and hence there are no unequivocal signals of depth–age relationships in the lower unit. However, the small but irregular trends of increasing total S and C/N values would be consistent with continuity of diagenesis through the iron oxide layer, and hence with a post-late Pleistocene origin for the whole core.

The iron-stained layer between the two units is a major chemical discontinuity whose temporal significance is unclear. No samples at this depth were analysed, but the small kink in the C/S ratio at 15–16 cm suggests a possible loss of S which, together with the presence of iron oxides, implies a discontinuity in sedimentation which allowed the oxidation of sulphidic sediments by exposure to oxygenated seawater. The oxidation of sulphides may proceed quite rapidly under these circumstances and numerical models for the oxidation of sulphidic sediments by the diffusive penetration of dissolved oxygen into the RISP sediments indicate that a 1-cm oxide layer could be produced in as little as 100 yr.

The diagenetic chemistry clearly demonstrates that the RISP sequence is not wholly Miocene and that the upper unit is probably of Recent origin. Unfortunately, there are no clear indications as to the age of the lower unit. Our observations can be reconciled with the presence of Miocene, also Pliocene and Pleistocene, fauna if the upper unit was formed by reworked material (similar to that in the lower unit) which was redeposited following suspension in the water column (which would allow an autochthonous organic matter contribution). There are no chemical features which are inconsistent with a post late-Pleistocene origin for the whole core.

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Late-Quaternary climatic change on the American North Pacific Coast

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Temporal changes in solar radiation caused by variations in the Earth's orbit figure prominently in current Quaternary climate theory[1–4]. Experimental design of atmospheric circulation models emphasizes the importance of solar radiation in the heating of interglacial land and ocean surfaces at different seasons of the year, whereby monsoon-type circulation—seasonal shifting of regional cyclonic and anticyclonic centres—develops, while during glacial ages, seasonality is greatly modified by the influence of ice sheets[5–7]. We investigated the late-Quaternary climate of the North Pacific, where according to modelling, solar radiation in the early Holocene at the time of the summer solstice is high and in the late Holocene is relatively low. We compared quantitative temperature and precipitation estimates from southern Alaska, obtained by application of transfer functions to fossil pollen records, with estimates from western Washington[8] and southern British Columbia[9]. Data extending over >10,000 years show a broadly consistent pattern of climatic change in general agreement with predicted variations in solar radiation and their effect on atmospheric circulation and seasonal duration of pressure systems over the North Pacific Ocean. In the early Holocene, as monsoon-type circulation became established with melting of glaciers, the subtropical North Pacific anticyclone annually regulated climate for a longer period at higher latitudes than at present, so that warmth and dryness (high summer radiation) increased in southern Alaska. The Aleutian low-pressure centre, locus of cyclonic storm activity, intensified during the late Holocene, resulting in colder and more humid coastal climate (low summer radiation) over much of the year and increased frequency of glacier growth in the cordillera.

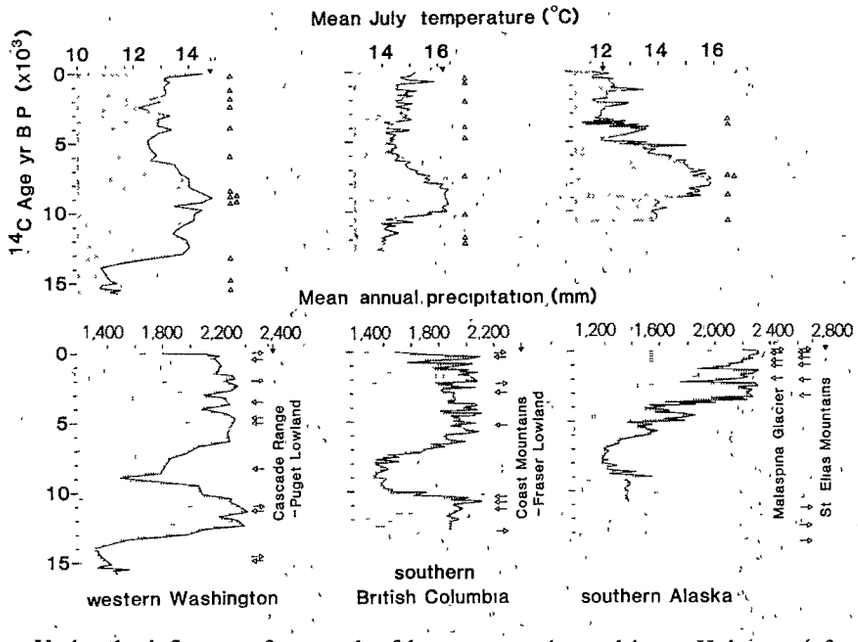


Fig. 1 Late-Quaternary mean July temperature and annual precipitation estimated by use of transfer functions for sectors of North America located in western Washington (47° 49′ N, 124° 12′ W)[8], southern British Columbia (49° 15′ N, 122° 20′ W)[9], and southern Alaska (60° 01′ N, 141° 57′ W). Data are drawn to a uniform time scale with chronology indicated by radiocarbon ages (△) and assuming constant sedimentation rates between ages. Markers on temperature and precipitation scales are modern means. Arrows pointing left next to precipitation curves signify times of glacier advance, whereas retreat is indicated by arrows pointing right; vertical lines between arrows indicate extended intervals of glacier activity.

Under the influence of a trough of low pressure located in the Aleutian/Gulf of Alaska region, climate today along the American north-west coast is mostly stormy, cold and rainy[10]. In summer, as the subtropical anticyclone develops over the North Pacific Ocean, the Aleutian low is weakened and displaced northwestward by the build up of high pressure. Some weeks of relatively stable conditions ensue in the western conterminous United States and southwestern Canada, providing a contrast with the weather during the colder part of the year. Although summer months in southern Alaska and northern coastal British Columbia are driest, influenced by the seasonal pressure change, cyclonic storms, accompanied by heavy precipitation and cloudiness, continue to strike this sector of the coast.

Seasonal duration and extent of pressure systems over the North Pacific were variable in the late Quaternary, as implied by quantified mean July temperature and annual precipitation estimates for three geographic areas (Fig. 1). Data from southern Alaska serve for comparison with data from the Olympic Peninsula of western Washington[8] and the Coast Mountains of southern British Columbia[9]. All three data sets were generated by two independent regression equations[8] that relate modern composition of pollen in surface samples from the Pacific coastal forest and tundra regions to recorded temperature and precipitation values at sites between the Aleutian Islands and California. Applied to radiocarbon-dated fossil pollen records in the three areas, the equations yield estimates of late-Quaternary climatic parameters. Transfer functions used were applied within the limits of the calibration data set.

Southern Alaska data (Fig. 1) are derived from fossil pollen contained in two sections of a muskeg located near Munday Creek on the Gulf of Alaska, ~15 km north-west of Icy Cape in the Malaspina Glacier district. One section extends to ~9,000 yr BP[11] whereas the other reaches beyond to 10,820 yr BP[12]. Mean July temperature is near 14 °C at the end of the Pleistocene (10,000 yr BP) and in the early Holocene, reaching a maximum close to 16 °C at ~8,000 yr BP and then decreasing to 12 °C at ~5,000 yr BP, with fluctuations around this value until the present. Annual precipitation, just under 1,400 mm at the beginning of the record, reached a minimum near 1,200 mm, coinciding with the temperature maximum at ~8,000 yr BP, and thereafter increased to almost twice as much during the past four millennia, while temperature fell to its lowest level of the entire time span.

Temperature and precipitation trends expressed by the curves represent a pattern, repeated to the south in British Columbia and Washington (Fig. 1). All sites show early Holocene temperature maxima and precipitation minima, whereas in the late Holocene (after 5,000 yr BP), as in the late Pleistocene, higher precipitation is coincident with lower temperature. Increased warmth and dryness during the early Holocene suggest extended dominance by the subtropical Pacific anticyclone; conversely, before and after this interval, when precipitation amounts are higher and temperature values lower, polar Pacific cyclones became effective in generating storms that increased in frequency over a greater part of the year. Changes associated with the centres of atmospheric pressure were influenced to a great extent by land–ocean temperature contrasts, latitudinal temperature gradients and topography. Changes follow closely predictive general circulation models in which monsoon-type climate is related to variations in seasonal solar radiation intensity[5–7].

Storm activity, implied by the fossil evidence, was most intense in the late Holocene in southern Alaska, where precipitation increased by some 1,200 mm and temperature fell ~4 °C. Particularly wet climate effecting generation of the muskeg studied near Icy Cape is indicated by an unusually high deposition rate (42 mm yr^{-1}, assuming no compaction) for 150 cm of unhumified peat formed between 3,860 and 3,500 yr ago; before this, under a warmer, drier climate between 9,000 and 7,700 yr ago, rate of deposition of 25 cm of highly humified peat was much lower (2 mm yr^{-1}). Rapid peat deposition close to 3,500 yr ago has been found elsewhere in southern Alaska[13].

This pronounced climate change occurred at the time of the Neoglaciation, during which colder wetter conditions, giving rise to excessive snowfall in the lofty St Elias Mountains, caused glacier regeneration (Fig. 1) between 3,300 and 2,100 yr ago, 1,250 and 1,030 yr ago and in recent centuries, after an extended interval of early Holocene recession[14–18]. During the Neoglaciation, ice flow from the St Elias Mountains contributed to the formation of the huge piedmont lobe of Malaspina Glacier, which spread across the coastal lowland and continental shelf to the Pacific. Episodes of ice advance in the Malaspina Glacier district occurred over the past two millennia, with maxima dated between 1,600–1,200 and 830–560 yr ago and in the past few centuries[19–23].

Malaspina Glacier, covering some 2,200 km^2, rests mostly below sea level[24] along a part of the Gulf of Alaska coast that, under the warmer, drier climate of the early Holocene, was probably an embayment. Located between the eastern end of the Robinson Mountains at Icy Bay and the Deception Hills at the mouth of the Alsek River, the embayment extended along >200 km of coastline, including present-day Icy and Yakutat Bays, before the Neoglaciation. Higher temperature and lower precipitation estimates make it unlikely that mass balance of Malaspina Glacier in its present lobate form could have been maintained in the early Holocene.

Greater frequency of storms passing across southern British Columbia and Washington over the past six or seven millennia is reflected by the estimates of increased precipitation in this part of the coast (Fig. 1). The increase, while temperature steadily decreased, inaugurated late Holocene glacial episodes at ~5,000 yr ago[25-31]. During the early Holocene, except for isolated instances of minor glacial advance in the Cascade Range[32-34], glaciers were inactive. Climate, under control of the subtropical anticyclone, was apparently too warm and dry for glacier growth. In the late Pleistocene (15,000–10,000 yr ago), a major advance occurred in the Puget Lowland in a cold, dry climate ~14,500 yr ago[35], followed under wetter but less cold conditions by minor advances in the Fraser Lowland and North Cascades close to 11,000 yr ago[36-38] and in the Coast Mountains ~10,500 yr ago[39]. Late Pleistocene glaciation of the Puget Lowland has been attributed to northward movement of storm tracks, causing heavy snowfall in the glacier source region in the Coast Mountains of British Columbia, coincident with decay of the Laurentide ice sheet[40]

In the long-term, late-Quaternary climate fluctuations along the American north-west coast, keyed to shifting dominance of atmospheric pressure cells over the North Pacific, are outlined by the pattern of temperature and precipitation estimates. They conform with temporal changes in seasonal radiation intensity predicted by experimental modelling[5-7], previously brought out by fossil pollen studies in north-west Canada[41]. In the absence of tighter radiocarbon-dated time control over extended intervals in the records, assessment of short-term fluctuations of the estimates (of the order of centuries) is not justified. Rates of response to climate change, as well as speed of migration northward following deglaciation of the British Columbia and Alaskan coasts, by plant species used in the equations, for example, are variables that must enter into the timing of events. These may be significant, but further dating and replication are required to define them.

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Evidence for an early Plio-Pleistocene rainforest expansion in East Africa

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The Plio-Pleistocene palaeoenvironmental history of the Turkana Basin and neighbouring areas in north Kenya and southern Ethiopia has been a matter of considerable interest due to the important palaeoanthropological, palaeoarchaeological and palaeontological discoveries that have been made in this region[1-3]. The consensus is that although environmental oscillations have certainly occurred over the past 4 Myr, Plio-Pleistocene environments were generally comparable to the modern environment of the area[4]. However, the recent recovery of two taxa presently characteristic of the central African rainforest—the anacardiacean tree *Antrocaryon* and the prosobranch gastropod *Potadoma*—from a restricted chronostratigraphic horizon dating at ~3.3–3.4 Myr, at widely separated sites in the Turkana region, suggests that the general pattern of environmental stability was punctuated at this time by a brief but significant rainforest extension in East Africa. This brief humid interval apparently coincides with certain important molluscan immigrations, and can be broadly correlated with the first major episode of climatic deterioration in the Northern Hemisphere.

Recent reviews of Plio-Pleistocene palaeoenvironments in the Omo Valley and Koobi Fora areas of the Turkana Basin, north Kenya, have drawn on sedimentological, palaeontological and palaeobotanical evidence (see, for example, refs 1–4). Although a relatively xeric interval between 2.5 and 2.0 Myr has been postulated[5], the evidence for significant environmental change at this time has been questioned[4]. The general consensus seems to be that throughout the past 4 Myr the Turkana region has been characterized by a suite of terrestrial environments similar to those found at present—dry tropical scrub and grasslands of the 'Sudan savanna' replaced locally by limited gallery forest in the neighbourhood of perennial drainages[4]. However, several recent lines of evidence suggest that the general pattern of overall environmental stability in the latest Cenozoic of East Africa may have been briefly but significantly perturbed by an important humid episode at 3.3–3.4 Myr, an episode which entailed a major expansion of the central African rainforest. The suggestion that a humid episode occurred at this time was originally prompted by the discovery of fruits of the anacardiacean rainforest genus *Antrocaryon*[6] in the 'Brown Sands' locality of the Usno Formation, a short stratigraphic distance above tuff U10, and from Member A1.1 of the Shungura Formation in the lower Omo Valley (see Fig. 1), immediately underlain by tuff A and overlain by tuff B. Tuff U10 is considered to be a lateral correlate of tuff B of the Shungura Formation (F. H. Brown, personal communication), and tuff B itself is now considered a lateral correlate of the Tulu Bor Tuff from the Koobi Fora area[7,8] The age of this latter horizon is considered to be ~3.35 Myr, on the basis of its stratigraphic position between the immediately overlying Toroto Tuff (firmly dated at 3.32∓0 02 Myr) and the underlying Lokochot Tuff[9]. On the basis of geochemical comparisons, the Lokochot has been correlated with tuff A of the Shungura, which is known to lie close to the boundary between

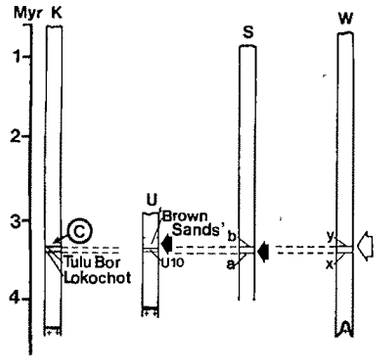


Fig. 1 Stratigraphic distribution of *Antrocaryon* (solid arrows) in the Usno (U) and Shungura (S) formations of the Omo Valley; occurrence of *Potadoma* (open arrow) in sections west of Lake Turkana (W) and the earliest African occurrence of *Corbicula* (C), in the Koobi Fora area (K). a, Tuff A of the Shungura Formation, which can be correlated with the Lokochot Tuff of the Koobi Fora area and also with a tuff (indicated here by x) in the areas west of Lake Turkana; b indicates tuff B of the Shungura Formation, which can be correlated with Tuff U10 of the Usno Formation, with the Tulu Bor Tuff of the Koobi Fora Formation, and with a tuff (indicated here by y) in the area west of Lake Turkana. Note the extremely restricted stratigraphic distribution of both *Potadoma* and *Antrocaryon*, and the fact that their occurrence coincides closely with the first African record of *Corbicula*. Data from various sources. The geographical distribution of these occurrences is indicated in Fig. 2. General chronostratigraphic framework after Brown *et al*[9]

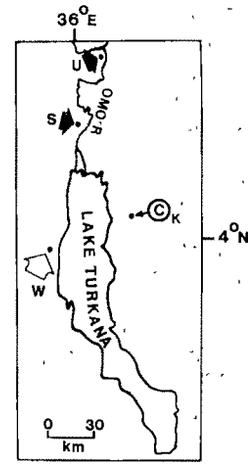


Fig. 2 Geographical location of occurrence of *Antrocaryon* (solid arrows) in the Usno (U) and Shungura (S) formations of the Omo Valley; *Potadoma* (open arrow) in the area of the Lomekwi drainage (W), west of Lake Turkana, and the first African occurrence of *Corbicula* C in area 129 of the Koobi Fora area (K) east of Lake Turkana.



Fig. 3 Present distribution of *Antrocaryon* and *Potadoma* in Africa. Black areas indicate present co-occurrence of *Antrocaryon* and *Potadoma*, except the small isolated area to the north, in which *Potadoma* occurs alone. Grey (stippled) areas indicate the present distribution of *Antrocaryon*. The circled area (T) indicates the location of the Turkana Basin Distribution of *Antrocaryon* after Bonnefille and Letouzey[6]; distribution of *Potadoma* after Brown[13].

the Gauss and Gilbert chrons; the Lokochot/tuff A horizon must accordingly date at ~3.42 Myr[9]. *Antrocaryon* in the lower Omo Valley is therefore confined to an interval from ~3.3 Myr to ~3.4 Myr. In addition to the occurrence of *Antrocaryon* in the Omo Valley deposits, the striking development of palaeosols in Member B has been considered evidence for humid conditions[10], and the mammal fauna from Member B10 contains rodents and prosimians (notably *Galago*) which have been interpreted as indicating a rainforest setting and forest connections with central Africa[11]. However, the various sites from which this evidence was obtained cover a relatively small area of the Omo Valley and all are fluvial deposits in close proximity to the presumed axis of the original Omo drainage[4]. Consequently, the evidence for rainforest conditions around the tuff B level could simply reflect a restricted local development of riverine gallery forest rather than a regionally significant rainforest expansion, as Brown[4] and others have pointed out[6].

The suggestion that a regionally significant humid episode and concomitant rainforest expansion occurred at 3.3–3.4 Myr in northern Kenya and southern Ethiopia is greatly strengthened by the recent discovery of the rainforest prosobranch *Potadoma*[12] from two faunas, separated stratigraphically by some 2 m, in the Lomekwi drainage area west of Lake Turkana some 60 km south of the Omo Valley (see Fig. 2). These faunas occur at the level of a tuff which has been correlated, on geochemical grounds, with the Tulu Bor (U10; tuff B) horizon[9] (see Fig. 1); this occurrence of *Potadoma* constitutes the only definite record of this west and central African genus in East Africa[12].

Both *Antrocaryon* and *Potadoma* are at present restricted to the central African rainforest[6,12,13]; indeed, their joint distribution essentially delimits the present distribution of rainforest in central Africa (see Fig. 3). The simultaneous occurrence of these distinctive taxa in sections up to 150 km apart in the Turkana region at 3.3–3.4 Myr strongly suggests, therefore, a dramatic expansion of humid rainforest conditions in the area at this time. However, the extremely restricted chronostratigraphic distribution of these taxa in the Turkana area deposits suggests that this period of enhanced humidity was relatively brief. The regional nature of this climatic change may also be reflected by the fact that a fauna some 8 m above the Tulu Bor Tuff in collection area 129 of the Koobi Fora area has recently provided the first African record of the primarily Eurasian bivalve *Corbicula*[14]. *Corbicula* has a long Cenozoic record in southern Eurasia, but is a comparatively recent addition to the African malacofauna[15]. *Corbicula* is unknown from any African malacofaunas definitely pre-dating its occurrence in area 129; it is absent from faunas lower in the Koobi Fora Formation[14], from the lower Usno or Mursi formations or the Basal Member of the Shungura in the Omo Valley[16], from the Lothagam or Kanapoi formations in the southern Turkana area[16], and from deposits such as the Kaiso Formation in the western rift[15]. However, the genus predominates in the majority of faunas in the Koobi Fora Formation subsequent to its first appearance above the Tulu Bor level in area 129 (ref. 14), and *Corbicula* is now one of the most abundant and widespread African freshwater bivalves[18]. A regional episode of enhanced humidity, as suggested by the occurrence of *Antrocaryon* and *Potadoma*, may well have encouraged the immigration of *Corbicula* from southern Eurasia by enhancing hydrographical connections between the latter area and northeastern Africa. However, it should be noted that in area 129 the Tulu Bor is overlain by a disconformity[8], and the first occurrence of *Corbicula* may therefore post-date the Tulu Bor by a greater interval than that indicated in Fig. 1, although no evidence of disconformity is apparent between the upper surface of the Tulu Bor and the fauna yielding *Corbicula*. Any molluscan interchange between north-east Africa and southern Eurasia is unlikely to have been strictly 'one-way', and it is therefore of interest that the only known Eurasian occurrence of the African prosobranch *Bellamya unicolor* (Olivier) is from the 'Erq el-Ahmar beds of the

central Jordan Valley[19]; these beds are not directly dated but immediately overlie a basalt (the 'cover basalt') recently dated at ~3.2 Myr[24].

A major humid episode and consequent expansion of rainforest in East Africa at 3.3–3.4 Myr should be reflected by palaeoclimatic events elsewhere. In fact, the regional episode of rainforest expansion discussed here appears to correspond broadly to the early Gauss 'ice-age transition' episode, long considered to signify the initial formation of significant Northern Hemisphere ice sheets[20–23]. However, this transition episode essentially represents a unidirectional palaeoclimatic deterioration reflecting the onset of ice-age conditions[23], whereas the extremely restricted chronostratigraphic distribution of *Antrocaryon* and *Potadoma* in East Africa suggests a palaeoclimatic 'punctuation' or 'overshoot' of relatively brief duration.

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Reconstruction of prehistoric plant production and cooking practices by a new isotopic method

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As archaeological research has focused increasingly on economic questions, archaeologists have collected data that reflect production and consumption of food. Macrobotanical remains, recovered by methods such as water flotation, often provide the most available and relevant data concerning production in archaeological contexts. However, these data do not necessarily reflect the proportion of crops that were consumed[1]. Here we present a new method, based on isotopic analysis of burnt organic matter, allowing the characterization of previously unidentifiable plant remains extracted from archaeological contexts. We used this method to reconstruct prehistoric production, preparation and consumption of plant foods, as well as the use of ceramic vessels, in the Upper Mantaro Valley region of the central Peruvian Andes[2]. The method will be of use to archaeologists studying these prehistoric activities in other areas of the world.

The basis for the isotopic method used here can be summarized as follows. Nitrogen isotope ratios separate modern plants into two groups: legumes, capable of fixing atmospheric nitrogen, and non-leguminous plants, which must use nitrogen from the soil[3,4]. Carbon isotope ratios also separate modern plants into two groups, C_3 plants and C_4 and crassulacean acid metabolism (CAM) plants, which differ in the enzymes that catalyse initial CO_2 fixation[5–7]. Because all legumes are C_3 plants and no C_4 or CAM plant is known to fix atmospheric nitrogen, combined C and N isotope analysis can be used to separate living plants into three groups: legumes, non-leguminous C_3 plants, or C_4 and CAM plants[3–7]. Prehistorically burnt domesticated plants, identified to the species level and assigned to one of the three plant groups on the basis of this taxonomic information[3–8], retain their isotopic identities during burning and fossilization (Fig. 1*a*). Thus, isotope ratios can be used to separate prehistoric burnt plant remains into the same three groups as modern plants.

To gain information on crop production, we used the isotopic method described above to classify prehistoric plant fragments, extracted from the soil by flotation techniques, that could not be identified by morphological criteria because they had been burned so extensively. These specimens came from three sequential occupation phases in the Upper Mantaro Valley region, namely the Early Intermediate Period/Middle Horizon (EIP/MH, 200 BC to AD 1000), the Huanca I period (H-I, AD 1000–1200) and the Huanca II period (H-II, AD 1200–1470)[2]. The identification of the three phases is based on changing settlement patterns, ceramic type frequencies and radiocarbon dates[9,10]. Results of the isotopic identification of the morphologically unidentifiable plant remains (Fig. 1*b*) were added to the much larger database of plant specimens identified by morphological analysis[10]. From these data we determined the changes that occurred in plant production during the three occupation phases in the Upper Mantaro Valley region (Table 1). (Because we have focused on plant production and consumption, we have included only the major agricultural crops of the region in constructing the table.) Among the indigenous crops, lupines (*Lupinus mutabilis*) and beans (*Phaseolus vulgaris*) are legumes, maize (*Zea mays*) is the only C_4 plant (there are no CAM plants) and the remaining plants are all non-leguminous C_3 plants—namely *quinoa* (*Chenopodium quinoa*) and the tubers, which include potatoes (*Solanum tuberosum, Solanum juzepczukii*), *oca* (*Oxalis tuberosa*), *olluco* (*Ullucus tuberosus*) and *mashua* (*Tropaeolum tuberosum*)[10]. The data in Table 1 indicate that during each phase, legumes and C_4 plants were produced in roughly equal amounts, but non-leguminous C_3 plants predominated as the major crops. During the H-I period there was also increased production of C_4 plants (maize) and legumes compared with the preceding and subsequent periods.

Table 1 Domestic plant group frequencies for three sequential occupation phases of the Upper Mantaro Valley

Period	Plant group frequencies (%) Non-leguminous C_3	C_4	Legumes	No. soil samples analysed
EIP/MH	74	29	23	171
H-I	83	43	43	175
H-II	69	13	20	265

Values for each period represent the percentage of soil samples weighing 6 kg that contained the indicated plant group. This measurement is based on presence/absence of specimens in each sample and not on internal density of the plant type. The frequencies[11–13] do not total 100% but show the relative presence of plants The provenences of the sites are given in ref 10

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To study prehistoric cooking practices and associated vessel function, we determined the isotope ratios of the organic matter encrusted on the inside surfaces of ceramic vessel shards excavated from habitations in the Upper Mantaro Valley region. Only the plainest self-slipped jar type had encrustations, although these encrustations did not occur on every self-slipped shard. From vessel reconstructions, we found that the encrustations covered the bottoms but not the upper walls of the vessels.

All shards with visible encrustations were scraped to collect the organic matter. The 71 shards whose encrustations were analysed isotopically were chosen at random from all encrusted shards collected at the five sites spanning the three time periods for which we have the production data described in Table 1.

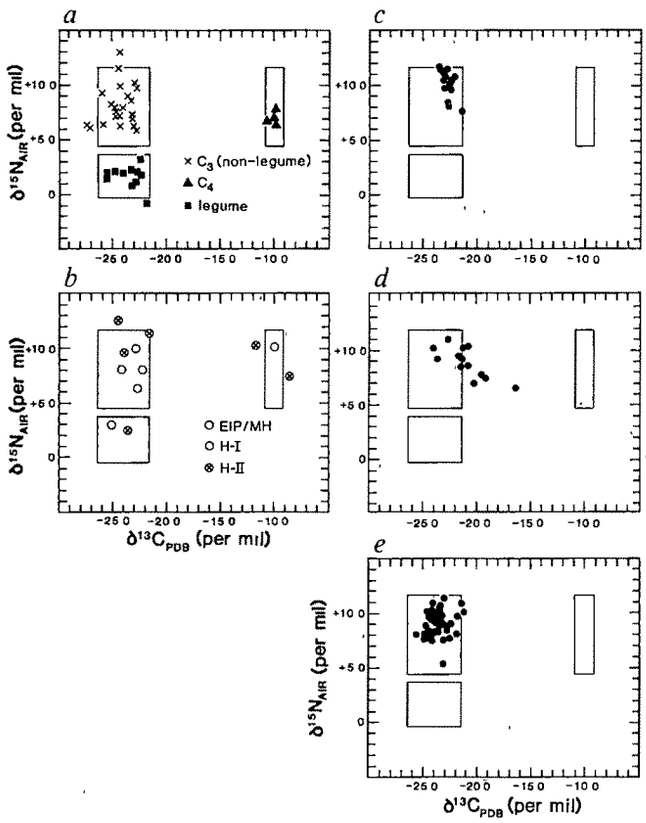


Fig. 1 C and N isotope ratios of burnt plants and of encrustations from the insides of ceramic vessel shards excavated from archaeological sites representing three continuous phases of occupation in the Upper Mantaro Valley area. The $\delta^{13}C$ and $\delta^{15}N$ values are given for: *a*, prehistoric burnt plant fragments assigned to the indicated groups based on morphological criteria; *b*, prehistoric plant fragments too extensively burnt to allow such identification; *c–e*, vessel shard encrustations from the Early Intermediate Period/Middle Horizon (*c*), the H-I period (*d*) and the H-II period (*e*) Plant fragments and shard encrustations were cleaned in an ultrasonic bath and freeze-dried before the determination of their isotope ratios by methods described in ref. 8. The identified prehistoric plants in *a* came from the three occupation phases and include all the local indigenous Andean crops discussed in the text[8]. The boxes in each graph indicate the means ±2 s.d. of the $\delta^{15}N$ values for the identified legumes and non-leguminous plants and of the $\delta^{13}C$ values for the identified C_3 and C_4 plants shown in *a* Isotope ratios of modern plants in the three groups[8] are similar to those indicated by the boxes. Isotope ratios are given as δ^*X values, where

$$\delta^*X = \left[\frac{(^*X/X)_{\text{sample}}}{(^*X/X)_{\text{standard}}} - 1\right] \times 1{,}000 \text{ per mil}$$

For $\delta^{13}C$ values, $^*X/X = {}^{13}C/{}^{12}C$ and the standard is the PDB carbonate (C_{PDB}). For $\delta^{15}N$ values, $^*X/X = {}^{15}N/{}^{14}N$ and the standard is atmospheric nitrogen (N_{AIR}). The precisions for determination of both the $\delta^{13}C$ and $\delta^{15}N$ values are ±0.2 per mil.

For the purposes of this analysis, we assume that our random sample is representative of the total population of encrusted shards.

Analysis of the encrusted organic matter by light and scanning electron microscopy (SEM) indicates that all morphological characters useful for identification had been destroyed during burning. Three lines of evidence indicate that these organic encrustations originated from plant material that was boiled and mashed before charring. First, SEM analysis of the encrustations did not reveal the presence of bone fragments, which would have survived the burning process. Second, the atomic C/N ratios of the prehistoric encrustations were similar to those of encrustations we made by cooking and then charring modern food plants from the Upper Mantaro Valley region onto ceramic vessels, but higher than those we produced by the same process using fresh meat. The ranges and means ±1 s.d. of the C/N ratios were as follows: prehistoric encrustations, 8.2–19.3/12.8 ± 2.1 ($n = 71$); modern plant encrustations, 8.2–38.8/23.4 ± 10.0 ($n = 8$); modern meat encrustations, 4.4–6.1/5.3 ± 0.8 ($n = 6$), where n is the sample size. Third, all of the prehistoric encrustations analysed were evenly distributed on the inside surfaces of the shards. The only way we could reproduce this type of distribution was by boiling and mashing fresh plants before burning them onto ceramic vessels. Dry-toasting before charring produced a spotty distribution of burnt material on the vessel. Experiments with modern plants demonstrate that isotope ratios are not substantially altered when plants are cooked and then burned onto ceramic vessels. Thus, isotope ratios of the shard encrustations indicate the type of plants cooked in these vessels.

The isotopic data for shard encrustations from the EIP/MH period (Fig. 1*c*) and the H-II period (Fig. 1*e*) show a strong clustering in the C_3 plant group box. For the intervening H-I period (Fig. 1*d*), however, the isotopic data provide evidence of C_4 plants mixed with C_3 plants in some of the encrusted samples. With the possible exception of a single sample from the H-II period (Fig. 1*e*), none of the shard encrustations shows isotopic evidence of legumes having been cooked in significant amounts in these vessels.

The ceramic shards with encrustations came from large self-slipped jars, from midden and floor excavation proveniences, suggesting that they were fragments of cooking vessels. The results from the isotopic analysis of these body shards support the hypothesis that these large jars were used for daily domestic food preparation. From this, we assume that food preparation practices reflect food consumption.

We conclude that the prehistoric inhabitants of the Upper Mantaro Valley region used these ceramic jars to cook C_3 plants alone or C_4 plants and C_3 plants together, either simultaneously or sequentially, but did not cook legumes, either alone or in combination with non-leguminous plants. These conclusions are consistent with modern cooking practices in this part of the Andes (C.A.H., unpublished). Today C_3 plants (*quinoa* or tubers) are often boiled alone for a meal, whereas when maize is boiled, it is generally prepared with C_3 plants and spices. Comparison of modern and prehistoric processing of legumes is complicated by the present-day use of fava beans (*Vicia fava*) and peas (*Pisum* sp.), which have been introduced from Europe[10]. Fava beans and peas are boiled as well as dry-toasted at present, whereas the indigenous legume *Lupinus mutabilis* is a casual food and tends to be toasted or eaten raw. *Phaseolus vulgaris*, although indigenous, is not in common use today. Although we do not know whether introduction of new plant foods influenced traditional cooking practices, our isotopic analysis of the shard encrustations indicates that present-day cooking patterns for indigenous plants also existed in the past.

Comparison of the consumption data (Fig. 1*c–e*) with the production data (Table 1) indicates that temporal changes in food preparation paralleled changes in food production for the C_3 and C_4 plant groups. The absence of parallel trends in the two activities for legumes and non-leguminous plants is probably related to how indigenous legumes were prepared. The evidence for maize on some H-I period shards (Fig. 1*d*), in association

with evidence of increased maize production at that time (Table 1), suggests that the additional maize available to the population during this period was eaten as a bulk carbohydrate. We suspect that during all three periods maize was being consumed as *chicha* (fermented beverage) and *cancha* (toasted corn). Because there is no evidence for significant differences in social status during the H-I period[10], differential access to resources based on social position cannot have helped to produce evidence for increased consumption of maize during this period.

The isotopic method described here can be used to characterize plant materials burned onto ceramic vessels (whether the vessels were everyday cooking wares, storage jars or ceremonial items) in areas that have plants from more than one of the three isotopically identifiable plant groups. This method should also be applicable to organic encrustations on vessel exteriors, identifying plant groups used prehistorically as fuel

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Population dynamics of *Schistosoma mansoni* in mice repeatedly exposed to infection

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Studies of host resistance to parasite infection are usually based on experimental designs involving a primary infection and subsequent challenge exposure, resistance being recorded as the percentage reduction in parasite establishment in challenged hosts when compared with that in uninfected animals[1-8]. Few studies have focused on the dynamic nature of helminth establishment and mortality (and their presumed dependency on the rate of current exposure and past experiences of infection) in hosts repeatedly exposed to low levels of infection[9-11]. Here, we report the results of population studies on the dynamics of resistance to *Schistosoma mansoni* infection (a helminth parasite) in mice repeatedly exposed to cercarial invasion. Parasite burdens created by different levels and durations of exposure to infection reflect a dynamic interplay between rates of helminth establishment and mortality. Depending on the intensity of exposure, changes in worm load with duration of host infection vary from monotonic growth to a stable average parasite burden to convex curves in which the average load attains a maximum value before decaying in old animals. These trends are similar to observed patterns of *S. mansoni* infection in human communities[12-14].

Under conditions of constant exposure to infection and constant worm mortality, and in the absence of acquired resistance to infection, average helminth burdens per host will rise monotonically as the duration of exposure increases to a stable equilibrium (an 'immigration-death process')[15]. Patterns of change in the intensity of *S. mansoni* infection in human communities with host age, however, often show a rise to a maximum value in teenage or young adult age groups and a decline thereafter[12-16]. It is not known whether the observed decline in older age groups reflects age dependency in the rate of exposure to infection (that is, an ecological process) or acquired resistance to worm establishment (an immunological process)[16,17]. We used a laboratory model to generate age-intensity curves of *S. mansoni* infection (a Puerto Rican strain) in inbred mice (CBA/Ca) by repeated exposure to constant numbers of cercariae, using four levels of cercarial exposure (Fig. 1*a*, *b*). Separate experiments revealed that ~50% of the cercariae to which a naive host was exposed became established as mature adult worms (5-week maturation period) (Fig. 1*d*).

The severity of the initial rise in mean worm burden with age of the host and the maximum worm burden attained are dependent on the density of cercariae to which the host is exposed At the lowest infection dose, mice survived infection well and the experiment ran for 50 weeks with worm burdens attaining a stable plateau of ~4-6 parasites per mouse. Burdens of this magnitude may produce disease that most closely resembles human hepatosplenic schistosomiasis[18]. At higher exposure levels some host mortality occurred (Fig 1*a*, *b*). In the two highest exposure experiments there was a decline in parasite burden in older mice, the severity of which was not accounted for by a dependence between host death rate and parasite burden.

As the rate of exposure of the host to infective stages was kept constant with time, these observations suggest that the acquisition of resistance to infection occurs as the duration and intensity of exposure increases. The convex pattern (Fig. 1*a*) recorded for the highest exposure rate could have arisen as a consequence of a time-delayed density-dependent mechanism created by limitation of resources in the host, as opposed to host defence mechanisms. This seems to be unlikely for two reasons. First, there is clear evidence for immunological response to *S. mansoni* infections[8]. Second, our experiments generated comparatively low burdens of mature and juvenile worms when compared with the much higher worm loads that can be harboured in the mesenteric veins[8].

Perfusion of infected mice led to the recovery of juvenile worms throughout all the experiments, which suggests that parasites can become established in hosts despite long durations of past experience of infection and the presence of mature adult worms. The observed patterns of infection represent a dynamic interplay between parasite establishment (at a rate $\bar{\Lambda}$) and worm mortality (at a rate per parasite of μ). The rate of change of the mean worm burden per mouse, $M(t)$ with respect to time can be described by an immigration-death process where

$$\frac{dM(t)}{dt} = \bar{\Lambda}(\bar{M}(t)) - \mu M(t) \qquad (1)$$

In agreement with current understanding of the mechanism of immunity to *S. mansoni* infection in mice[8], we assume that acquired resistance acts to decrease the rate of parasite establishment, $\bar{\Lambda}(\bar{M}(t))$, in a manner dependent on the accumulated experience of past infection, $\bar{M}(t)$, where $\bar{M}(t) = \int_0^t M(t')dt'$.

For simplicity we assume a linear dependency where

$$\bar{\Lambda}(\bar{M}(t)) = \Lambda\left[1 - \varepsilon\int_0^t M(t')e^{-\sigma(t-t')}\,dt'\right] \qquad (2)$$

with the constant ε denoting the efficacy of acquired resistance, described in more detail in the accompanying paper[19]. We further assume that the 'immunological memory' of past experience of infection is finite and decays as the time interval between the present and past experience increases with an average memory duration of $1/\sigma$ (ref. 19). The model contains four adjustable parameters, Λ, μ, ε and σ. Estimates are available for Λ and μ (see Fig 1*c*, *d*) on the basis of our experimental design, and a crude estimate can be obtained for ε (by noting

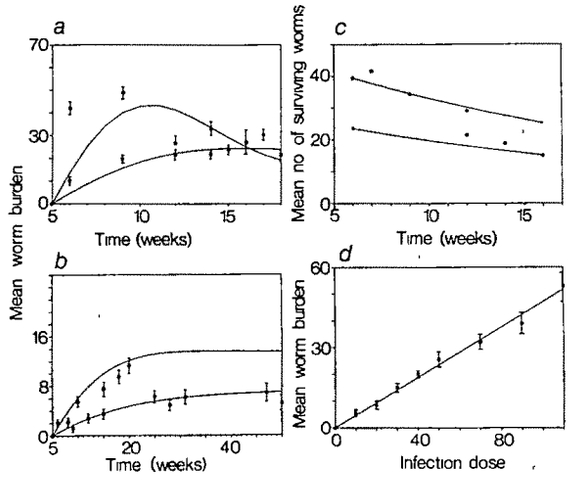


Fig. 1 *a*, *b*, Worm recovery through time from trickle infection experiments. Groups of 10 mice were exposed to infection at weekly intervals by paddling for 0.5 h in tanks containing a suspension of cercariae (of age <2.5 h) in 200 m dechlorinated tap water at 25 °C. Four infection densities were used: 10, 30, 100 and 300 cercariae per group per week. Worms were recovered at intervals post-infection by perfusion of the hepatic portal system with citrated saline and by general dissection. Symbols denote the mean worm recovery with standard error bars. Note that the abscissae start at 5 weeks because of the maturation delay (~5 weeks) of *S. mansoni* in laboratory mice. Solid line, predicted worm recovery calculated from the immigration-death model described in the text (equation (1)). For $(\mu-\sigma)^2>4\varepsilon\Lambda$ the solution of equation (1) is given by

$$M(t)=A+\frac{\Lambda}{\lambda}\left[\frac{(p_1+\sigma)e^{p_1 t}}{p_1}-\frac{(p_2+\sigma)e^{p_2 t}}{p_2}\right]$$

where $A=\Lambda\sigma/(\sigma\mu+\varepsilon\Lambda)$; $p_1=[-(\mu+\sigma)+\lambda]/2$, $p_2=-(\mu+\sigma+\lambda)/2$ and $\lambda=[(\mu-\sigma)^2-4\varepsilon\Lambda]^{1/2}$. When $4\varepsilon\Lambda>(\mu-\sigma)^2$ the solution adopts the form

$$M(t)=A+e^{-(\mu+\sigma)t/2}\left[\frac{2\Lambda}{\theta}\sin\left(\frac{t\theta}{2}\right)\left[1-A\left(\frac{\mu+\sigma}{2\Lambda}\right)\right]-A\cos\left(\frac{t\theta}{2}\right)\right]$$

where $\theta=[4\varepsilon\Lambda-(\mu-\sigma)^2]^{1/2}$. Equation (2) is a crude mimic of current understanding of acquired resistance mechanisms, as the value of ε must be small to prevent negative infection rates. The choice of this function was determined by analytical convenience and the observation that establishment decays slowly as the summed past experience of infection rises (negative infection rates prevented by a very small ε value). If the net infection rate in equation (1) <0 (that is, ε large), acquired immunity is assumed to influence parasite establishment and survival. ***a***, Record of the results for cercarial exposure levels of 10 and 30 infective stages per week (model parameter values $\varepsilon=0.0085$ per week, $\sigma=0.2$ per week and $\mu=0$ 045 per week (see *c*). The values of Λ calculated by means of the relationship shown in *d* were 4.711 and 14.13 per week, respectively. ***b***, Similar to *a* but the results for cercarial exposure levels of 1 and 3 infective stages per week ($\Lambda=0.4711$ and 1.413 per week, respectively, other parameter values as defined for *a*). ***c***, Adult worm survival over a 10-week period post-maturation. Two examples are shown where initial adult worm densities at week 6 of infection were ~40 and 25 parasites per mouse The symbols denote observed means (from 10 mice) and the solid lines denote the fit of an exponential decay survival model with constant death rate $\mu=0.045$ per week ***d***, Worm recovery 6 weeks post-infection of naive mice exposed to a single infection of varying intensity (0-110 cercariae). Symbols denote observed mean recoveries (10 mice) (the underlying distribution of worm numbers per inbred mouse was Poisson in form) and the solid line is the best-fit linear model with slope $b=0.4711$ (that is, 40% of invading cercariae established within the host at week 6) where $r^2=0.99$. Vertical bars denote 95% confidence limits The fit of the model to observed trends is poor in the early stages (weeks 6 and 9) of the cercariae/mouse/week experiment (*a*) as a consequence of higher than average established rates in these batches of experimental animals (higher than the average value of 47% of exposure dose recorded in *d*).

the decline in juvenile worm establishment as a function of accumulated past experience of infection). At present nothing is known about the duration of immunological memory, but model predictions crudely mimic observed changes in parasite burden as the duration of exposure to infection increases, with a fixed value of $1/\sigma=5$ weeks (obtained by trial and error) (Fig. 1*a*, *b*).

The model (with no adjustable parameters other than that (Λ) set by the experimental design) is able to reflect accurately monotonic growth to a stable parasite burden for low exposures to infection and convex curves for high rates of exposure. This provides tentative support for the view that convex age-intensity curves of *S. mansoni* infection in human communities may arise partly as a consequence of acquired resistance. Observed pattens in man are probably generated by a combination of ecological and immunological processes[20].

To explore the significance of immunological memory, curative chemotherapy was used in conjunction with repeated exposure to infection. For low and medium levels of exposure (1 and 10 cercariae per week), there were no significant differences following drug treatment in mice with 9, 12 and 15 weeks previous experience of continual infection, when compared with primary trickle infections (Fig. 2*a*, *b*, *c*, *d*). Resistance following chemotherapy has been the subject of previous studies but the

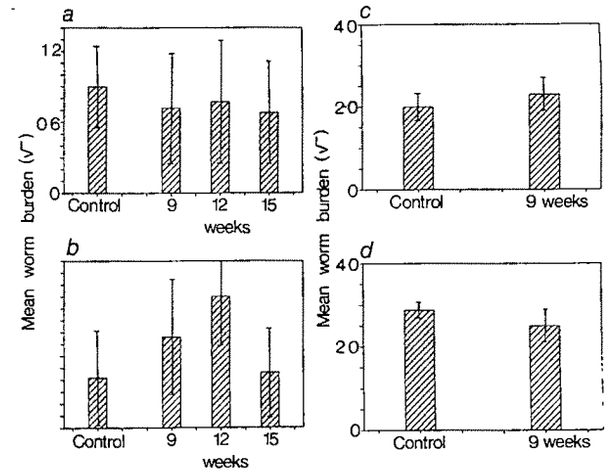


Fig. 2 Worm recovery following curative chemotherapy. Praziquantel was administered at a dose of 250 mg per kg divided into three applications given at 4-h intervals during a single day[21]. ***a***, The control was exposed to infections of 10 cercariae per group of mice per week for 6 weeks before autopsy. The experimental groups (10 mice per group) were exposed to an identical repeated infection for 9, 12 and 15 weeks before chemotherapy; autopsy was performed after 6 weeks of reinfection. One-way analysis of variance on transformed data (a square-root transformation based on the observed Poisson distributions of parasite numbers per mouse) showed no significant differences between treatments and control at the 5% level. Note, however, that the type II errors associated with this test (and those associated with the data presented in *b*, *c* and *d*) are large because of small sample sizes (10 mice per group). Studies involving much larger samples are required for future work. ***b***, As for *a* except that worm recovery was performed 9 weeks after initial infection in the control and after 9 weeks reinfection following chemotherapy in the experimental groups. One-way analysis of variance on transformed data ($\sqrt{x}$) showed no significant differences between treatments and control at the 5% level. ***c***, The control group was exposed to infections of 100 cercariae per group of mice per week for 6 weeks before autopsy The experimental group was exposed to 9 weeks infection before treatment (100 cercariae per week) and to 6 weeks reinfection post treatment. Student's *t*-test ($\sqrt{x}$) showed no significant differences at the 5% level. ***d***, As for *c* but with worm recovery performed 9 weeks after initial infection in the control group and after 9 weeks reinfection in the experimental group. No significant difference at the 5% level (*t*-test). *a*–*d*, Vertical lines show the 95% confidence limits. Note that the sample sizes of experimental animals (10 mice per group) were low and further studies are required to confirm these observations

results (based on primary and single challenge experiments) are inconclusive[10-12]; residual protective immunity was apparent in some studies but not in others. Our results from continual exposure experiments tentatively suggest that the expulsion of adult worm burdens eliminates the 'memory' of previous infections.

Studies of parasite establishment and mortality in hosts continually exposed to helminth infection have many advantages over more conventional experimental designs when seeking information to help interpretation of observed epidemiological trends in human communities. Where rates of parasite establishment depend on previous experience of infection, horizontal or longitudinal profiles of changes in parasite burden with age can vary from growth to a stable plateau in parasite load in areas of low transmission intensity to convex curves with marked declines in infection in older age classes in areas of high transmission intensity.

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Herd immunity to helminth infection and implications for parasite control

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Despite much research on immunological responses to helminth parasites, knowledge of the dynamic interplay between levels of herd immunity in humans and the rates of exposure, establishment and mortality of parasites remains limited[1-7]. We describe here a simple mathematical model for the population dynamics of helminth infections which mirrors the development of a degree of acquired immunity within populations which are genetically heterogeneous with respect to immunological responsiveness. We interpret observed patterns in the age-specific intensity of infection and attempt to understand the possible effects of control measures based on chemotherapy and vaccination. Mass chemotherapy can, in some circumstances, reduce the level of herd immunity such that average worm burdens in the adult age classes rise above their precontrol levels. When certain individuals or groups are predisposed to heavy infection[5,8,9], selective or targeted drug treatment can have significantly greater impact than mass or random application. Conversely, model predictions suggest that effective parasite control by vaccination (if and when vaccines become available) is difficult to achieve in communities that are genetically heterogeneous in their ability to mount protective responses to infection.

None of the existing epidemiological models of helminth transmission in human communities takes explicit account of acquired immunity, despite the growing volume of field[10-16] and laboratory-based studies[17-19] which attest to its significance as a determinant of parasite abundance. Infection with helminths and exposure to their diverse and complex antigens, generates varied and complex immune responses[4,7,18,20]. Acquired immunity seems to be characterized generally by a marked dependence on the degree of antigenic stimulation (the accumulated experience of infection over time), an ability to provide only partial protection against reinfection, and a transient efficacy[7,17-20]. Host responses seem to be most effective against the invading larval stages of helminth parasites and, as such, act to decrease the rate of parasite establishment within the host[7,18]. In endemic areas, humans seem continually to acquire parasites throughout life, although the average intensity of infection is often less in older than in younger age groups[21] (Fig. 1*a*, *b*). The degree to which intensity declines in the older people seems to be positively associated with the intensity of transmission (Fig. 1*a*, *b*). Much current debate centres on the relative roles of acquired immunity and age-specific changes in exposure to infection as determinants of such trends.

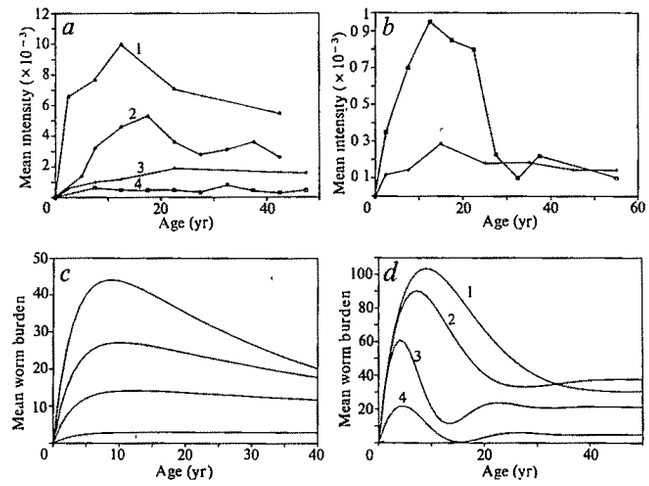


Fig. 1 Observed and predicted patterns of age-related changes in the average intensity of infection (horizontal cross-sectional profiles). *a*, Hookworm infection in communities with varying intensities of transmission. Data sources are: curves 1, 3, ref. 29; curve 2, ref. 30; curve 4, ref. 31. The indirect measure of hookworm burden (intensity) is mean eggs per g of faeces. *b*, Schistosome infection in areas of high[32] and low[33] transmission intensity. Intensity measured by eggs per g of faeces (Kato technique). Note how in both *a* and *b* the intensity profiles become convex in areas of high transmission intensity. *c*, Equilibrium/age-intensity curves ($\partial M(t, a)/\partial t = 0$) generated by the model defined in equation (1) (with $\Delta = 0$) with the following parameters (population assumed to be homogeneous): $\varepsilon = 0.0005$, $\sigma = 0.00001$, $\mu = 0.33$, $\Lambda = 17$ (top line); 10, 5 and 1 (bottom curve) (all rates are per yr). If the net infection rate in equation (1) < 0 (that is, ε large) acquired immunity is assumed to influence parasite establishment and survival. Note how the curves become convex in shape as the intensity of transmission increases (the value of Λ). *d*, Similar to *c* but with parameter values: curve 1, at age 10, $\Lambda = 10$, $\mu = 0.2$, $\varepsilon = 0.009$, $\sigma = 0.05$; curve 2, at age 10, $\Lambda = 30$, $\mu = 0.2$, $\varepsilon = 0.001$, $\sigma = 0.05$; curve 3, at age 10, $\Lambda = 30$, $\mu = 0.2$, $\varepsilon = 0.004$, $\sigma = 0.1$; curve 4, $\Lambda = 30$, $\mu = 0.2$, $\varepsilon = 0.005$, $\sigma = 0.02$ (all rates are per yr).

Current understanding of acquired immunity can be crudely captured by a model in which the rate of establishment of new parasites within individuals of a given age, *a*, is inversely correlated with some accumulated average experience of infection

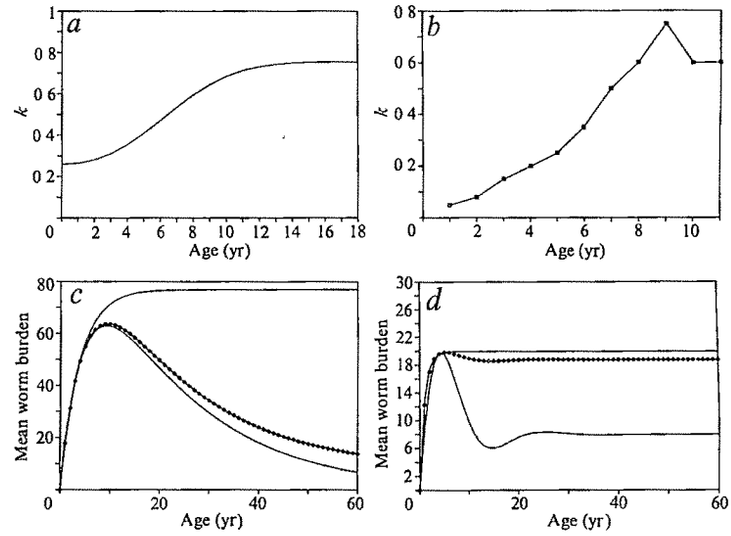
Fig. 2 Heterogeneity in 'immunological responsiveness' and exposure to infection. Model predictions for a population consisting of two types of individuals who represent proportions f_1 and f_2 of the total population, where $f_1+f_2=1$. where $M_1(t, a)$ is the mean worm lead of type I individuals (f_1) and $M_2(t, a)$ is the mean worm load of type II individuals (f_2) at time, t, of age, a, at equilibrium ($\partial M_1(t, a)/\partial t=\partial M_2(t, a)/\partial t=0$) the model takes the form

$$dM_1(a)/da=\Lambda_1\left[1-\varepsilon_1\int_0^a M_1(a')\,e^{-\sigma_1(a-a')}\,da'\right]-\mu_1 M_1(a)$$

$$dM_2(a)/da=\Lambda_2\left[1-\varepsilon_2\int_0^a M_2(a')\,e^{-\sigma_2(a-a')}\,da'\right]-\mu_2 M_2(a)$$

where the subscripts to the parameters denote differences in their values between type one and type two individuals. Here $\Lambda_i=R_{0i}(b+\sigma_i)\bar{M}$ where $1/b$ is the life expectancy of the human host, R_{0i} is the basic reproductive rate of the parasite in hosts of class i and $\bar{M}$ is the overall average worm burden in the entire population (average over all age classes and types),

$$\bar{M}=\sum_{i=1}^{2} f_i b\int_0^\infty M_i(a)\,e^{-ba}\,da$$



a, Predicted changes with age in the negative binomial parameter k (which varies inversely with the degree of parasite contagion within the host population)[5,21]. Model parameters: $\Lambda_1=1, f_1=0.9, \mu_1=\mu_2=1, \varepsilon_1=\varepsilon_2=0.01, \sigma_1=\sigma_2=0.1, \Lambda_2=20, f_2=0.1$; all are per yr. The differences in Λ reflect differing rates of exposure to infection (perhaps induced by behavioural differences between the two groups of individuals). *b*, Observed age-related changes in k for hookworm infection (*Necator americanus*) in a village community in India[34]. *c*, Predicted age-related changes in the mean worm burdens in type I individuals ($M_1(a)$) (top solid line), type II individuals ($M_2(a)$) (bottom solid line) and the total population $M(a)$ (diamond symbols). This example mirrors a population in which the wormy people (fraction f_1) are few in number ($f_1=0.1$) and are characterized by a short immunological memory ($\sigma_1=1$). The dominant proportion of the population ($f_2=0.9$) have a long-lasting memory ($\sigma_2=0.00001$, that is, lifelong). Parameter values: $\Lambda_1=\Lambda_2=20$, $\mu_1=\mu_2=0.25$, $\varepsilon_1=\varepsilon=0.05$; all per yr. *d*, Similar to *c* but the dominant proportion of the population are poor immunological responders to infection ($f_1=0.9$) with a low rate of acquisition of immunity ($\varepsilon_1=0.00001$) when compared with the small fraction of good responders ($\varepsilon_2=0.01$). Parameter values: $\Lambda_1=20$, $\Lambda_2=10$, $\mu_1=1$, $\mu_2=0.25$, $\sigma_1=\sigma_2=0.1$, all per yr.

through time. Laboratory studies of mice repeatedly exposed to helminth infection provide some support for this view (see accompanying paper[19]). We make the simple assumption that the rate of parasite establishment declines linearly as the accumulated experiences of exposure to infection and worm load increase with age. 'Immunological memory' of these past experiences is assumed to last only for a finite period of time. These assumptions can be expressed as a single partial differential equation denoting changes in the mean worm burden, $M(t, a)$, with respect to time t and age a:

$$\frac{\partial M(t,a)}{\partial t}+\frac{\partial M(t,a)}{\partial a}$$
$$=\Lambda(t)\left[1-\Delta\int_0^a \Lambda(t)\exp[-\sigma_2(a-a')]\,da'\right.$$
$$\left.-\varepsilon\int_0^a M(t,a')\exp[-\sigma_1(a-a')]\,da'-\mu M(t,a)\right] \quad (1)$$

Here $\Lambda(t)$ denotes that per capita rate of infection at time t; it is a function of the average fecundity of mature worms, infective stage life expectancy (for indirectly transmitted infection this includes the life expectancy of short-lived vectors or intermediate hosts), host density and the probability of host contact with infective stages or infected vectors[21]. The parameters Δ and ε record the strength of acquired immunity as it acts to decrease parasite establishment resulting from either exposure to infection (Δ) or adult parasite burdens (ε); $1/\sigma_2$ and $1/\sigma_1$ reflect the average duration of the immunolgical memory of past exposure and past worm loads, respectively (if $\sigma_i=0$, memory is life-long; if $\sigma_i=\infty$ memory is absent); and $1/\mu$ denotes the life expectancy of the adult parasites in man. Here, for simplicity, we set $\Delta=0$ because past worm burdens partly reflect past exposure. However, equation (1) has the flexibility to allow for the somewhat different ways in which immunity is influenced by exposure to infection as well as by the burden of established worms.

The acquisition of immunity to parasite establishment is assumed to be the only major constraint on parasite population growth (parasite fecundity and mortality are taken to be independent of parasite density in the present model; such effects are discussed elsewhere[5,9,21,22]). Equation (1) assumes that the human population is homogeneous in its immunological response to parasite invasion (that is, ε is a constant). This assumption can be relaxed by dividing the population into groups ($f_1 \ldots f_n$) in which ε, μ, Λ and σ adopt different values (Λ_i, ε_i, μ_i and σ_i). At equilibrium ($\partial M(t, a)/\partial t=0$), the mean worm burden at age a, $M(a)$, is simply (with $\Delta=0$ and λ real[19]),

$$M(a)=A+(\bar{\Lambda}/\lambda)\left[\frac{(p_1+\sigma)}{p_1}e^{p_1 a}-\frac{(p_2+\sigma)}{p_2}e^{p_2 a}\right] \quad (2)$$

where

$$A=\bar{\Lambda}\sigma/(\sigma\mu+\varepsilon\bar{\Lambda}); \qquad p_1=(\lambda-\mu-\sigma)/2;$$
$$p_2=-(\lambda+\mu+\sigma)/2; \qquad \lambda=([\mu-\sigma]^2-4\varepsilon\bar{\Lambda})^{\frac{1}{2}};$$
$$\bar{\Lambda}=(\sigma+b)(R_0-1)(b+\mu)/\varepsilon;$$

and $1/b=$ human life expectancy (assuming a type II survival curve). The quantity R_0 denotes the basic reproductive rate of the parasite[5,9,21,22] ($R_0>1$ for parasite persistence). Stochastic models for the distribution of worms among hosts in the equilibrium state reveal that the probability distribution of worm numbers per host is Poisson if ε, σ and μ are constant within the human community (the homogeneous case) but overdispersed if R_0, σ or μ vary within different segments of the population (heterogeneity in immunological responsiveness or exposure to infection). Although crude parameter estimates are available for Λ (from studies of reinfection following chemotherapeutic treatment) and μ, little is known about the possible values of ε and σ for the major helminth infections of man. However, the equilibrium/age-intensity curves generated by the model for various plausible values of ε and σ are similar to observed patterns (Fig. 1*a*–*d*). The model can mimic a range of patterns including: monotonic increase in $M(a)$ to a stable plateau; $M(a)$ peaking within childhood, teenage and early adult age groups, and then declining; and damped oscillations in intensity (Fig. 1*a*–*d*). For a fixed severity of the acquired response (ε), the worm burden curve tends to be convex if memory is long,

and/or transmission is intense, and/or worm life expectancy is long. The age at which a maximum worm load is attained depends primarily on the life expectancy of the worm ($1/\mu$) and the severity of acquired immunological response (ε). Certain of these predictions are in qualitative agreement with observed trends: a decline in $M(a)$ in older age groups is more apparent in areas of intense transmission (Fig. 1*a*, *b*). The damped oscillations in mean worm burden that can be produced within individuals under appropriate circumstances derive from essentially the same mechanisms that can produce oscillations in the incidence of microparasitic infections, such as measles, within populations[23].

Observed distributions of parasite number per person are invariably highly aggregated, with most people harbouring few worms and a few people harbouring most parasites (often 70% of the worms are harboured by <10% of the community)[5,8,9,21,22]. For certain helminths, these 'wormy' people seem to be predisposed to this state by as yet undetermined genetic, behavioural or social factors[8,9]. Under the assumption that predisposition to heavy (or light) infection is primarily under genetic[24–28] (via the immune system) or behavioural control, our model can be extended to encompass variability in the way different groups of individuals within a community either respond to parasitic invasion (different values of the parameters ε, σ and μ) or are exposed to infection (different values of Λ).

The range of patterns generated by such 'heterogeneous models' is bewilderingly large. Here, we simply focus on a model with two types of people; namely, high responders (resistive individuals who harbour low burdens) and low responders ('wormy' people). Figure 2 indicates a range of possible patterns for the change in intensity of infection with age. Heterogeneity between the two groups generates aggregation of parasite numbers within the total population, as measured inversely by the negative binomial dispersion parameter, k (Fig. 2*a*). Heterogeneity in exposure to infection (Λ), via its interaction with the rate of acquisition of immunity, can generate age-related changes in the degree of parasite contagion within age classes; these patterns are similar to observed trends (Fig. 2*a*, *b*). Oscillatory changes in worm load with age in high responders can be masked within the overall average burden if low responders predominate and, conversely, the severity of infection within 'wormy' people may be hidden by the overall average if high responders predominate (Fig. 2*c*, *d*). Therefore, average measures may hide interesting age-related trends in individuals predisposed to heavy or light infection. Such trends, if observable (by longitudinal studies of individual patients), could provide valuable insights into the nature of herd immunity to helminth infection.

The dynamics of naturally acquired immunity have important implications for control programmes. Repeated mass chemotherapy at a level less than that required to eradicate the parasites can significantly reduce the level of herd immunity and can raise average worm burdens in the older age classes above the levels pertaining before control (Fig. 3*a*). This trend will be most apparent if strong, long-lasting immunity is acquired (where σ is small and ε large) in areas where transmission is intense (especially relevant to schistosome and filarial infections). This danger can be avoided by targeting drug treatment to those predisposed to heavy infection (see, for example, Fig. 3*b*). Although helpful for community-based chemotherapy, predisposition to heavy infection as a consequence of poor immunological competence may present serious problems for parasite control by vaccination (if and when vaccines become available). A vaccine must be able to convert low responders (wormy people) into high responders if vaccination on a community scale is to be successful.

The model is a crude approximation to the true complexity of acquired immunity to helminths. Although it generates various epidemiological patterns that are similar to those actually observed, predictions must remain tentative; the main purpose of theoretical analysis is to highlight some of the possible population-level implications of acquired immunity to helminth infection. In particular, the model indicates how valuable insights may be gained from longitudinal epidemiological studies of fluctuations in parasite load within individual patients[10–16] and from experimental studies of long-term continual infection in laboratory animals, described in the accompanying paper[19].

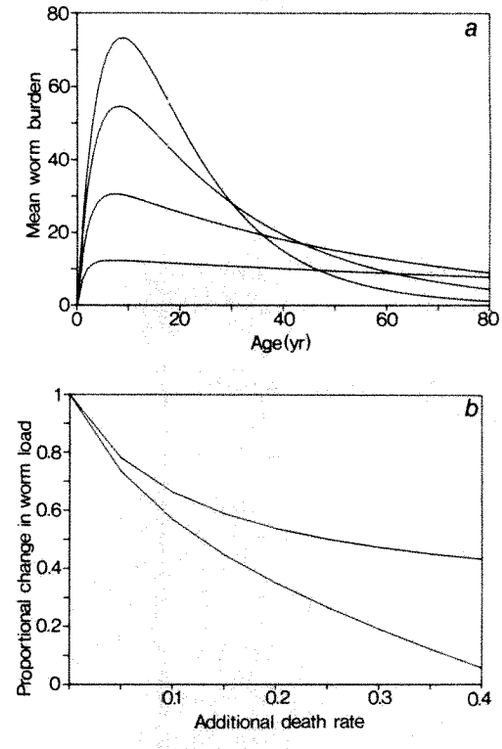


Fig. 3 Impact of chemotherapy on the intensity of infection. ***a***, Homogeneous population (with respect to immunological responsiveness and exposure to infection) in which drug treatment is applied randomly (independent of parasite load) and repeatedly. The four curves denote the equilibrium/age-intensity profile for the precontrol situation (curve 1, at age 10) and three levels of drug application which increase the overall death rate of the parasite within the population from $\mu = 0.25$ (precontrol) to $\mu = 0.33$ (curve 2, at age 10), $\mu = 0.5$ (curve 3, at age 10) and $\mu = 0.75$ (curve 4, at age 10). Parameter values: $\Lambda(\mu) = (\sigma + b)(b + \mu)[R_0 - 1]$, $b = 1/70$, μ = variable, $\varepsilon = 0.0005$, $\sigma = 0.00001$, $R_0 = 1.44$ (when $\mu = 0.75$), $R_0 = 2.13$ (when $\mu = 0.5$), $R_0 = 3.19$ (when $\mu = 0.33$) and $R_0 = 4.16$ (when $\mu = 0.25$). ***b***, Proportional change at equilibrium (over that pertaining before control) in the overall population worm load, $\bar{M}$ (over all age classes and all types of individual) in a population subject to varying degrees of treatment targeted at a 'wormy' fraction ($f_1 = 0.1$). The non-wormy people ($f_2 = 0.9$) are untreated. The value of $\bar{M}$ was calculated from the equation

$$1 = \frac{f_1 R_{01}(b+\sigma_1)}{[(b+\sigma_1)+\varepsilon_1 R_{01}\bar{M}]} + \frac{f_2 R_{02}(b+\sigma_2)}{[(b+\sigma_2)+\varepsilon_2 R_{02}\bar{M}]}$$

where $b = 1/70$, $\varepsilon_1 = 0.00001$, $\varepsilon_2 = 0.0075$, $\sigma_1 = \sigma_2 = 0.1$, $\mu_1 = 0.2$, $\mu_2 = 0.6$, $R_{01} = 4.67$, $R_{02} = 1.63$. Horizontal axis denotes the extra death rate (above the precontrol value of μ_1 and μ_2) resulting from mass chemotherapy. Curve 1 denotes the result of targeting in which only a fraction ($f_1 = 0.1$) are treated (the wormy people with a low ε_1 value, a low parasite death rate (μ_1) and a high parasite basic reproductive rate (R_{01}). Curve 2 denotes mass treatment of the entire population. Note that for an additional death rate of 0.3 per yr (on μ_1 in the case of targeted treatment, and on μ_1 and μ_2 in the case of mass application) the selective treatment of 10% of the population gives a reduction in average worm load ($\bar{M}$) to ~50% (curve 1). By treating the entire population, only an additional 30% change is attained (curve 2).

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Differential activity of maternally and paternally derived chromosome regions in mice

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Although both parental sexes contribute equivalent genetic information to the zygote, in mammals this information is not necessarily functionally equivalent. Diploid parthenotes possessing two maternal genomes are generally inviable[1], embryos possessing two paternal genomes in man may form hydatidiform moles[2], and nuclear transplantation experiments in mice have shown that both parental genomes are necessary for complete embryogenesis[3–6]. Not all of the genome is involved in these parental effects, however, because zygotes with maternal or paternal disomy for chromosomes 1, 4, 5, 9, 13, 14 and 15 of the mouse survive normally[7,8]. On the other hand, only the maternal X chromosome is active in mouse extraembryonic membranes[9], maternal disomy 6 is lethal[7], while non-complementation of maternal duplication/paternal deficiency or its reciprocal for regions of chromosome 2, 8 and 17 has been recognized[10–12]. We report that animals with maternal duplication/paternal deficiency and its reciprocal for each of two particular chromosome regions show anomalous phenotypes which depart from normal in opposite directions, suggesting a differential functioning of gene loci within these regions. A further example of non-complementation lethality is also reported.

The first set of opposite phenotypes was found in mice disomic for chromosome 11. These were produced, together with animals with disomy 13, by intercrossing heterozygotes for the robertsonian translocation, Rb(11.13)4Bnr, which typically exhibit high levels of non-disjunction of chromosomes 11 and 13 (ref. 13) (Fig. 1). Disomy 13 mice appeared normal in all respects, like other disomics previously produced[7,8], but disomy 11 mice were found to differ markedly in size from their sibs; significantly, when their chromosomes 11 were of maternal origin they were consistently smaller than their littermates, and when their chromosomes 11 were of paternal origin they were consistently larger. This was true for both sexes. The phenomenon was most evident within a few hours of birth (Fig. 2), although the size differences present at birth (ratio of weights, 0.606 ± 0.015 g, $n = 40$, and 1.408 ± 0.036 g, $n = 36$) usually persist to adulthood (small ♀♀, 0.576 ± 0.036 g, $n = 11$; small ♂♂, 0.627 ±

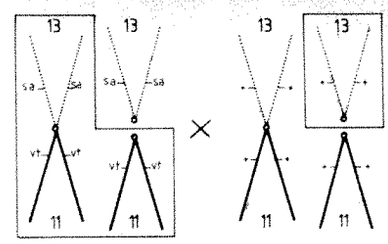


Fig. 1 Production and detection of disomy 11 and 13. Heterozygotes for the robertsonian translocation, Rb(11.13)4Bnr, exhibit high levels of non-disjunction of chromosomes 11 and 13. When intercrossed, normal disjunction in both parents results in the production of chromosomally balanced young; with non-disjunction in one parent, monosomy and trisomy for chromosomes 11 and/or 13 result; but when non-disjunction occurs in both parents and complementary products (as illustrated) unite, chromosomally balanced young disomic for chromosomes 11 (as shown) or 13 are produced. These are most readily detected when one parent is homozygous for a gene marker; marked young are then either maternal or paternal disomics according to which parent carries the marker. Disomy 11 is detected in this way with a frequency of about 1-2%[16] using the gene marker vestigial tail (*vt*). It has also been found with a dominant marker, Wavy coat (Re^{wc}). Marked maternal disomics are smaller than their sibs, marked paternal disomics are larger. Most unmarked young are of normal chromosome origin but unmarked disomic young are also found; in crosses producing large paternal disomy 11 progeny, small maternal disomy 11 young may also occur (*x* and *y*, Fig. 2) and their genotype can be confirmed by breeding tests. The reverse is also true. The size phenomenon is not therefore dependent on the marker genes present. In the same crosses, satin (*sa*) was the marker for detecting disomy 13.

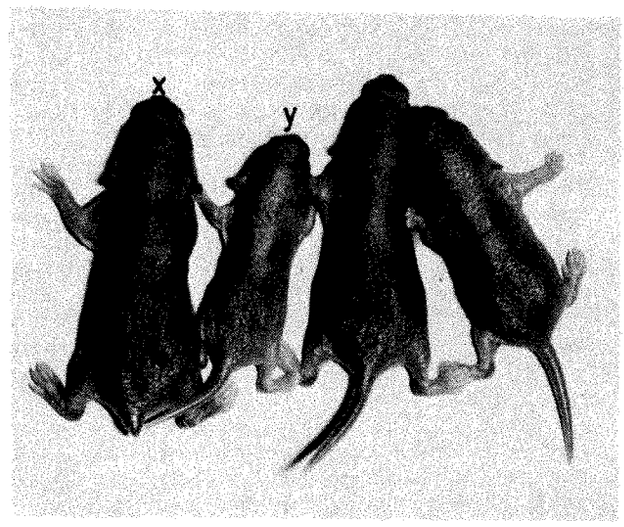


Fig. 2 Maternal and paternal disomy 11 in a single litter. Litter from an unmarked Rb4Bnr female heterozygote sired by a *vt* marked Rb4Bnr male heterozygote. The *vt* marked paternal disomic (*x*) is large, whereas one marked sib is clearly small (*y*). Breeding tests on such small animals show that most are not heterozygous for *vt*, but rather homozygous wild type and therefore maternal disomics. The reverse situation also occurs. Marked and unmarked disomics occur with near-equal frequencies, although seldom, as here, within single litters.

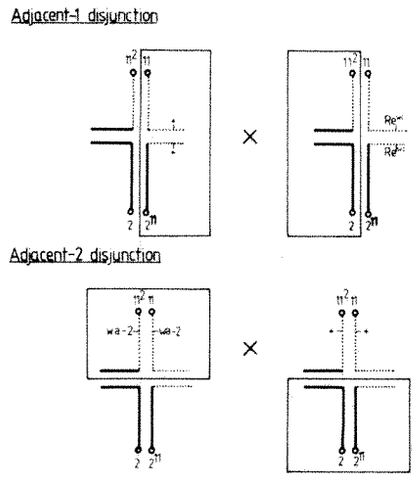


Fig. 3 Intercrosses of T(2; 11)30H heterozygotes. Alternate segregation in both parents leads to the production of chromosomally balanced young; adjacent-1 or adjacent-2 disjunction in one parent leads to the production of young with unbalanced, duplication/deficiency chromosomal constitutions. However, when adjacent-1 disjunction occurs in both parents and complementary products (as illustrated) unite, chromosomally balanced young appear which are either maternal duplication/paternal deficiency or maternal deficiency/paternal duplication for the distal region of chromosome 11 (and vice versa for chromosome 2). These can readily be detected when one parent is homozygous for a gene marking this chromosome region, Re^{wc}, in the present experiments. Each of the two duplication/deficiency types occurs with a frequency of about 11–14% ($n = 514$). When adjacent-2 disjunction occurs in both parents and complementary products (as illustrated) unite, chromosomally balanced young which are either maternal duplication/paternal deficiency or maternal deficiency/paternal duplication for the proximal region of chromosome 11 (and vice versa for chromosome 2) should be produced. These should be detectable with a proximal 11 chromosome marker, waved-2 (*wa-2*), in the present experiments. When the female parent carried the marker, *wa-2* progeny representing maternal duplication/paternal deficiency for proximal 11 were detected with a frequency of ~1.3% (2/153 classified for *wa-2* at 10 days of age); all were small like maternal disomy 11. No large unmarked young which could have represented maternal deficiency/paternal duplication for proximal 11 were detected, however, and, consistent with this, no *wa-2* progeny were recovered (0/248) from intercrosses in which the male parent carried the marker. The genotype may therefore be lethal (see text). However, small, unmarked young which proved to be maternal duplication/paternal deficiency types by breeding tests were recovered from the latter cross (3/248).

0.045 g, $n = 19$; large ♀♀, 1.328 ± 0.020 g, $n = 15$; but for large ♂♂, 1.234 ± 0.036 g, $n = 14$).

The large and small disomics were proportionately normal in all respects, they exhibited no viability problems and both sexes were fertile. The size differences did not persist into the next generation; the progeny of both large and small disomics of each sex and those of control Rb4Bnr heterozygotes were all of similar weight at birth and at 3 weeks of age. The absence of any indication of a maternal effect on progeny size may be attributed to the small litter sizes in all crosses, resulting from the high rates of translocation-associated non-disjunction.

Intercrosses between mice heterozygous for the reciprocal translocation, T(2; 11)30H, provided evidence that only the proximal region of chromosome 11 (proximal 11) was associated with the size phenomenon (Fig. 3). Neither maternal duplication/paternal deficiency for distal 11, nor the reciprocal, resulted in mice which were of abnormal size at birth. However, maternal duplication/paternal deficiency for proximal 11 resulted in mice which were small in size both at birth and subsequently, resembling maternal disomy 11 in all respects. No young representing maternal deficiency/paternal duplication for proximal 11 have been found and it seems probable that this genotype is lethal.

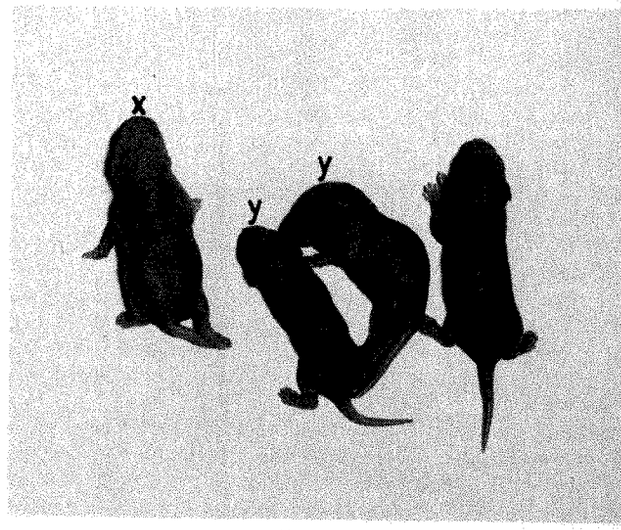


Fig. 4 Anomalous young produced from T(2; 11)30H intercrosses. *x*, Phenotype characterizing maternal deficiency/paternal duplication for the distal region of chromosome 2; *y*, phenotype characterizing maternal duplication/paternal deficiency for this region (see text).

As paternal disomy 11 is viable, the lethality may be reasonably attributed to the chromosome 2 constitution (maternal duplication/paternal deficiency for proximal 2) and represent a further example of the non-complementation phenomenon described by Searle and Beechey[10].

The second set of opposite phenotypes was derived from the T30H crosses. Although both maternal duplication/paternal deficiency for distal 11 and its reciprocal were detected, both genotypes proved to be postnatal lethals. Again, because both maternal and paternal disomy 11 are viable, the lethality may be attributed to the chromosome 2 constitutions, namely, maternal deficiency/paternal duplication for distal 2 and its reciprocal. A lethality for maternal duplication/paternal deficiency for this region of chromosome 2 has been reported previously[10], but a characteristic phenotype can now be described. Such mice have long flat-sided bodies with arched backs (Fig. 4). More strikingly, they become almost totally inactive within a few hours of birth and are killed by their mothers. Very few survive more than 24 h. A remarkable finding was that the reciprocal type, maternal deficiency/paternal duplication for distal 2, shows an effectively opposite phenotype. These mice have short square bodies with broad flat backs (Fig. 4) and are notably hyperkinetic. They usually survive for several days but fail to grow normally, and their behaviour becomes increasingly extreme, tremor and balance defects appearing frequently.

Because the anomalous phenotypes associated with maternal and paternal disomy 11 and corresponding types of duplication/deficiency for distal 2 can each occur among the progeny of single crosses, maternal cytoplasmic effects cannot be responsible for the abnormalities. Rather, a differential functioning of maternal and paternal chromosomes or chromosome regions is indicated, which suggests the existence of a form of chromosome imprinting[14,15] that affects gene activity. The contrasting phenotypes (small and large; hypokinetic and hyperkinetic) depart from the normal in opposite directions and this suggests excess versus shortage of gene activity. This could mean either single or earlier activity of the paternal chromosome regions in the cases cited, and inactivity or later activity of the corresponding maternal regions. Paternal duplication would then give excess/earlier activity and the reciprocal subnormal/later activity. The X-inactivation process[16] provides precedence for the activity of only one parental chromosome or chromosome

region[17]; precedence for an earlier activity of one chromosome or chromosome region over its homologue has not been found, but has been reported at the gene locus level in diverse species and inter-family hybrids, including the mouse[18–23].

The phenomena described could, of course, result from the differential regulation of single loci in the chromosome regions concerned. The size phenomena associated with proximal 11 might involve a growth hormone gene such as Ames dwarf (*df*)[24], which lies in this chromosome region. The *df* mutant is of normal size at birth despite a severe deficiency of pituitary hormones[25], but the homologous chromosome region in man[26] carries a cluster of growth hormone loci[27], and at least one of these acts in the placenta. Hormonal control of fetal growth mediated through the placenta is therefore suggested. For distal 2, the behavioural problems of the anomalous mice may mean they have neurological defects; therefore, the *wst* locus[28] may be involved.

Single or preferential activation of regulator loci or of receptor loci, to give the latter different affinities for regulator substances, may underlie the phenomenon presented here, and detailed studies of the anomalous mice may provide some answers. An interaction between loci in different chromosome regions is a further possibility and studies with translocations having other breakpoints in chromosomes 2 and 11 could be informative here. Whatever the mechanism operating, it may well be the basis of the non-complementation lethality phenomenon[10] and the lethality manifested by parthenogenic and androgenic embryos[1–6].

Since submission of this paper, it has been reported that an 180-base-pair DNA sequence thought to control pattern formation in early development, a homoeo box, is located on human chromosome 17 and mouse chromosome 11 (refs 29, 30) and therefore in the chromosome region responsible for the size phenomena described here. Another homoeo box has been located on chromosome 6 (ref. 31), which exhibits non-complementation lethality[7]. The occurrence of homoeo boxes in chromosomes which show differential activity according to parental origin could be fortuitous, but the association suggests a functional relationship. If true, homoeo boxes should also be found on chromosomes which show the parental origin phenomenon (2, 8 and 17), but perhaps not on those which do not (1, 4, 5, 9, 13, 14 and 15).

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Substance P raises neuronal membrane excitability by reducing inward rectification

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Much interest has recently centred on the properties of peptides that modulate the excitability of nerve cells. Such compounds include the undecapeptide substance P, which is particularly well established as an excitatory neurotransmitter[1,2], and we examine here its effects on magnocellular cholinergic neurones taken from the medial and ventral aspects of the globus pallidus of newborn rats and grown in dissociated culture[3]. These neurones have previously been shown to respond to substance P[3] and are analogous to the nucleus basalis of Meynert in man[4,5], which gives a diffuse projection to the cerebral cortex and whose degeneration is the likely cause of Alzheimer's disease[5]. Substance P depolarizes these cultured neurones by reducing an inwardly rectifying potassium conductance; this conductance has been found in several neuronal types[6–9] and has similar properties to those of certain other cells[10–13]. As discussed below, modulation of inward (or anomalous) rectification by substance P implies a self-reinforcing element to the depolarization caused by the peptide.

Neurones grown from 10 days to 3 weeks in culture were examined under whole cell recording[14,15] (for details, see Fig. 1 legend). When substance P (3 μM) was pressure ejected onto neurones examined in constant current conditions, a depolarization was produced (Fig. 1*a*) which lasted for 15 s to ≥5 min. Its amplitude was rarely as large as shown in Fig. 1*a*, but generally exceeded 1–2 mV. As described previously, this depolarization was associated with a fall in membrane conductance (for example, refs 16, 17), but in the cells examined in the present study, the fall in conductance was seen more clearly if the membrane was hyperpolarized (Figs 1*b*, 2*a*), implying that inward rectification[6–13] is the conductance affected.

In establishing that inward rectification is modulated, we have sought to satisfy three criteria; first, that a K^+ conductance with an inwardly rectifying current voltage relation is reduced; second, that the conductance depends on external K^+ concentration as well as on voltage[11–13,18]; and third, that the conductance mechanism is nearly impermeable to rubidium (refs 10, 12, 19), which should therefore abolish the response to substance P.

The nature of the response to substance P is illustrated in the current–voltage relations of Fig. 2*b*. The substance P-sensitive current reversed its polarity close to E_K, the equilibrium potential for K^+, reversal changing from -98.0 ± 1.9 mV ($n = 6$) to $-85.4 + 1.3$ mV ($n = 14$) to -57.9 ± 1.6 mV ($n = 7$) as external K^+ was changed from 2.5 to 5.0 to 12.5 mM (internal K^+, 128 mM).

Although it remains possible that conductances other than inward rectification are affected, neither the transient A-current[20] (Fig. 2*a*) nor the delayed K^+-current seem to be involved in the response of these cells. As Ca^{2+}-activated K^+ currents also become activated under depolarization[21,22], rather than under hyperpolarization as here, their involvement is unlikely. In addition, in our experiments internal Ca^{2+} was buffered to 10^{-7} M. The M-current, which is known to be involved in the response to substance P in certain cells[23], was not seen in the conditions of our experiments.

Our experimental external solutions contained tetrodotoxin (TTX, 1 μM) to block Na^+ currents, and blockage of Ca^{2+} currents with cadmium (0.5 mM, two cells) did not alter the response. Hence, the shape of the substance P-sensitive current-

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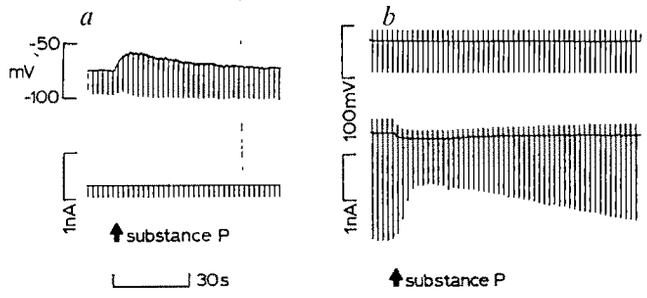


Fig. 1 Depolarization (*a*) and change in membrane current (*b*), measured under voltage clamp, resulting from application of 3 μM substance P to a neurone cultured from the magnocellular basal forebrain of a newborn rat The cell had been grown for 11 days in modified Eagle's medium with Earle's salt at 37 °C in a 10% CO_2 atmosphere. The methods of isolation, dissociation and culture have been briefly described previously[3], a detailed account of the methods will be published elsewhere. The initial resting potential of the cell was −65 mV; soma diameter, 28 μm, temperature, 31.2 °C. In *a*, a steady current of −75 pA was applied, which took the resting potential to approximately −75 mV; current pulses were −240 pA in amplitude and 100 ms long. In *b*, the holding potential was −76 mV The depolarizing pulse was 20 mV in amplitude and the hyperpolarization, 60 mV. Both were 100 ms long. E_K, measured from the substance P response, was −89 mV. Whole cell recording was used[14,15]. Recording pipettes were made from Blue Tip microhaematocrit capillary, and their tips were fire-polished but uncoated[14] The pipette used in the experiment shown here had a resistance of 3.0 MΩ. The pipette (intracellular) solution contained (mM)[15]. K-aspartate, 120; NaCl, 40; $MgCl_2$, 3; $CaCl_2$, 0.25 and EGTA, 0 5 (calculated ionized Ca^{2+}, 10^{-7} M); HEPES/KOH buffer, 5; Na_2ATP (vanadate-free), 2. The *p*H of this solution was adjusted to 7.2 with KOH. External solution (mM)· KCl, 5; NaCl, 145.5, $CaCl_2$, 2.4; $MgCl_2$, 1 3, (+)glucose, 11; HEPES/NaOH buffer (*p*H 7 4), 5. Internal and external solutions were designed to have the same osmolarity (~310 mosmol). The recording amplifier was a List electronic type EPC 7 patch clamp, and currents were filtered at 3 kHz, using the filter of this amplifier. Records of current, voltage and timing of substance P application were stored on a Hewlett-Packard 3520B tape recorder for later analysis Voltage commands were provided from the gated output of a Digitimer type D4030, divided appropriately. Application of substance P was made by pressure ejection. A pipette, tip diameter 5 μm, was filled with external solution containing substance P at 3 μM Timed pressure pulses (~0.2 p.s.i and 1 63 s long) ejected this solution onto the cell. To avoid excessive depolarization of neurites by substance P, this pipette was placed close to the soma of the neurone. In this experiment the distance was 12.8 μm from the edge of the soma, and single 'puffs' of substance P were applied at the times indicated by arrows.

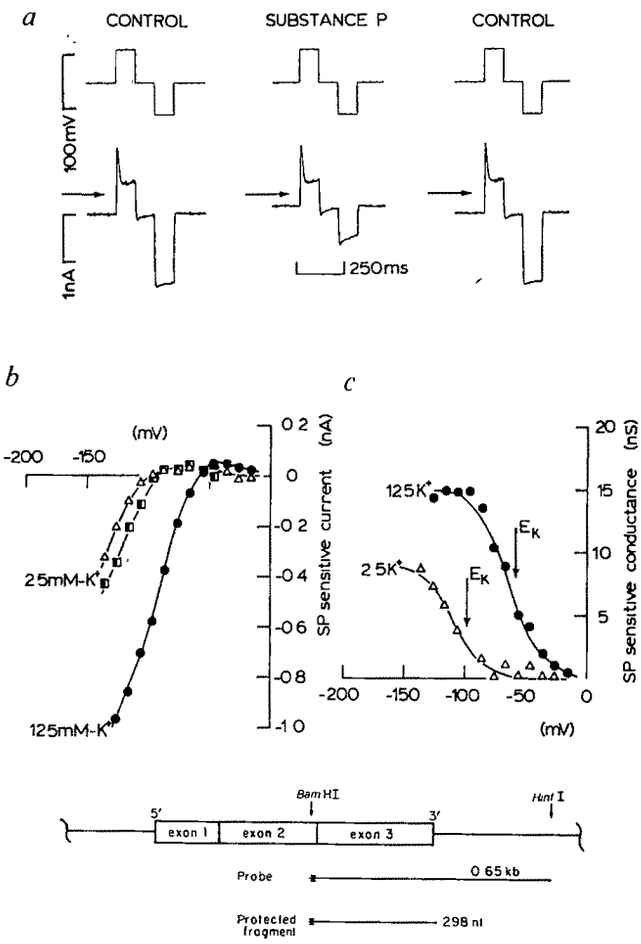


Fig. 2 *a*, Records of membrane potential (mp) (above) and membrane current (below) in 12.5 mM K^+ obtained before, during and after recovery from application of 3 μM substance P Holding potential, −76 mV, voltage steps depolarize and hyperpolarize by 60 mV for 100 ms. The pulses were applied 100 ms apart Horizontal arrows indicate the zero current level Note that the A-current is activated by depolarization, but is unaffected by substance P Inward currents are substantially reduced, and substance P seems to increase the rate of inactivation of these inward currents under hyperpolarization Three puffs (each 1 63 s long) were applied in rapid succession to produce a brief plateau in the response to the peptide, during this time a membrane current–voltage relation was measured with pulse pairs whose amplitude increased in 10-mV steps from ±10 to ±60 mV This procedure took 6 s to complete. *b*, Current–voltage relations from the same cell as in *a*, measured first in 2.5 mM (△), then 12.5 mM (●), then in 2 5 mM K^+ (◨). The current plotted is the substance P-sensitive current, measured by subtracting the current at the end of the 100-ms voltage steps applied during the action of substance P from the average of the control currents measured before and after recovery from substance P application During the final run in 2 5 mM K^+, no control was measured after substance P application, owing to breakdown of the seal between the recording pipette and the cell The initial resting potential of the cell was −77 mV, diameter of cell soma, 26 μm, temperature, 29.2 °C. In 2 5 mM K^+ solution, NaCl concentration was 148 mM, and in 12 5 mM K^+ it was 138 mM, otherwise the external solution had the composition given in Fig. 1 legend Ordinate, substance P-sensitive membrane current (nA), abscissa, membrane potential (mV). *c*, Chord conductance for the substance P-sensitive current The currents in 2 5 mM K^+ (△) and 12.5 mM K^+ (●) are divided by the driving force on K^+ ($V-E_K$, where E_K is measured from the reversal potential of the substance P-sensitive current–voltage relations) The relations are fitted by Boltzmann expressions, according to

$$g_K = \bar{g}_K\{1+\exp[(V-\bar{V})/k]\}^{-1}$$

where g_K is potassium conductance, $\bar{g}_K$ is limiting potassium conductance, $\bar{V}$ is the voltage at which $g_K = \bar{g}_K/2$, and k determines the steepness of the curve. For 2.5 mM K^+ $\bar{g}_K = 9.0$ nS; $\bar{V} = -109$ mV and $k = 11\ 5$ mV For 12.5 mM K^+, $\bar{g}_K = 15\ 0$ nS; $\bar{V} = -62$ mV and $k = 13\ 8$ mV. Inward rectification of egg cells[10] and skeletal muscle[18] has previously been fitted to an expression similar to the above equation, with a similar steepness for voltage dependence, and an approximately similar increase in $\bar{g}_K$ with $[K^+]_o$ The measured E_K (indicated by vertical arrows) shifts by 40 mV when $[K^+]_o$ is increased from 2 5 to 12 5 mM (theoretical, 42 mV), $\bar{V}$ shifts by 47 mV, close to the shift in E_K Ordinate, substance P-sensitive chord conductance (nS); abscissa, membrane potential (mV)

voltage relation is not influenced by altered activation of inward Na^+ or Ca^{2+} currents under depolarization.

The neurones we used possess long neurites whose membrane potential will not be fully controlled by the voltage clamp. Because this problem will affect the amount of membrane current we record, we minimized excitation of neurites by substance P by applying the peptide close to the cell body. We also attempted to test whether lack of complete control of neurites influences the shape of the relation between substance P-sensitive membrane current and membrane potential by applying tetraethylammonium ions (TEA^+, 50 mM), which reduce membrane potassium conductance[24] and may therefore be expected to alter voltage decrements along neurites. TEA^+ reduced the size of the response to substance P, but had no effect on the shape of the current–voltage relation (Fig 3*b*).

The second criterion for the dependence of the conductance on both external K^+ and voltage was demonstrated by measuring the response in the same cell in 2.5, 12.5 and 2.5 mM K^+ (Fig. 2). The dependence on external K^+ concentration ($[K^+]_o$) is seen more clearly if the K-chord conductance is computed (by dividing the substance P-sensitive membrane current by the driving force on K^+) and plotted against membrane potential (Fig. 2*c*). The relationship between conductance and potential was shifted

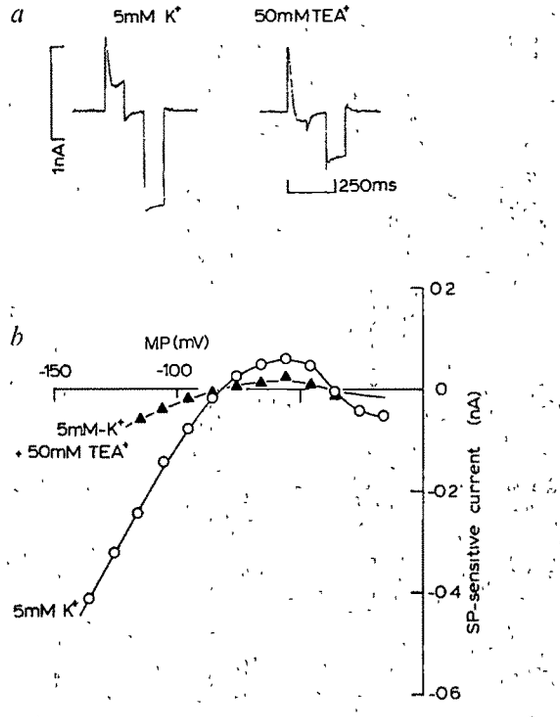


Fig. 3 *a*, Records of membrane current made under 50 mV depolarizations and hyperpolarizations from the holding potential of −76 mV, in a cell immersed successively in 5 mM K^+ solution and 5 mM K^+ + 50 mM TEA^+ solution. This concentration of TEA^+ leaves the A-current virtually unaffected, but abolishes delayed K^+ currents recorded under depolarization. Inward rectification is reduced, as indicated by the reduction in inward current under hyperpolarization and by the reduced response to substance P (*b*). *b*, Substance P-sensitive current (measured as described in Fig. 2 legend, but at the start of the voltage step) plotted against membrane potential in 5 mM K^+ (○) and 5 mM K^+ + 50 mM TEA^+ solution (▲). The TEA^+ solution contained 96 mM NaCl. The line to the TEA^+ results is that to the open circles, multiplied by 0.26, indicating that the form of the current–voltage relation is not altered by TEA^+, even though the current amplitude is reduced. Cell resting potential, −70 mV; diameter of cell soma, 24 μm; temperature, 29.9 °C; diameter of substance P pipettes, 5 μm; distance from cell, 12.8 μm; resistance of recording pipette, 3.0 MΩ.

to more positive levels at higher $[K^+]_o$, a property characteristic of inward rectification[11,13].

The third criterion that distinguishes inward rectification from certain other classes of K^+ conductance is its low permeability to Rb^+ (refs 10, 12, 19). Figure 4 shows that replacement of external K^+ by Rb^+ blocks inward rectification, reducing the membrane current that flows at potentials negative and slightly positive to the resting potential. Evidently, Rb^+ is itself impermeant and acts to block efflux of K^+ through the inward rectifier. The substance P-sensitive conductance of this cell had a current–voltage relation similar to that blocked by Rb^+, and Rb^+ abolished the response to substance P (Fig. 4*b*,*c*).

We have investigated certain other properties of this substance P-sensitive permeability. It is blocked by the addition of external Cs^+ (2 mM), as are other inward rectifiers[8,25–27]; it persists in the absence of external Na^+ (tetramethylammonium replacement), unlike that reported in hippocampal neurones[9]; it also persists in the absence of internal Na^+, whose replacement with K^+ does not abolish the response to substance P. This property (and its sensitivity to TEA^+ reported above) is shared with inward rectification of skeletal muscle[18,28], but not with that of egg cells, which is abolished in the absence of internal Na^+ (ref. 11).

Our results with substance P on magnocellular basal forebrain neurones of the rat are reminiscent of those obtained with serotonin on *Aplysia* (molluscan) neurones by Benson and Levitan[25]. In *Aplysia*, serotonin enhances inward rectification by phosphorylating the K^+ channels through cyclic AMP-dependent protein kinase. Here substance P reduces inward rectification. Although we have not investigated the steps between the binding of substance P to its receptor and the closure of K^+ channels, it seems likely that an intracellular second messenger system is involved[29], and that this is not washed out during whole cell recording for periods up to 1 h.

Substance P may have several effects on nerve cells. For example, in chromaffin cells, which secrete catecholamines, it acts indirectly, reducing the excitatory response to acetylcholine by increasing the rate of desensitization of nicotinic receptors for that transmitter[30]. In the central cholinergic neurones used in this study, excitability was increased, the more common physiological effect of the peptide[1,2]. As we have shown, this effect is achieved by reducing inward rectification, which contributes substantially to the resting membrane conductance (Fig. 4). Because this K^+ conductance decreases on depolariz-

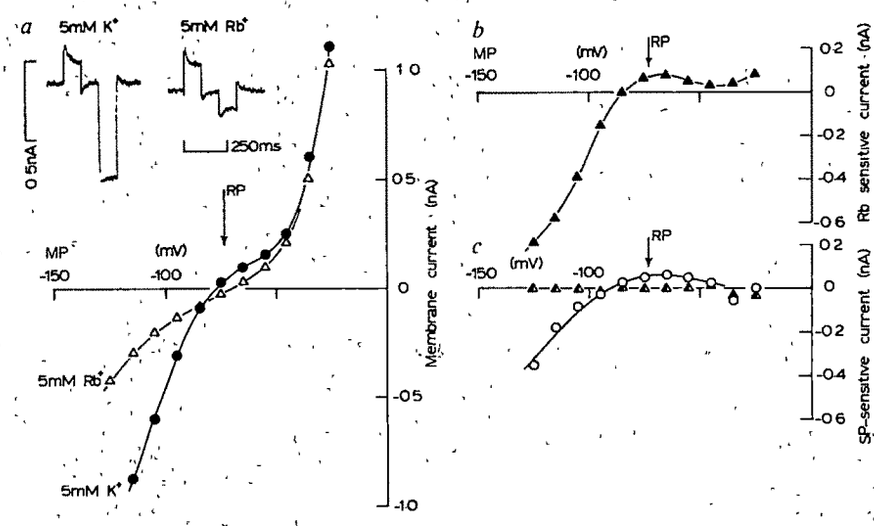


Fig. 4 Rb^+-induced block of inward rectification and of the effect of substance P. *a*, Membrane current–voltage relation obtained by plotting current at the beginning of the 100-ms potential step or at the peak of the A-current, which becomes activated at potentials positive to −50 mV. Currents were recorded in 5 mM K^+ (●) solution and then in 5 mM Rb^+ (zero K^+, △) solutions. These solutions also contained NaCl at 145.5 mM, but were otherwise identical with that listed in Fig. 1 legend. The vertical arrow denotes the resting potential (RP) of the cell (−74 mV) recorded immediately after sealing the recording pipette onto the cell and breaking the membrane patch for whole cell recording. The inset shows membrane currents obtained in response to 30 mV depolarizations and hyperpolarizations from the holding potential (−76 mV) in 5 mM K^+ and 5 mM Rb^+ solutions, 3.3 MΩ; diameter of cell soma, 24 μm; temperature, 30.3 °C. Ordinate, membrane current (nA); abscissa, membrane potential (mV). *b*, Membrane current removed by Rb^+ substitution. The current–voltage relation was obtained by subtracting the current in 5 mM Rb^+ from that in 5 mM K^+. Arrow denotes initial resting potential of the cell. *c*, Membrane current–voltage relation for the substance P-sensitive current in 5 mM K^+ (○) and in 5 mM Rb^+ (▲) solution. These relations were obtained as in Fig. 2 by subtracting the current during substance P action from the average of currents measured in controls before and after recovery from substance P application. Vertical arrow, initial resting potential of the cell. In this cell, whole cell recording failed during the return to 5 mM K^+ solution. However, we recovered the substance P effect after Rb^+ application in one other cell.

ation and increases as the membrane potential becomes more negative (Fig. 2c), substance P, by reducing K^+ conductance, will induce depolarization and this depolarization will further reduce K^+ conductance. Thus, by modulating inward rectification, substance P (and serotonin in neurones of *Aplysia*) will have a self-reinforcing element to its effect on excitability.

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Modulation of a transient outward current in serotonergic neurones by α_1-adrenoceptors

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The excitability of various neurones in the mammalian central nervous system (CNS), ranging from motoneurones to serotonergic neurones, is enhanced by α_1-adrenoceptor agonists[1]. Excitations mediated via α_1-adrenoceptors are associated with a slow depolarization and an increase in input resistance, probably resulting from a decrease in resting potassium conductance[1,2]. However, the involvement of voltage-dependent transient currents in mediating α_1 excitatory effects has not been evaluated. An early transient outward current has been described which is important in regulating the frequency of repetitive firing; it is activated by depolarizing voltage steps from potentials more negative than rest and blocked by 4-aminopyridine[3,4]. This current, which has been termed 'I_A', was found originally in invertebrates[3,4] and subsequently in various vertebrate neurones[5-8]. The present single-electrode voltage-clamp study demonstrates an early transient outward current (I_A) in serotonergic neurones which is suppressed by noradrenaline and the α_1-agonist phenylephrine; a suppression of I_A may account in part for the acceleration of pacemaker activity induced by α_1-agonists in serotonergic neurones.

Rat serotonergic dorsal raphe neurones, investigated in voltage-clamp conditions (see Fig. 1 legend), exhibited an early transient outward current with the characteristics of I_A as originally described in molluscan neurones[3,4]. This current could be evoked by depolarizations from holding potentials below resting potential and suppressed by 4-aminopyridine (4-AP; $n = 5$) (Fig. 1); in unclamped cells in the absence of tetrodotoxin (TTX), 4-AP (1 mM) produced a marked activation of firing

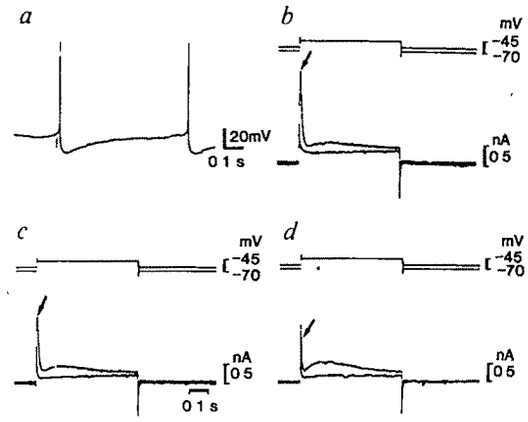


Fig. 1 A transient outward current in a serotonergic neurone and its suppression by 4-aminopyridine (4-AP). *a*, Spontaneous spikes of a typical serotonergic neurone in the dorsal raphe slice preparation; note the large AHPs, followed by a rapid phase of depolarization and then a plateau period before the subsequent spike. In *b*, the slice has been perfused with TTX (1 μM) to eliminate sodium spikes and the cell has been voltage clamped at −70 mV (holding current, −0.01 nA); a step depolarization to −45 mV elicits a transient outward current (arrow) which peaks approximately 8 ms after the depolarizing step; a superimposed trace shows the virtual absence of a transient outward current when the holding potential is −60 mV rather than −70 mV. In *c*, 10 min after the addition of 1 mM 4-AP to the perfusate, the transient outward current (arrow) is reduced by ~30% (leakage current was estimated by subtraction of instantaneous current after the −60 to −45 mV step). In *d*, 12 min after the 4-AP has been increased to 2 5 nM, the transient outward current (arrow) is reduced by more than 60%. In *b* and *c* the current traces are beneath the voltage traces; outward currents are upward going Photographs were taken on line from a storage oscilloscope.

Methods. Coronal midbrain slices (300-350 μm) through the dorsal raphe nucleus were obtained from 53 male albino rats (125-200 g; Charles River). As described previously[18], slices were placed on a lens paper strip at the fluid-gas interface of a slice chamber; the temperature was kept at 33 ± 0.5 °C. Artificial CSF, gassed with 95% O_2/5% CO_2 to give a final *p*H of 7.37-7.4, was composed of (in mM). NaCl, 130; KCl, 5.0; $CaCl_2$, 2.0; $MgSO_4$, 2.0, $NaHCO_3$, 25.7; NaH_2PO_4, 1 25; (+)glucose, 15. Drugs were administered in known concentrations in the perfusate; ascorbic acid (0.1 mM) was added to solutions of noradrenaline to retard oxidation. There was a continuous flow of humidified 95% O_2/5% CO_2 through the chamber. To facilitate the flow of fluid over the tissue, slices were covered with a monolayer of cotton gauze. To minimize capacitance and resistance, short stubby electrodes (7-8 mm, shank to tip; 30-50 MΩ) were pulled with a Brown-Flaming puller (Sutter Institute) using Microstar tubing (1.4 mm; Radnoti Glass Inc.); filling was with 2 M KCl. Intracellular recording and single-electrode voltage clamping were conducted using an Axoclamp-2 voltage clamp (Axon Instruments); electrode settling time was within 50-75 μs, allowing switching frequencies of 4-6 kHz and a loop gain of at least 5 nA mV^{-1} (30% duty cycle). Although phase lag was used (to prevent oscillations), false clamping was avoided by using optimal capacitance neutralization and by selecting a switching frequency which allowed full settling to a horizontal baseline as determined by continuously monitoring input voltage[19]. As an operational criterion of the adequacy of the space clamp, loop gain was increased until high-threshold calcium spikes, induced by step depolarizations, were completely clamped; this was carried out in the presence of TTX (1 μM; Sigma) to block sodium spikes. The dorsal raphe nucleus was visualized by its surface markings in incident light; serotonergic neurones within the dorsal raphe nucleus were identified by their characteristic long-duration action potentials (2 ms), high input resistance (200-400 MΩ), large AHPs, and pacemaker potentials[11,18].

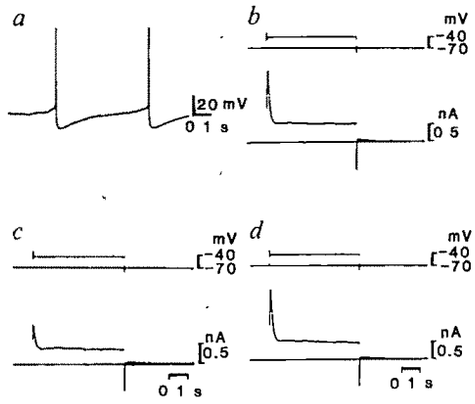


Fig. 2 Suppression by phenylephrine of the transient outward current (I_A) in a serotonergic neurone and the reversal of this effect by prazosin. *a* Shows the spikes of a neurone before clamping and treatment with TTX; this cell was not spontaneously active and spikes were evoked by 0.02 nA of depolarizing current. In *b*, TTX (1 μM) has been added to the perfusate and the cell has been clamped at −70 mV (holding current, −0 01 nA); a step depolarization to −40 mV elicits a typical I_A. As in Fig. 1, leakage current was estimated by holding at −60 mV (where I_A is inactivated; see Fig. 3*e*) and measuring instantaneous current after stepping to −40 mV (trace not shown). In *c*, the amplitude of I_A is markedly reduced after perfusion (15 min) with artificial CSF containing phenylephrine (25 μM); note that the holding current has shifted to −0.07 nA, indicating an increase in resting inward current. Although not readily discernible at this gain and holding potential, there is also a prolongation of the outward tail current (thicker trace) with little or no change in maximal amplitude during phenylephrine administration; this effect was seen consistently for both phenylephrine and noradrenaline. In *d*, 12 min after the addition of prazosin (5 μM) in the continued presence of phenylephrine, there is a total reversal of the suppression in I_A.

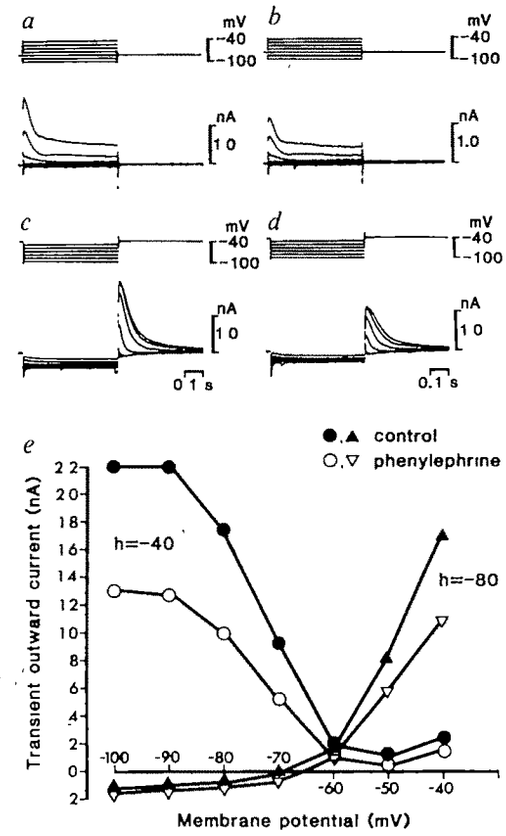


Fig. 3 The effect of phenylephrine on I_A activation/inactivation curves. In *a* and *b*, holding potential (h) is at −80 mV (holding current in *a*, −0 07 nA), current responses (lower traces) are to voltage steps (upper traces) ranging from −40 to −100 mV. Note that I_A first becomes activated at −60 mV and increases progressively up to the −40 mV step. In *b*, the administration of phenylephrine (25 μM; 18 min) results in a decrease in amplitude at each step. In *c* and *d*, the holding potential is at −40 mV (holding current in *c*; +0 25 nA) with hyperpolarizing prepulse steps ranging from −50 to −100 mV; note that I_A is de-inactivated only by hyperpolarizing steps below −60 mV. In *d*, following the administration of phenylephrine (15 min), the holding current shifts to +0 16 nA and there is a reduction in the amplitude of I_A elicited by a return to the holding potential (−40 mV). Note the decrease in leakage current in the prepulse series (−50 to −100 mV) during phenylephrine perfusion as compared with the control series in *c*. In *e*, control and phenylephrine traces for holding potentials of h = −40 mV and h = 80 mV are plotted as direct current-voltage (*I-V*) curves (no leakage current subtraction), as can be seen, at all voltage steps where I_A is activated, phenylephrine reduces the maximum amplitude. Also note that phenylephrine induces a small increase in 'resting' inward current. The slice was perfused with artificial CSF containing TTX (1 μM) throughout the experiment to block sodium spikes.

rate (not shown). A further similarity to I_A is the fact that in artificial cerebrospinal fluid (CSF) containing 5 mM KCl, tail currents of this early transient outward current had a reversal potential of approximately −82 mV (±3 s.d.; n = 4 cells); this shifted to −68 mV (±3 s.d.) in 10 mM KCl, as would be predicted by the Nernst equation for a current carried by potassium ions. Based on these criteria, the early outward current of serotonergic neurones is hereafter designated I_A. To achieve rapid responses, the effects of noradrenaline or phenylephrine on the amplitude of I_A were tested at a concentration of 25 μM, which is approximately 10 times their ED_{50} for activating the firing of serotonergic neurones[11]. Phenylephrine (25 μM) produced a marked reduction in the amplitude of I_A (Fig. 2). For the total group of serotonergic cells tested in this manner, phenylephrine and noradrenaline produced a 38% (±10 s.d.; n = 8) and 34% (±13, s.d., n = 8) reduction in I_A, respectively (leakage current was subtracted as described in Fig. 1 legend); increasing the concentration of phenylephrine or noradrenaline produced little or no further change. Activation/inactivation curves showed that suppressions of I_A by phenylephrine (Fig. 3; n = 5) and noradrenaline (n = 5) were present throughout a wide range of voltages, with initial activation occurring between −60 and −50 mV; in addition, phenylephrine reduced the leakage current (Fig. 3*d*), an effect which corresponds to the increase in input resistance detected by current-clamp methods[1].

The complex time course of current offset (see, for example, Fig. 3*a*, *b*) suggests the presence of transient currents in addition to I_A; these may include rapidly activating Ca^{2+} and Ca^{2+}-dependent potassium currents (I_{Ca} and $I_{K(Ca)}$)[9,10]. To evaluate the possible involvement of Ca^{2+}-dependent currents, the Ca^{2+}-channel blocker cadmium (1 nM) was perfused until an early inward current (arrow, Fig. 4*a*) and a delayed outward current (including an outward tail current; double arrows, Fig. 4*a*) were blocked (Fig. 4*b*); these currents, which were elicited from a holding potential at which I_A is inactivated, presumably represent components of I_{Ca} and $I_{K(Ca)}$, respectively. When I_A was once again evoked, it was found to be undiminished by Cd^{2+}; in these conditions, phenylephrine had its usual suppressant effect (Fig. 4*c*, *d*; n = 4).

Pharmacological studies have indicated that the noradrenergic activation of serotonergic neurones is mediated specifically by an α_1-adrenoceptor[11,12]. Accordingly, prazosin, a highly specific α_1-antagonist in electrophysiological studies[13], completely reversed the noradrenaline- or phenylephrine-induced depression of I_A (Fig 2*d*; n = 4 and 5, respectively).

The results of the present study show that serotonergic dorsal raphe neurones possess an early transient outward current (I_A) which can be elicited by depolarizations from potentials below resting levels and is suppressed by noradrenaline or phenylephrine. As serotonergic neurones in the dorsal raphe nucleus receive a direct noradrenergic synaptic input[14], the present

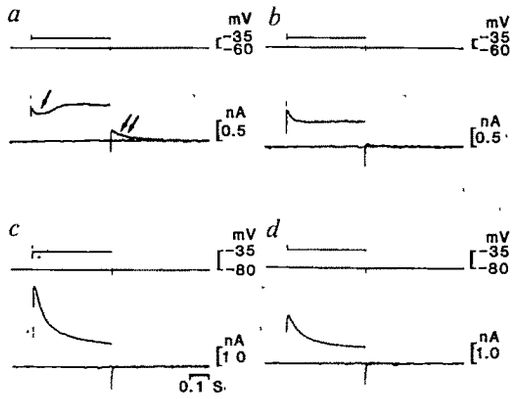


Fig. 4 Effect of the Ca^{2+}-channel blocker Cd^{2+} on the phenylephrine induced reduction of I_A. In *a*, a depolarizing step ($h = -60$ mV; holding current, +0.02 nA) to the command potential (−35 mV) induced a slow inward current (arrow) followed by a delayed outward current (note the outward tail current, double arrows); also note that at this holding potential I_A is largely inactivated. In *b*, after perfusion with Cd^{2+} (1 mM) for 20 min, the slow inward current and the subsequent outward currents are virtually abolished. In *c*, Cd^{2+} perfusion was continued but here the holding potential was changed to −80 mV to elicit I_A (note the change in current scale). I_A during Cd^{2+} was not diminished relative to its amplitude before Cd^{2+} (elicited from −80 mV, not shown). In *d*, phenylephrine (applied for 9 min) produces its usual reduction in I_A; full recovery from the effect of phenylephrine occurred within a 20-min washout period. After prolonged exposures to Cd^{2+} (for example, >1 h, not shown), it was no longer feasible to test for the response to phenylephrine because of increased membrane instability. In these experiments the artificial CSF was modified by substituting equimolar HEPES for NaH_2PO_4 to prevent the precipitation of Cd^{2+}.

results suggest that the suppression of I_A by noradrenaline represents a true neurotransmitter-induced modulation of this voltage-dependent current.

Intracellular recordings both *in vivo*[15] and *in vitro*[11] show that serotonergic neurones are tonic pacemaker cells whose spikes arise from gradual interspike depolarizations rather than discrete synaptic potentials. The interspike behaviour of unclamped serotonergic neurones is characterized by a large afterhyperpolarization (AHP), then a short period of relatively rapid depolarization, and finally a plateau region lasting several hundred milliseconds which precedes spike initiation[11]. α_1 Stimulation markedly shortens the plateau period but does not reduce the initial amplitude of the AHP[11]. Similarly, in preliminary voltage-clamp experiments, the amplitude of $I_{K(Ca)}$ was not reduced and, in fact, its duration was increased by phenylephrine and noradrenaline (Fig. 2*c* legend). This contrasts with β-adrenoceptor effects on hippocampal pyramidal neurones which are characterized by an inhibition of the Ca^{2+}-dependent AHP, resulting in increased excitability due to reduced spike frequency accommodation[16,17]. The plateau period of the pacemaker potential in serotonergic neurones occurs between −60 to −50 mV; this is precisely the range in which I_A becomes activated (Fig. 3*e*). Thus, by suppressing I_A, α_1-adrenoceptors would abbreviate this plateau period and thereby, together with a reduction in resting potassium conductance[1], accelerate pacemaker activity. It remains to be determined whether increases in neuronal excitability induced by α_1-receptors elsewhere in the CNS also involve a suppression of I_A.

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Modulation of single Ca^{2+}-dependent K^+-channel activity by protein phosphorylation

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There is considerable evidence that cyclic AMP can modulate the electrical activity of excitable cells[1–10] and that protein phosphorylation by the catalytic subunit (CS) of cAMP-dependent protein kinase is a necessary step in these modulatory effects[11–21]. In analogy to alterations in enzyme activities following phosphorylation[12], it seems possible that direct phosphorylation of ion-channel proteins may alter their gating properties, giving rise to the observed changes in electrical activity. However, the results obtained so far do not indicate whether it is ion channels themselves that are phosphorylated, or whether phosphorylation is simply an early step in some cascade of events which leads ultimately to modulation of channel activity. The development of single-channel recording techniques[22] has provided a way to investigate this question. Here we describe effects of CS on the activity of individual Ca^{2+}-dependent K^+ channels from the nervous system of the land snail *Helix* measured in isolated membrane patches and in artificial phospholipid bilayers. The results demonstrate that cAMP-dependent protein phosphorylation produces long-lasting changes in the activity of individual channels, and indicate that the relevant phosphorylation site is closely associated with the channel.

Helix ganglia contain an identified group of neurones (the F cluster) in which the outward current is predominantly Ca^{2+}-dependent[23], and DePeyer *et al.*[19] found that intracellular perfusion of CS could selectively enhance this Ca^{2+}-dependent K^+ current in such neurones. Using detached patches from these neurones in the 'inside-out' configuration[22], we have observed Ca^{2+}-dependent K^+ channels with Ca^{2+} and voltage dependences as shown in Fig. 1. The maximal single channel conductance, measured using symmetrical K^+ solutions in the detached patch configuration, is 40–60 pS. Thus, this channel is distinct from the large conductance (200–300 pS) Ca^{2+}-dependent K^+ channels which have been described in several systems[24]. Most of our experiments have been performed using 1 mM K^+ in the electrode (the outside of the membrane) and 120 mM K^+ in the solution bathing the inner membrane surface, to approximate the K^+ gradient seen by the channel in the normal cell membrane. In these conditions the apparent single-channel conductance measured at 0 mV is ~20 pS, similar to that reported by Lux *et al.*[25] for this channel in cell attached patches. Furthermore, the channel is K^+ selective, and thus is distinct from a Ca^{2+}-dependent non-selective cation channel which we have observed in *Helix* neurones (D.A.E. and I.B.L., unpublished) and which has been described previously in several cell types[26–28].

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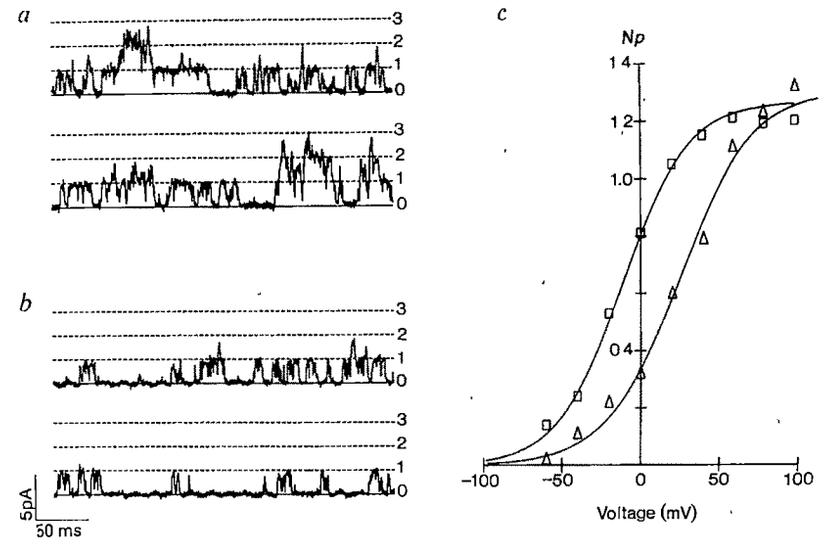


Fig. 1 A Ca^{2+}-dependent K^+ channel in an isolated patch of *Helix aspersa* neurone membrane. *a*, Continuous record of channel activity at 0 mV in a patch with its inner surface exposed to 10^{-7} M free Ca^{2+}. There are several channels in this patch (the dotted lines and the numbers to the right of the traces indicate the number of open channels). The single channel current is ~2.2 pA at the Na^+ plus K^+ reversal potential, and approaches zero at the K^+ reversal potential, indicating that the channels are K^+ selective. *b*, The same patch exposed to 10^{-8} M free Ca^{2+}. Channel activity is decreased substantially by this 10-fold decrease in free Ca^{2+}, indicating that the channels are Ca^{2+} dependent. There is no indication of any activity of other types of channels in this patch. In the experimental conditions described below, we often obtained patches such as this where the activity over a wide potential range was entirely due to this type of channel Channel activity in such patches was analysed by integrating the activity over baseline for a certain length of time and dividing by the integral of one channel being open for that length of time. Thus, channel activity is expressed in units of *Np* where *N* is the number of active channels in the patch and *p* is the probability of opening of a single channel. *c*, Analysis of channel activity in this patch as a function of membrane voltage for both 10^{-7} M (□) and 10^{-8} M (△) free Ca^{2+}. The data were fitted by eye with the approximation to a Boltzmann distribution given by $(Np)_{max}(e^{(V-V_{mid})/slope}/1+e^{(V-V_{mid})/slope})$ where *V* is membrane voltage and V_{mid} is the midpoint of the curve along the voltage axis. The fitted curves for 10^{-7} M and 10^{-8} M free Ca^{2+} had virtually the same maximum *Np* (1.25 and 1.30 respectively) and slope (*e*-fold change per 20.7 mV and 23.6 mV respectively), but the midpoints of the curves were about 30 mV apart. This shift is typical of our results in other experiments.

Methods. The *Helix* central nervous system was desheathed and decapsulated in *Helix* saline containing 80 mM NaCl, 4 mM KCl, 10 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM glucose, and 5 mM HEPES (*p*H 7.4). The exposed neurones were then bathed for 50 min at 37 °C in *Helix* saline containing 10 mg ml^{-1} trypsin. After 25 min the trypsin solution was taken up in a Pasteur pipette and gently flushed over the preparation for 2 min. Enzyme treatment was terminated with six rinses of 37 °C saline. Standard patch clamp recording techniques[22] were used to form 'inside-out' patches with seal resistances of 10 GΩ or more from neurones in the F cluster. The patch pipette solution contained 60 mM NaCl, 1 mM KCl, 40 mM $CaCl_2$, 5 mM $MgCl_2$ and 5 mM HEPES (*p*H 7.4). The inner surface of the membrane was bathed in EGTA-buffered Ca^{2+} solutions containing 5 mM EGTA, 120 mM KCl, 1 mM $MgCl_2$, 0.25 mM ATP and 5 mM HEPES (*p*H 7.4). The total concentration of $CaCl_2$ was either 1.13 mM or 3.72 mM to give free Ca^{2+} concentrations of ~10^{-8} M and 10^{-7} M, respectively. The K^+ and Na^+ concentrations in these pipette and bath solutions correspond to a K^+ reversal potential of −120 mV and a Na^+ plus K^+ reversal potential of −20 mV.

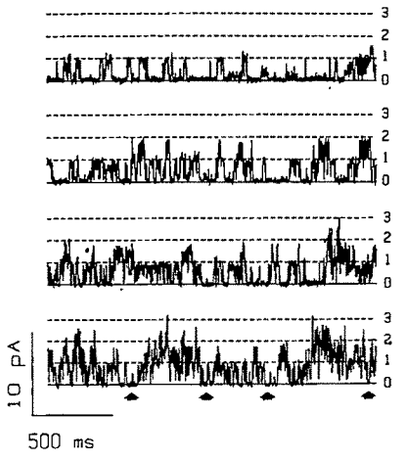


Fig. 2 Modulation of Ca^{2+}-dependent K^+ channel activity in an isolated membrane patch by catalytic subunit of cAMP-dependent protein kinase (CS) CS was purified to homogeneity by the method of Peters *et al.*[34] and stored at −20 °C. The preparation was diluted 100-fold with 10^{-8} M free Ca^{2+} bath solution immediately before use. The patch shown here was held at 0 mV in 10^{-8} M free Ca^{2+} and contained at least five Ca^{2+}-dependent K^+ channels. This continuous record of channel activity begins 50 s after exchanging the 0.2-ml chamber with 0.4 ml of 10^{-8} M free Ca^{2+} bath solution containing 0.4 μM CS. Channel activity increased abruptly during the 8 s of this record and remained at this heightened level for the remainder of the patch lifetime (5 min). Arrows below the bottom trace indicate examples of occasional time periods when all channels are closed amidst this heightened activity

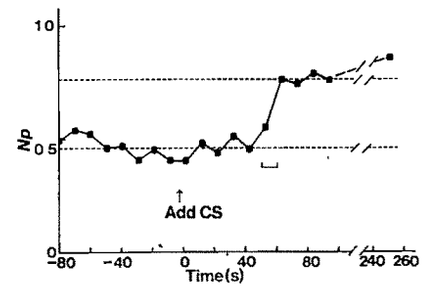


Fig. 3 Time course of the increase in Ca^{2+}-dependent K^+ channel activity produced by CS. Each point is the *Np* value for a 10.24-s interval during the 80 s before and 100 s after the addition of 0.4 μM CS (vertical arrow). The patch was held at 0 mV during this time. The traces in Fig. 2 were taken at the time indicated by the bracket, during the abrupt increase in channel activity resulting from CS addition. Dashed lines are drawn at the average *Np* levels before (*Np* = 0.45) and after (*Np* = 0.76) the abrupt increase. At 100 s, a set of voltage steps was begun which ended at 250 s, and the *Np* value at 0 mV between 250 and 260 s is also shown. In the six other experiments performed in our laboratory, the onset of the effect was similarly abrupt and in six out of seven cases the increase in channel activity lasted for at least 5 min. In four additional experiments performed in collaboration with J. DePeyer and H. Reuter, similar abrupt and long-lasting increases in the activity of this channel were seen. In all three experiments in which patches with this channel were exposed to heat-inactivated CS, no changes in channel activity were seen for at least 10 min. In one of these experiments the patch was subsequently exposed to active CS and there was an abrupt increase in channel activity within 4 min

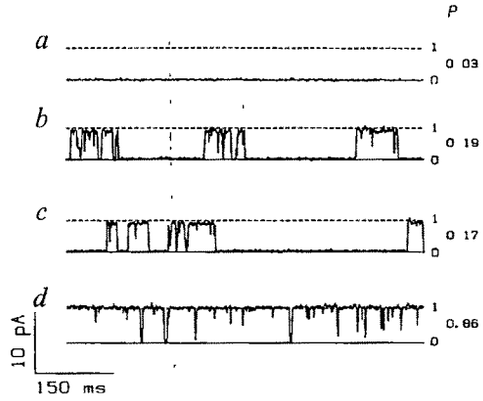


Fig. 4 Modulation of a reconstituted Ca^{2+}-dependent K^+ channel in a phospholipid bilayer formed on the tip of a patch electrode. A single channel which had been observed immediately after formation of the bilayer remained closed virtually all the time in a well containing 1.13 mM $CaCl_2$ to give a free Ca^{2+} concentration of about 10^{-8} M (*a*). When the electrode was moved to a well containing about 10^{-6} M free Ca^{2+} (4.84 mM $CaCl_2$), channel openings and closings were observed (*b*). When the bath solution contained either more or less KCl than the electrode, the single channel currents reversed at the calculated K^+ equilibrium potential (−17 mV in this experiment); furthermore addition of Na^+ to either the bath or the electrode solution had no effect on the reversal potential, indicating that the channel is K^+ selective. In some experiments the channel exhibited openings even at free Ca^{2+} concentrations as low as 1 nM, whereas in others it opened only in the presence of ≥10 μM free Ca^{2+}. Furthermore, this Ca^{2+}-dependent K^+ channel was only observed in about 30% of our membrane preparations. We cannot explain this variability between experiments However, the effects of protein phosphorylation on the activity of this channel (in this case in the presence of ~10^{-6} M free Ca^{2+}) were reliable and consistent from experiment to experiment. Treatment with 0.7 μM DTNB-inactivated CS[34] for 13 min had no effect on channel open probability (*c*). In contrast, treatment with 0.1 μM active CS led to a dramatic increase in open probability (*d*). The values to the right of each trace are channel open probabilities for each experimental condition, calculated from at least 30 s of record containing many opening and closing transitions. The voltage across the bilayer was +40 mV (corresponding to depolarization of the cell membrane) for the traces shown here.

Methods. F clusters were dissected from *Helix* central nervous system, and were rinsed sequentially with Listerine, then with 10 mM EGTA (*p*H 7.4), and finally with 20 mM HEPES (*p*H 7 4). They were then homogenized in the latter buffer (clusters from five *Helix* per ml of homogenizing buffer) using five strokes of a glass-glass homogenizer The homogenate was centrifuged at 10,000*g* for 15 min and the pellet was discarded The supernatant was centrifuged at 100,000*g* for 60 min; the resulting supernatant was discarded and the pellet was taken as the source of membranes. The pellet was resuspended in 200 μl of 200 mM KCl, 20 mM HEPES (*p*H 7.4) by homogenization. To this was added 200 μl of a mixture of brain phosphatidylethanolamine/phosphatidylserine (PE/PS, 3/1; Avanti) which had been suspended in 20 mM HEPES and sonicated until the suspension was clear. $CaCl_2$ was added to a final concentration of 0.1 mM, and the membrane/phospholipid mixture was frozen and thawed three times The reconstituted preparation contained, in a total volume of 400 μl, 300–500 μg membrane protein ml^{-1}, 4 mg phospholipid ml^{-1}, 20 mM HEPES, 100 mM KCl and 0 1 mM Ca^{2+} This preparation (100 μl) was placed in a well of a sterile microtitre plate (Falcon). A fresh plate was used for each experiment and all solutions were filtered through 0.45 μm filters to avoid spurious channels arising from bacterial contamination. The preparation was left for at least 5 min at room temperature to allow a phospholipid monolayer to form at the air/liquid interface. A fire polished patch electrode containing 50 mM KCl, 20 mM HEPES (*p*H 7.4) was pulled through the monolayer twice to form a bilayer on the electrode tip[32,33]. The seal resistance for this experiment was 17 GΩ, and the bandwidth was 1 kHz. Current across the bilayer was monitored, and when single-channel currents were detected the electrode was moved to an adjacent chamber containing 100 mM KCl, 20 mM HEPES (*p*H 7.4), 1 mM $MgCl_2$, 0.25 mM ATP, 5 mM EGTA, and $CaCl_2$ and enzymes as described above

Figure 1*c* shows the voltage dependence of channel opening at two different Ca^{2+} concentrations. Because we never obtain patches which contain only one Ca^{2+}-dependent K^+ channel, channel activity is expressed in units of *Np*, where *N* is the number of active channels in the patch and *p* is the open probability for an individual channel. Note that increasing the Ca^{2+} concentration shifts the *Np*-voltage curve to the left, with little change in slope or in the maximum *Np* value. The shift in the mid-point of the curve is ~30 mV for a 10-fold change in Ca^{2+} concentration.

We have investigated the possibility that the activity of this channel can be regulated by cAMP-dependent protein phosphorylation, by applying CS together with Mg^{2+} and ATP directly to the inner membrane surface of inside-out patches. The results of one such experiment are illustrated in Fig. 2. At the beginning of the record (top trace) the channel activity is that normally observed in the presence of 10^{-8} M Ca^{2+} at this voltage (0 mV, compare with Fig. 1*b*). During the 8-s period shown, channel activity increases dramatically, so that by the end of the record (bottom trace) several channels are open a large portion of the time.

Figure 3 shows the time course of the onset of this response. It can be seen that the *Np* value was stable before and for almost 1 min after addition of CS (vertical arrow), and then abruptly increased to reach a new stable level. Such abrupt increases in activity were observed in all experiments in which active CS was added ($n = 7$), with a latency ranging from 12 s to 4 min. In all but one experiment the activity remained elevated for many minutes, often for as long as we could hold the patches, although on occasion there did seem to be some reversal with time. In contrast to these results with active CS, we have never seen such changes in channel activity either spontaneously, or following addition of heat-inactivated CS.

A consistent finding in all our experiments is that CS causes an increase in *Np* at all voltages between −60 and +100 mV. Although the reasons are unclear, it seems likely that CS is having more than one effect. The results are consistent with the possibility that phosphorylation leads to an increase in the affinity of Ca^{2+}-dependent K^+ channels for Ca^{2+}, which shifts the *Np*-voltage curve to the left at negative voltages. The maximum *Np* at positive voltages is also increased, which is consistent with an increase in the number of functional channels in the patch, or with an increase in the maximum open probability for a given channel.

These and other isolated patch experiments[29,30] have provided direct evidence that the activity of individual channels can be modulated by cAMP-dependent protein phosphorylation, and demonstrate that the phosphorylation target must be something which remains associated with the patch of membrane when it is detached from the cell. This target may be the channel itself, or it may be some other membrane-associated element (for example, a cytoskeletal component) which comes away with the patch. To study this further, we have examined channel activity in a reconstituted system using homogenized F cluster neurones dissected from *Helix* ganglia and a crude membrane vesicle fraction prepared by differential centrifugation. When these vesicles are allowed to fuse with artificial phospholipid bilayers in conditions which permit the measurement of single channel currents across the bilayer[31], we find at least five separate K^+ channels which can be distinguished on the basis of their kinetics and single channel conductances (I.B.L. and A.W., in preparation). At least one of these K^+ channels is Ca^{2+}-dependent. These experiments have been performed in several different configurations, including large planar bilayers (80–200 μm diameter) formed across an opening between two chambers[31], and in bilayers formed on the tip of a patch electrode ('tip-dipping')[32,33]. Figure 4 illustrates an experiment in which a single Ca^{2+}-dependent K^+ channel was present in the artificial membrane on the tip of a patch pipette. This channel has a single-channel conductance of 70–100 pS, somewhat larger than that of the Ca^{2+}-dependent K^+ channel observed in membrane patches. It also seems to be less sensitive to Ca^{2+}, although the

Ca^{2+} sensitivity of the bilayer channel varies markedly between experiments. It remains to be determined whether the channels in the patch and the bilayer are distinct, or whether the apparent differences in some of their properties result simply from differences in their membrane environment.

The bilayer channel is closed virtually all of the time in 10^{-8} M Ca^{2+} at +40 mV (Fig. 4*a*), but is open for a significant time when the Ca^{2+} concentration is raised to 10^{-6} M (Fig. 4*b*). This degree of Ca^{2+} sensitivity is in the middle of the wide range that we observed. There is no effect on channel activity after prolonged treatment (in this case 13 min) with dithionitrobenzene (DTNB)-inactivated or heat-inactivated CS (Fig. 4*c*). However, the opening and closing kinetics of the channel change dramatically within 20 s of the addition of active CS, with a large increase in open probability (Fig. 4*d*). The latency for the onset of the response ranged from too fast to measure (that is, <5 s) to as long as 7 min. The *p* values to the right of each trace in Fig. 4 demonstrate the magnitude of the probability change at +40 mV produced by phosphorylation with active CS; such changes were observed in all the experiments ($n = 11$) carried out with either the large planar bilayers or the 'tip-dip' technique. Investigation of the change in probability elicited at various voltages by CS treatment produced results (not shown) entirely consistent with a shift in channel affinity for Ca^{2+}.

Previous studies from our own and other laboratories have provided evidence that protein phosphorylation can regulate the membrane properties of excitable cells. The present results extend these findings by demonstrating directly that the activity of individual Ca^{2+}-dependent K^+ channels can be modulated by cAMP-dependent protein phosphorylation. In addition, the results put the phosphorylation site considerably closer to the channel; indeed, from the reconstitution experiments, in which individual channels are effectively at infinite dilution in the bilayer, we conclude that the phosphorylation target is either the K^+ channel itself or something intimately associated with the channel. Finally, clues have been provided about those aspects of channel function which are modulated by phosphorylation, and it will be of great interest to extend these approaches to investigate the detailed molecular mechanisms of ion-channel regulation.

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Drastic change in idiotypic but not antigen-binding specificity of an antibody by a single amino-acid substitution

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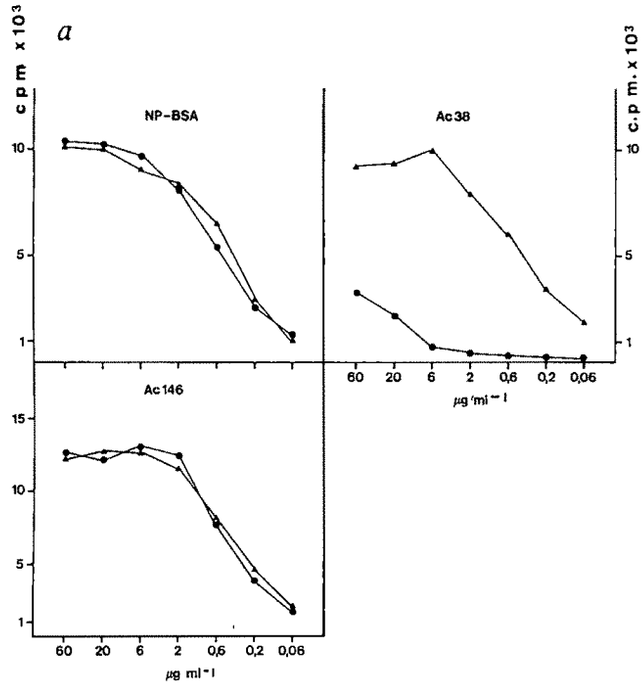
In proliferating B lymphocytes, somatic mutation of rearranged antibody variable (V)-region genes occurs at high frequency and may have a key role in the selection of these cells[1–3]. It is of interest in this context to learn in which way single mutations can affect antigen binding and/or idiotypic specificity of an antibody. Previous investigations have analysed spontaneous mutants of myeloma and hybridoma cells in which the mutation affected the antigen-binding specificity of the antibody[4–8]. Here we describe an antibody mutant that has fully retained antigen-binding specificity but has lost or drastically changed all V-region antigenic determinants (idiotopes) of the wild type as defined by monoclonal anti-idiotope antibodies. The mutant phenotype is generated by a glycine to arginine exchange in the middle of the diversity (D) element, at position 103 of the heavy chain.

The mutant was derived from the monoclonal antibody B1-8.δ1, which binds the hapten 4-hydroxy-3-nitro-5-iodophenylacetyl (NIP) with high affinity[9,10]. From a series of monoclonal anti-idiotope antibodies raised against antibody B1-8 (refs 11, 12), two were used for mutant selection. Antibody Ac38 defines an idiotope (idiotope Ac38) outside the hapten-binding cleft. In contrast, idiotope Ac146, recognized by antibody Ac146, is presumably located close to the hapten-binding site because hapten and antibody Ac146 compete for binding to the B1-8 V region. Spontaneously arising idiotope loss mutants of B1-8.δ1 were isolated by fluorescence-activated cell sorting. It has been shown previously that an $Ac146^-Ac38^+$ mutant has lost hapten-binding specificity together with the binding site-related idiotope Ac146[4]. The present experiments describe a mutant selected for the expression of the latter idiotope and against expression of idiotope Ac38.

Table 1 Affinity and fine specificity of hapten binding to wild-type and mutant antibody

	IC_{50} NIP-cap (M)	IC_{50} NP-cap (M)	Fine specificity
B1-8.δ1	1×10^{-7}	2×10^{-5}	NNP = NIP > NCLP = NP
B1-8 δ1V3	1.1×10^{-7}	1.8×10^{-5}	NNP = NIP > NCLP = NP

The IC_{50} value is the molar hapten concentration resulting in 50% inhibition of binding of antibody (60 μg ml^{-1}) to N^{125}IP-cap (4×10^{-8} M), determined by Farr assay as described in ref. 9. For unknown reasons, IC_{50} values for NP-cap differed reproducibly by an order of magnitude from those published previously[10]. The order of haptens according to affinity was: NNP, (4-hydroxy-3,5-dinitrophenyl)acetyl; NIP, (4-hydroxy-5-iodo-3-nitrophenyl)acetyl; NCLP, (4-hydroxy-5-chloro-3-nitrophenyl)acetyl; NP, (4-hydroxy-3-nitrophenyl)acetyl



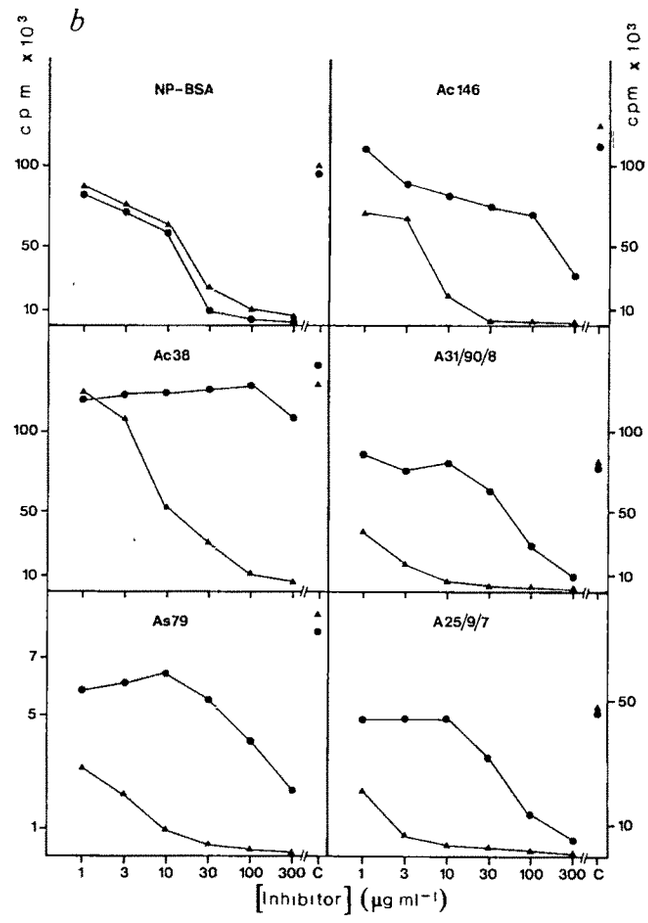


Fig. 1 Serological analysis of B1-8.δ1 (▲) and B1-8 δ1V3 (●) *a*, Direct binding in a solid-phase radioimmunoassay[26]. Plastic plates were coated with NIP-BSA or anti-idiotope antibody at a concentration of 50 μg ml^{-1} After saturating the plastic surface with BSA, B1-8 δ1 or B1-8.δ1V3 antibody was added to the coated wells at the concentrations indicated on the abscissa, and bound antibody detected with ^{125}I-labelled monoclonal anti-mouse λ1-chain antibody Ls136 (0.5 μg ml^{-1}; refs 11, 27). Results are shown for NIP-BSA and the anti-idiotopes Ac146 and Ac38. The anti-idiotope antibodies Ac106, As79, A25 9.7 and A31 90.8 behaved like antibody Ac38 in that they failed to bind the mutant B1-8.δ1V3 (data not shown). *b*, Binding inhibition The binding of iodinated antibody B1-8.δ1 to NIP-BSA or anti-idiotope-coated plates was inhibited by graded concentrations (abscissa) of unlabelled antibody B1-8 δ1 (▲) or B1-8.δ1V3 (●). Control binding without inhibitor is indicated (C)

B1-8.δ1 cells were incubated with fluoresceinated antibody Ac146 (50 μg ml^{-1}) in the presence of a 100-fold excess of unlabelled antibody Ac38. In these conditions, only cells that have retained the expression of idiotope Ac146 but lost that of idiotope Ac38 are stained. Stained cells were then enriched in eight successive rounds of sorting, expanding the separated cells after each sort. The mutant cells were finally isolated by cloning and identified by a radioimmunoassay of culture supernatant. Fifteen clones produced antibodies of mutant phenotype, that is, antibodies binding to antibody Ac146 but not to antibody Ac38. The frequency of the variant cells in the original B1-8.δ1 population is presumably $>2\times10^{-8}$, as about 5×10^{7} cells were used in the initial sorts. On the other hand, Ac146^{+}Ac38^{-} cells were not detected in the original population by fluorescence microscopy, staining the cells simultaneously with the two anti-idiotope antibodies coupled to different fluorochromes[4] and repeatedly screening 1×10^{5} cells. These results are consistent with previous data[4] and indicate that the frequency of a given idiotype variant in B1-8.δ1 cells is at least one order of magnitude lower than the frequency of variants of the myeloma S107 which have lost the property of antigen binding.

Because of the rare occurrence of the variants and the fact that three of the variant cell clones behaved identically when tested with a variety of other anti-idiotopes, it seemed unlikely that we had isolated a series of independent mutants. Therefore, we chose only one clone, B1-8.δ1V3, for a detailed characterization. The antibody produced by this clone (antibody B1-8.δ1V3) was assayed for binding to a NIP-conjugate of bovine serum albumin (NIP_{14}-BSA) and the anti-idiotope antibodies

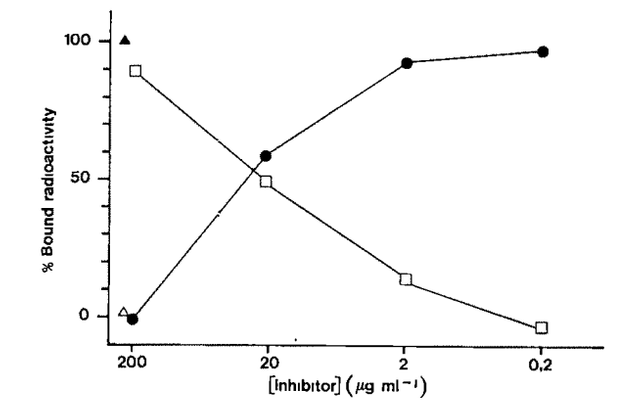


Fig. 2 Binding of λ1 light chain to variant and wild-type heavy chain. The myeloma protein HOPC1 (IgG2a, λ1) and IgD from B1-8.δ1 and B1-8.δV3 were mildly reduced with 10 mM dithioerythrol (150 min, room temperature, in the dark) and light and heavy chains separated on a Sephadex G-150 column (Pharmacia) in 1 M acetic acid with 10 mM dithioerythrol. The various fractions were dialysed first against 10 mM sodium acetate *p*H 5.5 and then against phosphate-buffered saline (PBS). When tested on a 7 5% SDS-polyacrylamide electrophoresis gel, the separated chains were >95% pure HOPC1 λ1 chains were iodinated[27], re-associated at 20 μg ml^{-1} in PBS with 1% BSA at 4 °C overnight to either B1-8.δ1 (▲) or B1-8.δ1V3 (△) heavy chains (20 μg ml^{-1}) and tested for binding to an Ac38 (30 μg ml^{-1}) anti-idiotope-coated plastic plate. The λ1 chains can bind to the anti-idiotope only when associated with a B1-8 δ1 chain. Bound λ1 chains were counted in a γ-counter. The re-association of λ1 to B1-8.δ1 or B1-8.δ1V3 heavy chains was inhibited by increasing amounts (abscissa) of B1-8.δ1V3 (●) or B1-8 δ1 (□) heavy chains, respectively The two curves cross each other at roughly 50% binding, indicating a similar affinity of the two heavy chains for the λ1 light chain

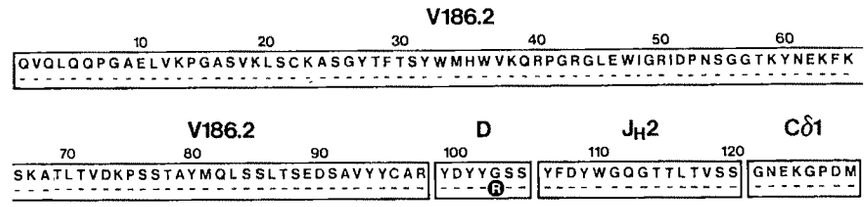


Fig. 3 Amino-acid sequence of the B1-8.δ1V3 heavy-chain variable region. The B1-8 δ1V3 antibody was cleaved by cyanogen bromide and the three fragments of the heavy-chain variable region isolated as described for B1-8 δ1 and B1-8.δ1V3 (refs 5, 28). Because H1 (residues 1–34) and H3 (residues 82–128) are blocked by the presence of N-terminal pyroglutamic acid[8], only H2 (residues 35–81) was sequenced directly by automated Edman degradation in a spinning-cup sequencer. Fragments H1, H2 and H3 were cleaved with trypsin or/and thermolysine and the fragments isolated by reverse-phase HPLC. The peptides were identified by amino-acid analysis and compared with the B1-8.δ1 sequence (upper line, single-letter code). The peptides covering residues 20–34 and 99–128 were sequenced in a gas-phase sequencer[29]. The amino-acid compositions of the peptides covering residues 1–21 and 82–98 isolated from B1-8.δ1 and B1-8.δ1V3 were indistinguishable, suggesting that for these parts both heavy chains are identical. The sequence of B1-8.δ1V3 (lower, dashed line) shows at position 103 an arginine residue instead of the glycine found for B1-8.δ1. The region of the variable regions encoded by the *D* gene (D), J_H2 gene (J_H2) and the J_H2 border to the first constant domain ($C_\delta1$) are indicated by vertical lines.

Ac146, A25.9.7, Ac38, A31.90.8, A6.24, As79 and Ac106. Efficient binding was observed only in the case of NIP-BSA and antibody Ac146 (Fig. 1*a*). The Ac146 determinant, however, is also affected by the mutation, as the B1-8.δ1V3 antibody was about 100-fold less efficient than the wild type in inhibiting the binding of radiolabelled antibody B1-8.δ1 to antibody Ac146 (Fig. 1*b*). In the case of the anti-idiotopes Ac38, A25.9.7, As79 and A31.90.8, binding inhibition by the mutant was even less efficient.

Despite its drastic idiotypic alteration, the mutant antibody inhibited the binding of the B1-8.δ1 wild type to NIP-BSA with the same efficiency as the wild-type antibody itself (Fig. 1*b*), and the affinities of both antibodies to NIP and some related haptens were identical within the limits of the analysis (Table 1).

The mutation in B1-8.δ1V3 was localized to the heavy chain. In chain re-association experiments, mutant and wild-type heavy chains, when re-associated to λ1 chains of the myeloma HOPC1, gave rise to mutant and wild-type immunoglobulin, respectively. The mutation does not interfere with the capacity of the heavy chain to bind light chain, as the two heavy chains competed with equal efficiency for re-association to λ1 (Fig. 2). Isoelectric focusing of Fv fragments revealed a small but distinct difference between the C-terminal heavy-chain variable-region fragments (relative molecular mass 19,000) of mutant and wild-type immunoglobulin (data not shown).

The amino-acid sequence of the V_H, D and joining (J) segments of the B1-8.δ1V3 heavy chain, determined by the strategy developed previously for the sequencing of B1-8.δ1V1 (ref. 5), revealed a single amino-acid difference between variant and wild type; at position 103 a glycine residue is changed to an arginine residue (Fig. 3).

In the previously isolated mutant, B1-8.δ1V1 (ref. 4), recombination (conversion) between the expressed and a non-rearranged V_H gene had generated the mutant phenotype[5,6]. Although we cannot exclude such an event in the case of B1-8.δ1V3, it seems more likely that in this antibody a point mutation has occurred, changing the glycine-encoding triplet GGT at position 103 (ref. 13) into CGT, encoding arginine. Point mutation is also the simplest explanation for the generation of mutants of the S107 myeloma line with changed antigen-binding specificity[8,14].

The observations that single amino-acid substitutions can profoundly affect idiotypic and/or antigen-binding specificity and that the D element has a key role in determining idiotypic specificity are not new[3,15–20]. The main point of the present analysis is the demonstration that a single amino-acid exchange in D can essentially eliminate the reactivity of an antibody with the majority of the anti-idiotopes raised against it, while leaving hapten-binding specificity intact. The conservation of NIP-binding specificity in the mutant, its reactivity with the NIP group on a macromolecular carrier (BSA), and the chain re-association data argue that the mutation has left the overall structure of the antibody and the hapten-binding cleft intact and selectively changed a surface structure (arginine 103 is a surface residue according to ref. 21) of key importance for idiotypic recognition. This structure may be directly involved in the interaction of the various anti-idiotope antibodies with the B1-8 V region. Like other protein interactions, idiotypic interactions seem in general to involve extended areas on the protein surface[3,19]. In the present system it has been shown that, in addition to D, residues in V_H and the V region of the light chain all contribute to the reactivity of antibody B1-8 with various anti-idiotopes[3,4,22]. It is therefore not surprising that, as far as analysed, the anti-idiotope antibodies compete with each other in binding to antibody B1-8 (ref. 11). Nevertheless, the points of contact differ for the various antibodies, as none of the target idiotopes is necessarily co-expressed with any other (refs 12, 23, 24 and our unpublished observations). Only some of the idiotopes are intimately associated with the hapten-binding site[11,12].

In biological terms, the B1-8.δV3 mutant demonstrates that a single amino-acid exchange can make an antibody essentially invisible for a variety of anti-idiotypic antibodies. Thus, somatic mutation can easily mediate escape from idiotypic control[25].

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Ca^{2+}-dependent and Ca^{2+}-independent phagocytosis in human neutrophils

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The phagocytic function of neutrophils is a crucial element in host defence against invading microorganisms. Two main specific receptor-mediated mechanisms operate in the phagocyte plasma membrane, one recognizing the C3b/bi fragment of complement and the other the Fc domain of immunoglobulin G (ref. 1). There is evidence that phagocytosis mediated by these receptors differs in the number and nature of the intracellular signals generated[2–5]. However, the mechanism by which receptor binding is transduced into a signal that generates the formation of the phagocyte pseudopod is not known, although extensive biochemical evidence has allowed the postulate that calcium ion gradients in the peripheral cytoplasm, by interacting with calcium-sensitive contractile proteins, initiate the process of engulfment[6]. Using the high-affinity fluorescent calcium indicator quin2 both to measure and to buffer intracellular calcium ($[Ca^{2+}]_i$), we show here that in human neutrophils two mechanisms of phagocytosis coexist: a $[Ca^{2+}]_i$-dependent and modulated phagocytosis, triggered by activation of the Fc receptor, and a $[Ca^{2+}]_i$-independent mechanism triggered by the activation of the C3b/bi receptors.

To monitor changes in $[Ca^{2+}]_i$ during phagocytosis, human neutrophils loaded with quin2 were exposd to yeast (*Saccharomyces cerevisiae*) particles coated with IgG, C3bi or heat-inactivated serum. Figure 1 shows how $[Ca^{2+}]_i$ changes during the phagocytosis of different particles and also the kinetics of phagocytosis, expressed as a percentage of phagocytosing cells, in the same batch of quin2-loaded neutrophils. In contrast to the observations of Campbell *et al.*[7] with latex particles, we found that with both IgG- and C3bi-coated yeasts, $[Ca^{2+}]_i$ rises after a lag time of 2–3 min, reaching a plateau at about 4–6 min and remaining elevated for >10 min. Both the lag time and the maximum rise in $[Ca^{2+}]_i$ was dependent on the yeast to cell ratio and the intracellular quin2 concentration (see below). No $[Ca^{2+}]_i$ increase was observed with unopsonized yeast that was not ingested (Fig. 1*c*). In several experiments, with both IgG- and C3bi-coated yeast, phagocytosis began slightly before the rise in $[Ca^{2+}]_i$ became detectable. With cells loaded with 10 μM quin2 (same conditions as for Fig. 1), the rise in $[Ca^{2+}]_i$ was detected on average within 1.8 ± 0.4 ($n = 9$) for C3bi-coated yeast and 2.8 ± 0.5 min ($n = 12$) for IgG-coated yeast whereas phagocytosis was already detectable at 1 min for both IgG-coated yeast ($7 \pm 3\%$ ingesting cells, $n = 5$) and C3bi-coated yeast ($27 \pm 8\%$, $n = 5$).

This kinetic discrepancy between the increase in $[Ca^{2+}]_i$ and phagocytosis may be explained in two ways. First, the kinetics of the $[Ca^{2+}]_i$ increase and that of yeast ingestion are similar, but because the rise in $[Ca^{2+}]_i$ occurs initially in a small proportion of cells, possibly in the cortical cytoplasm, the average value of $[Ca^{2+}]_i$ for the whole cell population, which is what quin2 actually measures, would be affected only to a small extent; only when a large number of cells becomes engaged in phagocytosis, and perhaps the increase in $[Ca^{2+}]_i$ spreads throughout the cytoplasm, is a significant average increase in $[Ca^{2+}]_i$ revealed by quin2. Furthermore, for a process like phagocytosis, which occurs as discrete events in a proportion of the whole cell population, the quin2 technique, by definition, underestimates the real rise in $[Ca^{2+}]_i$ in the cells actually engaged in particle ingestion. Figure 1 (inset) shows that soluble

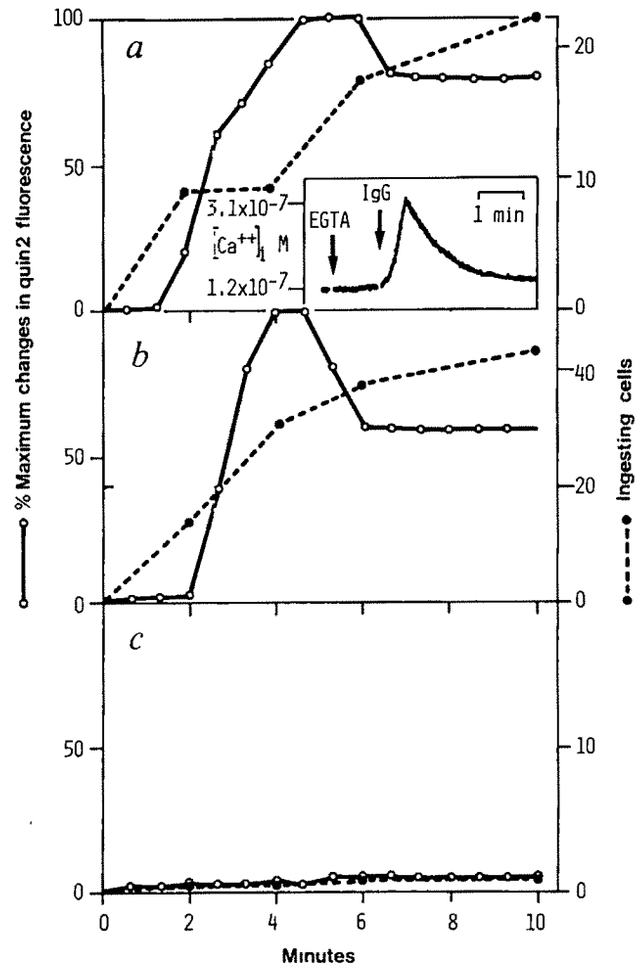


Fig. 1 Changes in $[Ca^{2+}]_i$ and phagocytosis of yeast particles by neutrophils. *a*, An experiment using yeast particles coated with rabbit anti-yeast IgG. The inset shows the effect of heat-aggregated (63 °C, 30 min) human IgG (500 μg ml^{-1}) on $[Ca^{2+}]_i$ in cells incubated in Ca^{2+}-free medium + 1 mM EGTA. *b*, *c*, Experiments using C3bi-coated yeast particles (*b*) and unopsonized yeast particles (*c*). **Methods.** Purified neutrophils were loaded with the fluorescent probe quin2/AM as described previously[11]. Loaded cells were incubated in a spectrofluorometer (Perkin-Elmer LS3) at 37 °C with a stirring system, and $[Ca^{2+}]_i$ as well as phagocytosis in suspension were measured. Because the phagocytic assay uses fluorescein isothiocyanate (FITC)-labelled yeast particles[2], both experiments were performed in parallel with different aliquots from the same batch of cells. Intracellular quin2 was 0.1 nmol per 10^6 cells. Cells (5×10^6) were incubated with yeast particles (2.5×10^7) in a total volume of 2.9 ml buffer containing 138 mM NaCl, 6 mM KCl, 1 mM $MgSO_4$, 1.1 mM $CaCl_2$, 5 mM $NaHCO_3$, 100 μM EGTA, 5.5 mM glucose, 20 mM HEPES, *p*H 7.4. The IgG-coated particles were opsonized by incubating yeast with purified anti-yeast IgG (20 μg ml^{-1}) in the presence of 20% heat-inactivated (56 °C, 30 min) human serum; C3bi-coated particles were opsonized by incubating the yeast with 20% fresh normal serum and unopsonized particles were incubated with 20% heat-inactivated serum. All particles were incubated at 37 °C, 30 min and washed before use. No IgG was detected on the surface of the yeast incubated with serum[2]. Phagocytosis of FITC-labelled yeast particles was monitored by the fluorescent quenching method[2], where the extracellular particles are quenched by adding trypan blue. Phagocytosis is expressed as the percentage of phagocytosing cells. The uptake of C3bi-coated particles was furthermore blocked by $F(ab')_2$ anti-C3c (J. H. and O.S., unpublished observation). All the above experiments were performed on the same batch of quin2-loaded neutrophils. For total change in quin2 fluorescence, see Fig. 2 showing similar results.

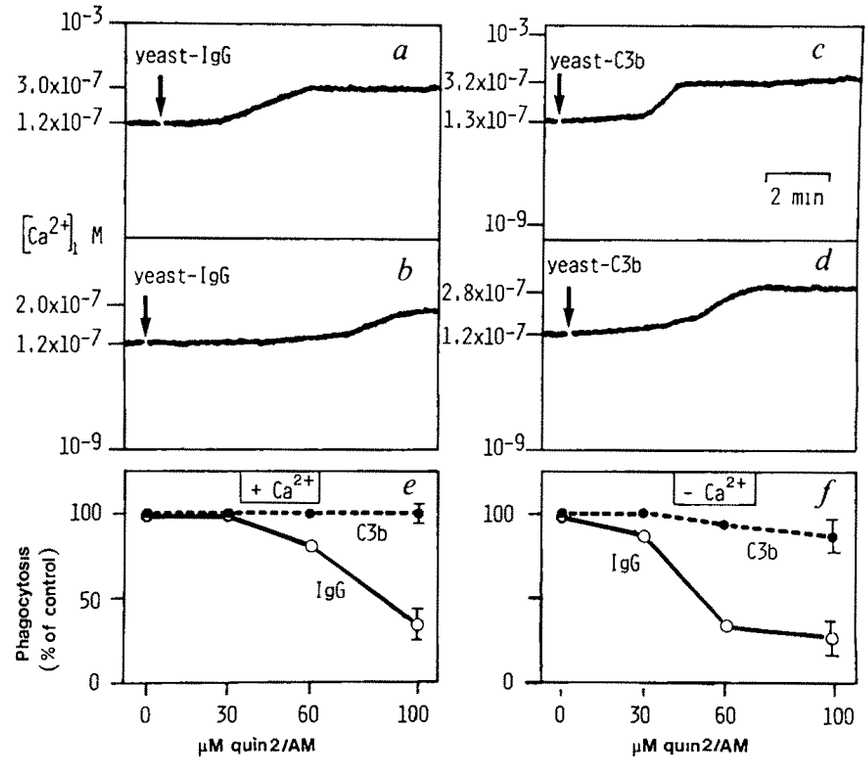


Fig. 2 Changes in $[Ca^{2+}]_i$ and phagocytosis of C3bi- and IgG-coated yeast particles by neutrophils loaded with different concentrations of quin2. *a–d*, Neutrophils were loaded with 10 or 30 μM quin2/AM and $[Ca^{2+}]_i$ was monitored. Intracellular quin2 concentration per 10^6 cells were 0.11 and 0.34 nmol, respectively *a*, *c*, Cells loaded with 10 μM; *b*, *d*, cells loaded with 30 μM quin2/AM The number of neutrophils and yeast particles were as for Fig. 1 legend. *e*, *f*, The effect of increasing cytosolic calcium buffering capacity by increasing intracellular quin2, on IgG- and C3bi-mediated phagocytosis. *e*, Cells incubated with various concentrations of quin2/AM in the presence of 1 mM Ca^{2+} for 60 min at 37 °C. The phagocytosis assay was performed with washed cells in the same medium containing 1 mM Ca^{2+} *f*, Phagocytosis by cells incubated with various concentrations of quin2/AM in the absence of extracellular Ca^{2+} + 1 mM EGTA ($[Ca^{2+}]_0 = 10^{-9}$ M). This procedure depletes the cells of intracellular calcium[12]. The cells were then incubated in Ca^{2+}-free medium at 37 °C for 5 min before yeast particles and Ca^{2+} (final concentration 200 μM) were added. This concentration of calcium is necessary to establish good binding of the yeast particles to the neutrophils Phagocytosis was monitored as described in Fig. 1, and expressed as the % of phagocytosing cells after 10 min. 100% = % of phagocytosing cells in the absence of quin2. The values for IgG- and C3b-coated yeast phagocytosis in the absence of quin2 were 30% and 64%, respectively.

aggregated IgG, presumably by interacting with several receptors on most cells, initiates and mediates a rapid increase in $[Ca^{2+}]_i$ from intracellular stores, as also shown by others[8,9]. (For further discussion of the problem of cell heterogeneity, see refs 10 and 11.) A second possibility is that phagocytosis itself does not require an increase in $[Ca^{2+}]_i$ which, if correct, would indicate that the increase in $[Ca^{2+}]_i$ observed with IgG- and C3bi-coated yeast is an event that is secondary to phagocytosis and/or receptor triggering, and not directly related to the process of particle ingestion.

To determine the role of the increase in $[Ca^{2+}]_i$ in phagocytosis, we used the fact that quin2 not only measures $[Ca^{2+}]_i$, but can also act as a high-affinity calcium buffer inside the cells. Thus, increasing the intracellular quin2 concentration, $[quin2]_i$, should decrease the amplitude and/or delay the increase in $[Ca^{2+}]_i$. The presence of increasing concentrations of quin2 inside the cells should, furthermore, counteract the formation of large $[Ca^{2+}]_i$ gradients within the cell (see also ref. 12). Thus, it can be predicted that if phagocytosis casually depends on a local, or average, rise in $[Ca^{2+}]_i$, it should be inhibited and/or delayed by increasing $[quin2]_i$. Figure 2*a–d* shows that increasing $[quin2]_i$ decreases the amplitude and delays the appearance of the yeast-induced elevation of $[Ca^{2+}]_i$. However, although the effect of quin2 loading on $[Ca^{2+}]_i$ is similar for both IgG- and C3bi-coated particles, its effect on phagocytosis is strikingly different. Figure 2*e*, *f* shows that phagocytosis of IgG-coated yeast is decreased markedly as a function of quin2 loading whereas ingestion of C3bi-coated particles is unaffected by increasing the intracellular calcium-buffering capacity; the percentage of ingesting cells (Fig. 2) and the number of ingested IgG-coated yeast molecules per cell are reduced to the same extent. The inhibitory effect of $[quin2]_i$ on IgG-mediated phagocytosis was more pronounced in cells that had been loaded with quin2 in the absence of extracellular Ca^{2+} ($[Ca^{2+}]_0$) + EGTA ($[Ca^{2+}]_0 = 10^{-9}$ M), a condition which decreases basal $[Ca^{2+}]_i$ and depletes intracellular Ca^{2+} stores[12]. In contrast, C3bi-coated yeast particles were ingested to the same extent and with the same rate independently of the loading conditions. In cells loaded with quin2 in Ca^{2+}-free buffer + EGTA, resting $[Ca^{2+}]_i$ was reduced to below 10 nM; the addition of Ca^{2+} (200 μM) produced a slow (5 min) increase in $[Ca^{2+}]_i$ towards basal levels, the rate of the rise being unaffected by the simultaneous addition of Ca^{2+} and opsonized yeast.

Taken together, these data suggest that Fc receptor-triggered phagocytosis depends on $[Ca^{2+}]_i$ transients, possibly localized to some specific regions of the cytoplasm, whereas C3bi receptor-mediated phagocytosis occurs at the same speed and to the same extent independently of $[Ca^{2+}]_i$ A major problem in considering C3bi-mediated phagocytosis as a completely $[Ca^{2+}]_i$-independent process, however, is the finding that at least 100 μM Ca^{2+} must be present in the medium before yeast particle ingestion is observed. This probably does not result from an effect on $[Ca^{2+}]_i$, but reflects a role for $[Ca^{2+}]_0$ in the interaction between the surface of the cell and the yeast. It remains possible, however, that $[Ca^{2+}]_0$ is required to induce local changes in $[Ca^{2+}]_i$ or that receptor triggering sensitizes the phagocyte machinery to resting levels of $[Ca^{2+}]_i$. To exclude these two possibilities, it would be necessary to reduce $[Ca^{2+}]_i$ to 10 nM and maintain it at this low concentration throughout phagocytosis. At such a low Ca^{2+} concentration, all known Ca^{2+}-dependent processes are almost completely inhibited[13–16]. To study phagocytosis in the absence of extracellular calcium, C3b-opsonized endotoxin-coated oil particles were used which are efficiently ingested even at $[Ca^{2+}]_0 = 1$ nM[17]. Figure 3 (right panel) shows initial ingestion of C3b-opsonized oil particles by neutrophils loaded either in Ca^{2+}-containing medium ($[Ca^{2+}]_i = 120$ nM) or in Ca^{2+}-free medium + EGTA ($[Ca^{2+}]_i = 10$ nM), and tested in Ca^{2+}-free medium + EGTA. Ingestion of C3b-opsonized oil droplets, at $[Ca^{2+}]_0 = 1$ nM, was identical whether the $[Ca^{2+}]_i$ was 10 nM, where no Ca^{2+} would be released by ionophore, or $[Ca^{2+}]_i$ = 120 nM and in the presence of ionophore-releasable pools. The oil particles are, unfortunately, strongly fluorescent at the quin2 wavelength, which prevents us from monitoring $[Ca^{2+}]_i$ during oil droplet phagocytosis.

The experiments shown in Fig. 3 support the $[Ca^{2+}]_i$ dependency and independency of Fc- and C3b/bi-mediated

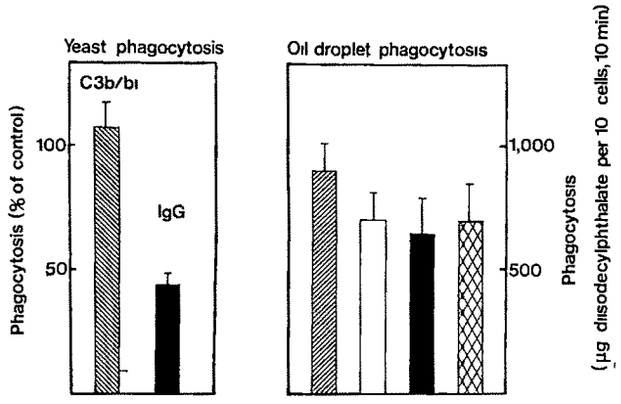


Fig. 3 *a*, Effect of the calcium ionophore ionomycin (1 μM) on phagocytosis of C3bi- or IgG-coated yeast in Ca^{2+}-containing medium. The ionophore was added 1 min before the yeast particles. Incubation time was 10 min. Phagocytosis was monitored as described in Fig. 1 legend and expressed as % of controls (=neutrophils + 0.1% dimethyl sulphoxide without ionophore). *b*, Effect of manipulation of $[Ca^{2+}]_i$ on the initial rates of ingestion of C3b-opsonized endotoxin-coated oil particles in Ca^{2+} free medium + 1 mM EGTA ($[Ca^{2+}]_0 = 10^{-9}$ M) The oil particles were prepared and opsonized as described previously[18]; at the end of opsonization (15 min, 37 °C), 2 mM EGTA was added to the oil particles. The phagocytosis experiments were performed in the above medium containing 1 mM Mg^{2+}, 1 mM EGTA but without Ca^{2+} ($[Ca^{2+}]_0 = 10^{-9}$ M). Phagocytosis was stopped by ice-cold isotonic NaCl + 1 mM *N*-ethylmaleimide + 2 mM EDTA, and the cells were washed twice in the same buffer. ▨, Phagocytosis in the presence of calcium by cells that were not loaded with quin2/AM. □, Phagocytosis by unloaded cells in the absence of extracellular calcium (Ca^{2+}-free medium + 1 mM EGTA) ($[Ca^{2+}]_0 = 10^{-9}$ M). It was 77% of the values obtained in medium containing 1 mM Ca^{2+}, and 72% of the cells ingested oil particles. ■, Phagocytosis by cells loaded with quin2/AM in Ca^{2+}-containing medium and tested in Ca^{2+}-free medium + 1 mM EGTA. ▩, Phagocytosis by quin2/AM cells, loaded and tested in Ca^{2+}-free medium + 1 mM EGTA. The intracellular quin2 concentration was 0.7 nmol per 10^6 cells. $[Ca^{2+}]_i$ in this last batch of cells was 8 nM, and no calcium could be released from intracellular stores by ionomycin. About 70% of the neutrophils contained oil droplets at the end of these four experiments after 10 min incubation.

phagocytosis, respectively. The IgG-coated yeast phagocytosis was inhibited by ~60% by preincubation of the neutrophils with the Ca^{2+} ionophore ionomycin, whereas C3bi-mediated yeast phagocytosis was completely unaffected by this treatment. Ionomycin at this concentration increases $[Ca^{2+}]_i$ to >1 μM (not shown, see ref. 13) and should abolish any existing localized $[Ca^{2+}]_i$ transient.

Our main conclusion is that two main mechanisms of phagocytosis seem to operate in human neutrophils First, C3b/bi-mediated phagocytosis, which occurs independently of $[Ca^{2+}]_i$, enabling phagocytosis, a motile event requiring a rearrangement of contractile proteins[6], to occur without the involvement of calcium ions. Other signals which trigger the contractile machinery must therefore exist. Whether latex particle ingestion occurs through a similar mechanism remains to be established. The elevation in $[Ca^{2+}]_i$ induced by C3bi-coated yeast should thus be considered a secondary event probably not directly related to phagocytosis. Second, Fc receptor-mediated phagocytosis, which possibly requires localized changes in $[Ca^{2+}]_i$ and depends on the presence of this ion at least at physiological concentrations in the cytosol. The reduction, but not complete inhibition, of IgG-mediated phagocytosis by high quin2 loading or ionomycin in Ca^{2+}-containing medium suggests that Fc-mediated phagocytosis may occur, at least in part, through a mechanism that does not require major changes in $[Ca^{2+}]_i$. However, we cannot eliminate the possibility that discrete local changes in $[Ca^{2+}]_i$ close to the plasma membrane are sufficient to trigger some ingestion, or that some IgG-independent ingestion of the yeast particles may occur.

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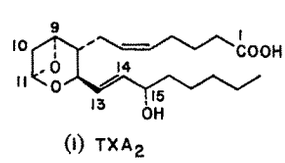
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Synthesis and structure of the platelet aggregation factor thromboxane A_2

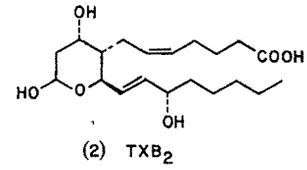
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In 1975, Hamberg *et al.*[1] reported evidence for the existence of an unstable platelet-aggregating factor which they named thromboxane A_2 (TXA_2) and for which they proposed a novel bicyclic oxetane structure (1, below) based on the short half-life of the factor ($t_{1/2}$(37 °C) = 32 s at *p*H 7.4) and the isolation of degradation products related to thromboxane (TXB_2) (2, below). As natural TXA_2 has not yet been isolated and characterized as a pure compound, we have synthesized the proposed structure (1) from TXB_2 and compared its biological properties with those of authentic, biologically generated material. Here we present evidence that synthetic material having structure (1) is indistinguishable from platelet-derived TXA_2 in various biological assays and that the proposed structure (1) for TXA_2 is correct.



(1) TXA_2



(2) TXB_2

The acid-lability of TXA_2 is believed to be a result of the highly strained nature of the bicyclic oxetane acetal nucleus and it was only recently that the hetero-unsubstituted ring system, a 2,6-dioxabicyclo[3.1.1]heptane, was isolated[2]. (References 3-6 describe the preparation of heterosubstituted derivatives.) Bhagwat *et al.*[2] prepared the TXA_2 model (3) by a modified Mitsunobu[7] etherification [$(MeO)_3P$, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2] of compound (4), followed by mild free-radical reduction (Bu_3SnH, C_6D_6, sun-lamp irradiation) and, auspiciously, (3)

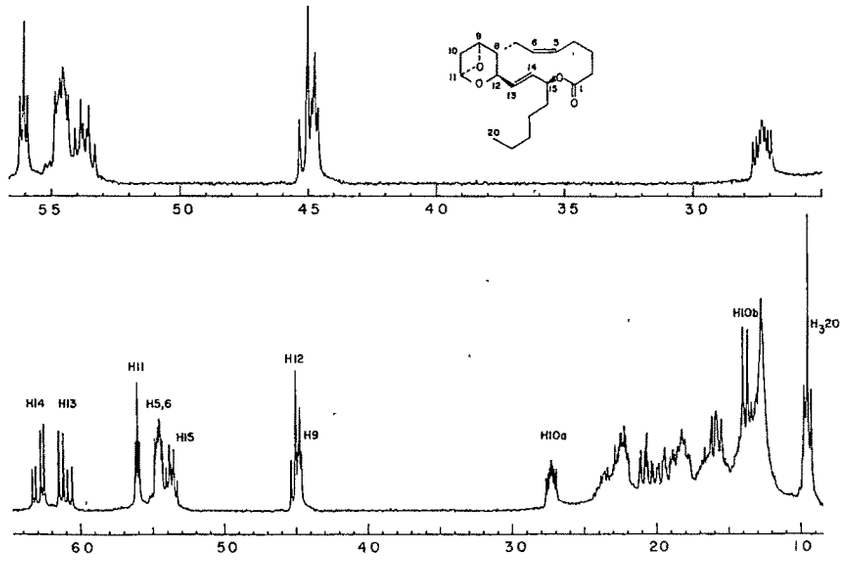
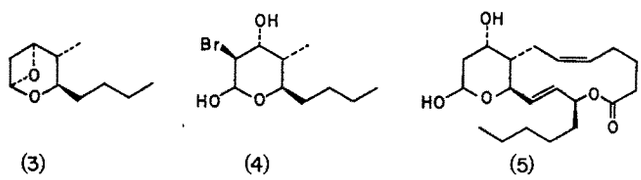


Fig. 1 270-MHz ^{1}H-NMR spectrum of synthetic 1,15-anhydro-TXA_2 in benzene-D_6. The lower trace shows the complete spectrum, with assignments being confirmed by double-resonance decoupling studies. The upper trace shows the expanded spectrum segment spanning 2.5–5.7 p.p.m.

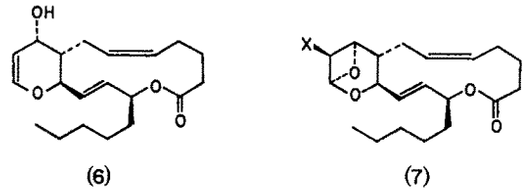
was found to undergo hydrolysis in the Krebs medium[8] at a rate comparable with that reported for TXA_2.

To prepare compound (1) by an analogous procedure, problematic C_{10} free-radical cyclization onto the C_{13}, C_{14} olefin in the penultimate precursor of (1) had to be avoided. This undesired reaction pathway could be eliminated by forming a conformationally restricted 1,15-macrolactone which prevented approach of the C_{13}, C_{14} olefinic linkage to the C_{10} free-radical intermediate in the debromination reaction. Thus, TXB_2 was converted to the 1,15-macrolactone (5) using the Corey-Nicolaou procedure (refs 9, 10 and see also refs 11–13), then dehydrated to the β-hydroxy enol ether (6) using 2-chloro-1-methylpyridinium iodide[14]. Formation of bromohydrin (*N*-bromosuccinimide, 1% $NaHCO_3$, H_2O/Et_2O; 51% yield overall from TXB_2) and oxetane [$(MeO)_3P$, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2; 20% yield] then yielded 1,15-anhydro-10-bromo-TXA_2 (7, X=Br). Finally, polymer-bound tin hydride[15] reduction and filtration of the tin reagent gave the crystalline, acid-labile 1,15-anhydro-TXA_2 structure (7, X=H, melting point (pentane) = 81–82 °C) in high yield. Compound (7) (X=H) displayed the characteristic bicyclic oxetane ^{1}H- and ^{13}C-NMR resonances which closely matched those of compound (3) (the 270-MHz ^{1}H-NMR spectrum is shown in Fig. 1). The novel bicyclic oxetane structure of compound (7) was confirmed by single-crystal X-ray crystallography.

Although macrolide (7, X=H) did not show the biological properties of TXA_2, it was saponified in $NaOD/D_2O/CD_3OD$ (30 min, 22 °C) as evidenced by ^{1}H-NMR, which showed opening of the 1,15-macrolactone with retention of the bicyclic oxetane nucleus, to yield the synthetic compound (1) (TXA_2S) as its sodium salt, which did show the biological properties of



(3) (4) (5)



(6) (7)

TXA_2. TXA_2S was also prepared by saponification of compound (7) (X=H) with Me_3SiOK[16] in tetrahydrofuran and isolated as an amorphous solid by solvent evaporation. Although TXA_2S underwent rapid decomposition in neutral or acidic solutions, it was relatively stable in the basic saponification solutions described and could be stored for up to 1 week at −20 °C without significant loss of activity.

TXA_2S at concentrations of 16–100 ng ml^{-1} caused a concentration-dependent aggregation of human platelet-rich plasma (PRP). Irreversible aggregation occurred with 50–100 ng TXA_2S per ml for all cases examined. TXA_2S was found to be ~10 times more potent than the prostaglandin endoperoxide PGH_2 at inducing aggregation of human PRP. These comparative potencies (shown in Fig. 2) agree with published data[17,18]. TXA_2S (10 μg ml^{-1}) decayed in human plasma with first-order kinetics, with a half-life of 3.9 min at 37 °C, consistent with values of 3 min[19] and 5–6 min[20] determined by indirect methods for biologically generated material. The aggregatory effects of TXA_2S were confined to platelets. At 2 μg ml^{-1} it did not aggregate human neutrophils nor did it stimulate formation of leukotriene B_4.

A selective thromboxane synthetase inhibitor with no accompanying receptor-level antagonist properties (9,11-iminoepoxy-prosta-5,13-dienoic acid, U-54701) at 5–15 μg ml^{-1} had no effect on platelet aggregation induced by TXA_2S. In contrast, a related compound with receptor-level antagonist properties (9,11-diazo-prosta-5,13-dienoic acid, U-51605) at 1–3 μg ml^{-1} inhibited TXA_2-induced aggregation in a concentration-dependent manner[21].

Hydrolysis of TXA_2S in 0.1 M phosphate buffer, *p*H 7.4, eliminated its biological activity and produced a substance that was immunologically indistinguishable from synthetic TXB_2. Radioimmunoassay determinations of hydrolysed TXA_2S yielded values of 84±21, 60±16, 39±11 and 25±8 pg TXB_2 (mean±s.d. at 95% confidence level, $n=3$) which correspond to the predicted values of 125, 62, 31 and 15 pg, respectively. The half-life of TXA_2S at 37 °C in Krebs solution, measured by bioassay on vascular tissue, was 30 s, which corresponds closely to previously reported half-lives for biologically generated material[1,17].

The substance that induces contraction of rabbit aorta, detected by Piper and Vane[22] in the effluent of perfused guinea pig lung during anaphylaxis, was shown to be a mixture of TXA_2 and prostaglandin endoperoxides. Similarly, TXA_2 generated by platelets or a partially purified platelet enzyme, has potent vascular contracting activity. Thus, both natural and synthetic TXA_2 contracted all vascular tissue tested including Krebs-superfused[23] rabbit aorta, pulmonary, mesenteric and coeliac arteries and bovine coronary arteries. On most tissues,

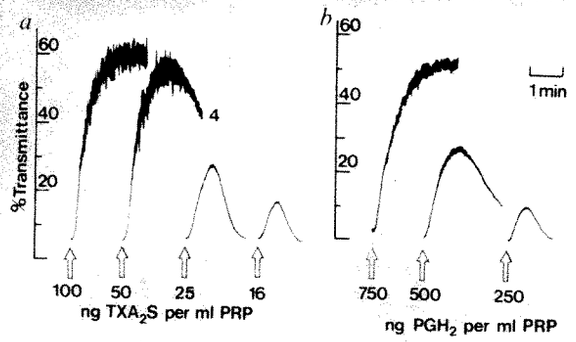


Fig. 2 Dose-response curves for aggregation of platelets in human platelet-rich plasma (PRP) for synthetic (*a*) and biologically generated (*b*) TXA_2. Whole blood (45 ml) was collected over 3.8% w/v trisodium citrate (5 ml). PRP (20 ml) was isolated from blood after centrifugation at 200*g* for 10 min at 25 °C. PRP suspensions (1.0 ml) were incubated at 37 °C for 2 min, then transferred to an aggregometer cuvette containing TXA_2S (10–200 ng) or prostaglandin endoperoxide PGH_2 (100–1,000 ng). Platelet suspensions were stirred at 1,100 r.p.m. at 37 °C and aggregation was monitored as described by Born[25]. To verify that the effects were attributable solely to exogenous TXA_2 or PGH_2, and to exclude any contribution to aggregation from activation of endogenous arachidonic acid metabolism, PRP containing 250 μM aspirin was used.

2–3 ng of TXA_2S was the threshold dose for contraction, generating ~200 mg of tension, with higher doses generating more than 2 g of tension in rabbit aorta and 4 g of tension in bovine coronary arteries. The potency of TXA_2S compared well with the activity of biologically produced TXA_2, calculated from the amount of PGH_2 originally in the incubation (assuming 100% conversion). Rabbit mesenteric and coeliac arteries respond to PGH_2 with a small, short-lived contraction, followed by larger, longer-lasting relaxation[24], whereas TXA_2S and biologically generated TXA_2 gave only constriction.

Although these experiments do not exclude the formal possibility that compound (1) is not TXA_2 itself but is converted *in vivo* to a different material which is the endogenous TXA_2, the qualitative and quantitative activity and the half-life of TXA_2S provide strong evidence that the Samuelsson[1] oxetane structure (1) for TXA_2 is correct.

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Tissue localization and chromosomal assignment of a serum protein that tracks the cystic fibrosis gene

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The basic gene defect in the autosomal recessive disorder cystic fibrosis has not been identified, and no firm linkage of the disorder to any other marker has been reported. However, a serum protein abnormality present in unaffected heterozygotes as well as in affected homozygotes has been described[1], and immunological quantitation of this protein, termed cystic fibrosis antigen, allows the three genotypes to be distinguished[2,3]. We show here that an immunologically indistinguishable protein is present at high concentrations in granulocytes from normal and cystic fibrosis individuals as well as in myeloid leukaemia cells. Somatic cell hybrids between the mouse myeloid stem-cell line WEHI-TG and myeloid leukaemia cells express cystic fibrosis antigen only when human chromosome 1 is present.

Cystic fibrosis is one of the most common serious genetic diseases in Caucasian populations: 1 in 2,000 live births produces an affected child. Assuming that homozygosity for mutant allele(s) at a single locus gives rise to affected individuals[4], the expected heterozygote frequency in these populations is about 1 in 22. The major abnormalities are those of exocrine secretion[5]. Raised sweat sodium and chloride concentration is currently the best diagnostic test for the disease[6], but clinical problems arise because of chronic obstructive lung disease and exocrine pancreatic insufficiency leading to malabsorption and neonatal intestinal blockage[5]. Despite much effort to define the basic gene defect, no obvious candidate has been found among the many, mainly secondary, observed metabolic abnormalities. Therefore, the study of any abnormality which is manifested in a gene dosage-dependent manner in the symptomless obligate heterozygote as well as in the affected homozygote may lead to the identification of a primary defect and hence to the aberrant gene in cystic fibrosis[7].

We have followed a serum protein first described by Wilson *et al.*[1] as a doublet band at *p*I 8.4 on isoelectric focusing (IEF) in the presence of urea. This doublet band is present in serum from cystic fibrosis homozygotes and heterozygotes but not in normal serum, a finding confirmed by several other groups[8–10]. Manson and Brock[2] produced a guinea pig antiserum to the doublet band cut out of IEF gels. When used in rocket immunoelectrophoresis, this antiserum distinguishes all three genotypes: homozygotes have high, heterozygotes intermediate and normals very low (or absent) concentrations of this protein, cystic fibrosis antigen. But the exact method of serum collection is critical for accurate quantitation of the antigen and hence for heterozygote detection. The performance of the rocket assay on optimally collected serum samples is described by Bullock *et al.*[3]. Blood from normal individuals collected in the presence of the serine protease inhibitor benzamidine exhibits high levels of cystic fibrosis antigen. Heparin- or EDTA-collected plasma samples, on the other hand, possess antigen levels expected on the basis of genotype, but produce poor rockets.

Attempts have been made to purify the antigen to identify it biochemically and to produce monoclonal antibodies which would improve and simplify the heterozygote detection technique, but with increasing purification the antigen was repeatedly lost. Partial purification by gel-permeation on Sephacryl S-300, however, is possible. The antigen can be recovered in fractions strongly contaminated with albumin, suggesting that it has a relative molecular mass of ~70,000. To attempt to eliminate this contamination, several probable tissue and cell

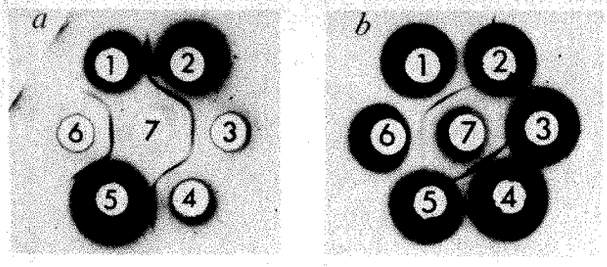


Fig. 1 Ouchterlony double-diffusion analysis for the presence of cystic fibrosis antigen. *a*, 1, Pooled sera from 15 cystic fibrosis homozygotes; 2, guinea pig antiserum raised against material cut out of the cystic fibrosis-specific doublet band region of IEF gels[1] (GP anti-IEF); 3, guinea pig antiserum raised against partially purified antigen from myeloid leukaemia cells (GP anti-CML); 4, antiserum raised as in 3, but in rabbit; 5, citrated plasma from a cystic fibrosis patient; 6, GP anti-CML; 7, freeze-thawed, spun lysate in Dulbecco A buffer of peripheral leukocytes (at 10^8 cells per ml) from a CML patient who was the human parent in the WEMAD hybrids. *b*; 1–5, Cell lysates prepared as for the CML cells above. 1, WEMAD 1.1.9.1; 2, WEMAD 1.1.6.3; 3, NYX 3.1 (see text); 4, WEH 3127.6.1; 5, WEP 1342.2.3; 6, cystic fibrosis serum pool; 7, GP anti-IEF. For assessment of antigen status see Fig. 2. Wells contained 10 μl of appropriately diluted antiserum or antigen. Diffusion was carried out for 24 h at room temperature in 1.5% Difco Noble agar in Dulbecco A buffer containing 0.02% azide; the gels were washed extensively in buffer with 0.05% Tween 20, dried down on Gelbond and stained with Coomassie Blue.

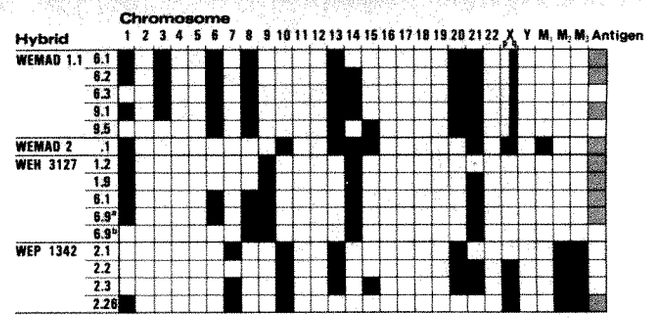


Fig. 2 The segregation of human chromosomes and cystic fibrosis antigen in subclones from a series of four independent hybrid lines between the mouse myeloid stem cell line WEHI-TG (ref. 11) and human myeloid leukaemia cells. Filled squares denote the presence of human chromosome in >30% of karyotyped cells (>16 cells for each clone). Open squares denote the absence of the chromosome in >95% of cells. Each series of marked subclones (WEMAD 1, WEMAD 2, WEH and WEP) is derived from a hybrid line which arose from an independent fusion event. Fusion was effected between leukaemic cells and WEHI-TG (at 10:1 ratio) with 0.5 ml 25% polyethylene glycol 1500 containing 1,250 haemagglutinating units of β-propriolactone-inactivated Sendai virus. Hybrid cells were selected for by selection using hypoxanthine-aminopterin-thymidine[13]. Independent hybrids arose in separate wells. Subcloning was by limiting dilution and carried out repeatedly until reasonable chromosomal stability was established. Subclones were then grown up so that cells within a few transfer generations were preserved in liquid nitrogen, pelleted for antigen assay and karyotyped by sequential trypsin banding[14] and Giemsa-11 staining[15]. The subclone WEH 3127.6.9 contained cystic fibrosis antigen and chromosome 1 was present in 32% of cells on first testing (a). Six transfer generations later (b) both the antigen and chromosome 1 were absent from WEH 3127.6.9. Unidentifiable human marker chromosomes M_1, M_2 and M_3 were found in some hybrids. None seemed to be derived from chromosome 1.

types were examined as potential antigen sources[5] by rocket immunoelectrophoresis and by immunodiffusion. Peripheral leukocytes from chronic myeloid leukaemia (CML) patients proved consistently to be strongly positive for cystic fibrosis antigen, and antigen from normal granulocytes was immunologically identical. It remains to be seen whether the presence of antigen in tissue lysates from adult liver, kidney and brain is caused by blood contamination of these tissues or whether it is intrinsic to tissue-specific cells. Lymphoblastoid cell lines from normal and cystic fibrosis individuals are antigen negative. Ouchterlony double diffusion demonstrates the immunological identity of cystic fibrosis antigen from serum and CML cells (Fig. 1*a*), and also shows that antiserum raised in rabbit and guinea pig against partially purified antigen from CML lysates detects the antigen in appropriate serum samples.

It has been shown previously that at least some human granulocyte-specific products are expressed in somatic cell hybrids between the mouse myeloid stem-cell line WEHI-TG and CML cells[11]. To observe segregation of cystic fibrosis antigen with a human chromosome, we produced a series of independent hybrid lines between WEHI-TG and cells from four different myeloid leukaemia patients. As soon as sufficient cells were available from each hybrid line, cell lysates were tested by Ouchterlony assay for the presence of antigen. (WEHI-TG and mature mouse granulocytes are negative in this assay). Negative and positive hybrids were subcloned repeatedly to achieve reasonable chromosomal stability. Subclones were assayed for cystic fibrosis antigen and analysed for the presence of human chromosomes within the space of one to three transfer generations. Figure 1*b* shows the clear-cut results seen with these hybrid lines as long as the equivalence point between each cell lysate and the guinea pig antiserum was predetermined by previous titration. The results of the complete segregation analysis on 15 subclones from 4 independent hybrid lines are summarized in Fig. 2. Antigen segregates with human chromosome 1 with complete concordance. No other chromosome is consistently present with this antigen, suggesting that the structural gene for this protein is on chromosome 1. Note that NYX 3.1 (a mouse myeloma × human B-lymphoblastoid line somatic-cell hybrid containing chromosome 1 at high frequency) does not express the antigen. This observation agrees with the notion that this serum protein is a differentiated cell product.

As with other autosomal recessive diseases, the defect in cystic fibrosis presumably results from the absence of a normal biological function which arises only if both copies of a gene are aberrant. Assuming that the accumulation of cystic fibrosis antigen is closely related to the basic genetic defect, what sort of mutation could account for the observed pattern: high, intermediate and zero serum levels, in cystic fibrosis homozygotes, heterozygotes and normals respectively, of a protein immunologically indistinguishable (with available antisera) from a normal granulocyte product?

Increased granulocyte fragility which might result from a membrane defect is an unlikely explanation because heterozygotes have increased antigen levels and yet are symptom-free. Suggestions that homozygote and heterozygote sera contain factor(s) which induce increased leukocyte degranulation[12] are not supported by our observation that serum levels of lactoferrin (a specific granule protein) are unaffected by cystic fibrosis gene status (D. Brock, S. Chung and V. van H., unpublished). Another possibility is that a protein that is routinely released into the circulation from granulocytes needs to be processed (for example, by proteolytic cleavage) to fulfil a normal biological function and in the course of this it becomes antigenically unrecognizable. The gene dose dependence of antigen level again makes it unlikely that the mutation resides in a processing enzyme as this would require the aberrant step in the processing sequence to be rate-limiting. The simplest explanation is that the accumulated protein itself is the product of the cystic fibrosis gene and it is altered so that it cannot be normally processed.

The probable site for the mutation, then, is a region of polypeptide chain which acts as a site for a specific proteolytic cleavage step, probably one of several sequential steps involved in releasing a biologically active product from a precursor. This scheme would explain the presence of the high level of antigen and the absence of an essential function in affected homozygotes. Heterozygotes, on the other hand, would possess one normally

processed copy of the cystic fibrosis gene and one mutant copy which would give rise to a half dose of the antigen.

Resolving the biochemical identity of the cystic fibrosis antigen should provide insight into the nature of the disease and allow critical assessment of the genetic hypothesis described here. We are attempting the immunological purification of the antigen, using low-affinity monoclonal antibodies, and are also screening for high-affinity antibodies which will permit the development of a more reliable heterozygote detection assay. Attempts are also in progress to isolate the cystic fibrosis antigen gene by complementary DNA cloning.

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Human transforming growth factor-α causes precocious eyelid opening in newborn mice

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Both murine and human epidermal growth factors (EGFs) are known to cause precocious opening of the eyelids in newborn mice[1-3]. Another set of peptides that are structurally and functionally homologous to murine and human EGFs are the murine and human type-α transforming growth factors (TGF-αs)[4-7]. TGF-αs have been found in many cancer cells and it has been suggested that their autocrine action may play an important part in malignant transformation[8-11]. In several *in vitro* systems murine and human TGF-αs are functionally interchangeable with murine and human EGFs[6,12-14]. However, the *in vivo* activity of the TGF-αs has not been characterized, as only small amounts of these peptides were available until recently. The cloning of the gene for human TGF-α and its expression in *Escherichia coli*[6] now allow us to demonstrate that human TGF-α is as active as murine EGF in promoting eyelid opening in newborn mice. Furthermore, we show in a dose-dependent eyelid opening assay that human EGF is as potent as its murine homologue with respect to this biological property.

The original discovery and purification of murine EGF[1] was based on the measurement of the intrinsic ability of this molecule to accelerate eyelid opening as its primary activity. This property formed the basis for the discovery of the identity of human urogastrone and human EGF and their functional as well as structural homology to murine EGF[2,3]. TGF-αs were first described in retrovirus-transformed cells[8] and have subsequently been found to be secreted by a wide variety of rodent and human tumour cells, suggesting that aberrant expression of these peptides has a role in transformation. It has been speculated that TGF-α functions as an embryonic form of EGF, but it is uncertain what physiological role, if any, TGF-α has in post-embryonic life. Both rat and human TGF-αs have recently been isolated as pure substances and their amino-acid sequences determined[4-7]; they have significant homologies with mouse and human EGFs, particularly with regard to the location of their six cysteine residues, which are important determinants of molecular shape through the formation of disulphide bridges. Human and murine EGF and TGF-α all bind to a common receptor, generally referred to as the EGF receptor, and the qualitative and quantitative activity of all four peptides in many *in vitro* assays is essentially the same[6,13-16].

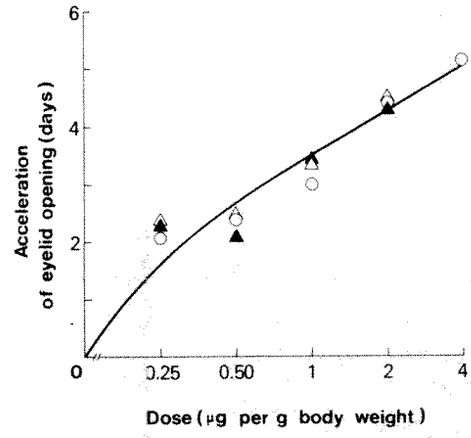


Fig. 1 Dose-response curve for activity of human TGF-α (▲), human EGF (△) and mouse EGF (○) in accelerating eyelid opening in newborn Swiss mice.

In the experiments reported here, 315 newborn mice were injected with human TGF-α, human EGF, mouse EGF or the vehicle for the growth factors (phosphate-buffered isotonic saline, PBS) as a control. Each of the three growth factors was shown to be pure by its unique amino-acid composition, particularly by the absence of specific amino acids. Human TGF-α

Table 1 Acceleration of eyelid opening by TGF-α and EGF

Dose (μg)	Mouse EGF	Human EGF	Human TGF-α
4.0	8.8 ± 0.2 (4)		
2.0	9.6 ± 0.1 (35)	9.4 ± 0.1 (21)	9.6 ± 0.3 (18)
1.0	10.9 ± 0.1 (20)	10.6 ± 0.1 (18)	10.5 ± 0.2 (21)
0.50	11.5 ± 0.1 (16)	11.4 ± 0.1 (18)	11.8 ± 0.2 (18)
0.25	11.8 ± 0.2 (12)	11.5 ± 0.1 (15)	11.6 ± 0.1 (18)
Control	13.9 ± 0.1 (81)	13.9 ± 0.1 (81)	13.9 ± 0.1 (81)

Values shown are the mean day of eyelid opening ±s.e.; values in parentheses are the numbers of mice treated at each dose level. The doses are per g body weight. The data from four experiments, all of which yielded similar results, were pooled. Newborn mice were treated with growth factors dissolved in PBS or with PBS alone, as described in the text. The doses for the three growth factors were adjusted to be equivalent to a murine EGF standard, as measured[18] by the ability of each peptide to compete with ^{125}I-labelled EGF for binding to cell-surface receptors in normal rat kidney fibroblasts (NRK 49F). Based on amino-acid analysis, the amount of TGF-α per binding equivalent was fourfold greater than that of either mouse or human EGF. The effects of all dosages of TGF-α and both EGFs are significantly different from the controls (Student's t-test; $P < 0.001$). There are no significant differences between the effects of the three peptides themselves at any given dose; $P > 0.5$.

was purified by passage through Sephadex G-75 and HPLC after expression of the cloned gene in *E. coli*[6]; the material used was essentially free of other proteins, as shown by the absence of isoleucine and methionine on amino-acid analysis. Human EGF was obtained by chemical synthesis of the gene for this peptide[17], followed by expression of the gene in *E. coli*[17]. The expressed precursor protein (provided by G. D. Searle) was cleaved with trypsin to give the exact human sequence, and the cleaved protein was isolated by ion-exchange and gel chromatography. The purity of this peptide was demonstrated by HPLC in three systems, by acrylamide gel electrophoresis, and by the absence of threonine and phenylalanine on amino-acid analysis. Mouse EGF was prepared by acid/ethanol extraction of salivary glands, followed by gel filtration, DEAE-cellulose chromatography, and HPLC[18], which is a modification of the procedure of Savage and Cohen[19]. The purity of the murine EGF was shown by the absence of alanine, lysine and phenylalanine from the final material.

Newborn Swiss mice were injected daily, beginning at 1 day old, with one of the three growth factors or with a PBS control. All injections were made subcutaneously, in the nape of the neck, in a volume of 10 μl per g body weight. Each litter of nine mice was divided into three groups, which received different doses of the same growth factor, or the same dose of each of the three growth factors. Each mouse was treated for eight consecutive days; the doses used are shown in Table 1. Figure 1 and Table 1 show that human TGF-α, and human and murine EGF all accelerate normal eyelid opening, and that the effect is dose-responsive, except at the very lowest dose used. Moreover, the response of the newborn mouse to the three different peptides is essentially identical.

In another set of experiments (data not shown) we found that transforming growth factor-β (TGF-β), isolated from human blood platelets[20] and used at a dose of 400 ng per g body weight, either alone or in combination with murine EGF, had no activity in the eyelid opening assay. TGF-β has been shown to be a potent synergist of EGF in promotion of the growth of non-neoplastic rat fibroblasts in soft agar[7,12,20].

Although the activity of human EGF in this eyelid opening assay has been described previously in tests involving small numbers of mice[2,3], the present data show definitively for the first time that the respective human and murine peptides are essentially identical in this regard. Furthermore, the almost identical activity of TGF-α and the two types of EGF suggests that the *in vivo* response to all three peptides involves a common, receptor-mediated pathway, much as has been shown for their effects *in vitro*. The equivalence of these peptides *in vivo* is all the more remarkable considering that pharmacokinetic phenomena such as absorption, transport and half-life are also involved. Finally, the data are of interest in that they show that a defined peptide growth factor, generally associated with the malignant behaviour of cells, may have a benign physiological activity that is indistinguishable from related molecules found in non-malignant tissues.

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Expression of c-*mos* proto-oncogene transcripts in mouse tissues

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Valuable information about proto-oncogenes and their physiological function has been obtained by studying their expression in normal cells. However, expression of the c-*mos* gene, the cellular homologue of the transforming gene of Moloney murine sarcoma virus[1,2], has not been detected in normal mouse cells or tissues[3–6]. The conservation of the c-*mos* open reading frame[7–9] strongly indicates that the gene must function during some portion of the animal life cycle, and other lines of evidence[10–15] suggested to us that the c-*mos* proto-oncogene may be expressed at very low levels in normal tissues. We have used a sensitive S_1 nuclease assay to screen RNA preparations from mouse tissues and describe here the detection of c-*mos*-related transcripts especially in mouse embryos, testes and ovaries. The transcripts found in testis RNA are estimated to be ~1.7 kilobases (kb) long by Northern analysis. S_1 analysis demonstrates that the entire *mos* open reading frame is present. In contrast, we detect ~1.4-kb transcripts in ovary RNA and at least two major transcripts, ~2.3 and ~1.3 kb, in embryo RNA. The latter transcripts have in common sequences of at least 1 kb, representing most of the c-*mos* open reading frame. The variation in size of the *mos* transcript in different tissues suggests a novel regulatory mechanism for the expression of this proto-oncogene.

In the present study, we have examined mouse tissues for c-*mos* expression using S_1 nuclease[16,17]. An internal fragment

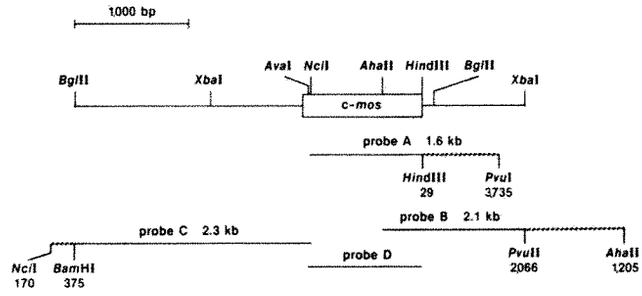


Fig. 1 Restriction fragment probes derived from the mouse genomic c-*mos* locus. Relevant restriction sites are shown. Wavy lines represent pBR322 sequences (restriction sites and their sequence position are given below the line). Probe A, 3′ end-labelled at *Ava*I; probe B, 3′ end-labelled at *Aha*II; probe C, 5′ end-labelled at *Nci*I; and probe D, *Ava*I/*Hin*dIII fragment labelled by nick-translation.

Methods. All probes were derived from appropriate subclones of c-*mos* sequences in pBR322; 3′ end-labelling was carried out with the Klenow fragment of *Escherichia coli* DNA polymerase (Boehringer) in the presence of the appropriate [α-^{32}P]-labelled deoxynucleotides (3,200 Ci mmol^{-1}; NEN) required to fill-in the respective restriction site as described elsewhere[20], except that labelling was done in the presence of 240 mM NaCl. 5′ End-labelling was carried out using polynucleotide kinase (Boehringer)[20,23] and [γ-^{32}P]ATP (7,000 Ci mmol^{-1}, NEN). The *Ava*I/*Hin*dIII fragment for probe D was gel-purified and nick-translated as described elsewhere[24].

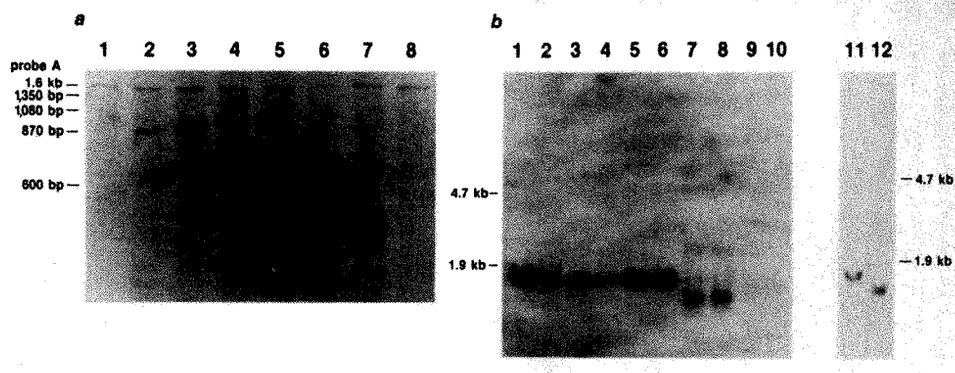


Fig. 2 Detection of transcripts homologous to c-*mos*. *a*, S_1 analysis using probe A. Lane 1, 25 μg of yeast transfer RNA; lane 2, 2.5 μg of total RNA from a NIH 3T3 cell line transformed by pm13 plus 25 μg of total NIH 3T3 cell RNA; lane 3, 50 μg of total embryonic RNA; lane 4, 5 μg of embryonic poly(A)$^+$ RNA plus 25 μg of yeast RNA; lanes 5–8, 25 μg of total RNA from, respectively, liver, kidney, brain and skin. The samples were hybridized to probe A at 60 °C overnight as described [16,17], followed by digestion at 37 °C for 45 min[16,17] with single-strand-specific S_1 nuclease (10,000 U ml^{-1} final concentration; Boehringer). Samples were then analysed by electrophoresis on a 5% denaturing polyacrylamide gel, which was then dried and exposed to X-ray film (XAR-5; Kodak) at −70 °C for 20 h in the presence of an intensifying screen. In this analysis a small amount of self-annealing probe is detected at 1.6 kb. *b*, Northern analysis using probe D. Lane 1, 25 μg total testis RNA; lane 2, 25 μg testis RNA (fraction not bound to oligo(dT) cycle 1); lane 3, 2 μg testis RNA (fraction bound, cycle 1); lane 4, 2 μg testis RNA (fraction not bound, cycle 2); lane 5, 2 μg testis RNA (fraction bound, cycle 2); lane 6, 2 μg testis RNA (fraction bound, cycle 3); lane 7, 10 μg male embryo poly(A)$^+$ RNA; lane 8, 10 μg female embryo poly(A)$^+$ RNA; lane 9, 25 μg total male embryo RNA; lane 10, 25 μg total female embryo RNA; lane 11, 25 μg total testis RNA; lane 12, 25 μg total ovary RNA. Samples 1–10 and 11–12, respectively, were electrophoresed on separate agarose formaldehyde gels and transferred to nitrocellulose as described elsewhere[25–27]. Hybridization was carried out at 42 °C in 50% formamide[20], then the blots were exposed to X-ray film (XAR-5; Kodak) for 3 days. RNA preparation: Animals were killed by cervical dislocation and the various organs were removed. Embryos for the experiment in *a* (and in Fig. 3*a*) were a mixture from nine different inbred strains removed 1 day before birth. The BALB/c embryos for the experiment in *b* were removed at day 19 of gestation. In each case the embryo was completely separated from extra-embryonal membranes and the placenta. For the experiment in *b*, embryos were sexed by microscopic examination of the gonads. Testes and ovaries were taken from 34–39-week-old BALB/c breeders. Routinely, specific organs from several animals were pooled before homogenization, for example, testes from 10–15 mice. The tissue was homogenized immediately or frozen on dry ice and kept at −70 °C for later homogenization (using a mortar and pestle) to a fine powder. Fresh tissue and the frozen powder were then homogenized in a Dounce homogenizer in 5 M guanidinium isothiocyanide, 0.1 M mercaptoethanol and total RNA was prepared as described elsewhere[20,28,29]. In *a*, poly(A)$^+$ RNA was prepared as described elsewhere[20]; the same poly(A)$^+$ RNA selection procedure was used for *b*, except that oligo(dT) columns were not washed with low-salt buffer after loading.

of mouse c-*mos* was radioactively end-labelled with all four deoxynucleotides and used as a probe (probe A, Fig. 1). This probe was then used to screen for *mos*-containing transcripts in total or poly(A)$^+$ RNA extracted from a variety of adult mouse tissues and embryos (Fig. 2*a*). As a positive control for the S_1 assay, we used total RNA extracted from NIH 3T3 cells transformed by pm13, a recombinant DNA clone that contains v-*mos*; this cell line was estimated to contain 1–10 copies of v-*mos* RNA per cell[10,11]. *mos*-Specific transcripts were detected in a 10-fold dilution of total RNA prepared from these transformed cells (Fig. 2*a*, lane 2).

Probe protection was clearly observed in RNA prepared from mouse testes, ovaries (see below) and embryos (Fig. 2*a*, lanes 3 and 4), the size of the protected fragment corresponding to the distance between the *Ava*I and *Hin*dIII sites (925 base pairs, bp) in c-*mos*. RNA from pm13-transformed NIH 3T3 cells protects a somewhat smaller fragment (Fig. 2*a*, lane 2) because the 3′ end of this particular v-*mos* gene differs from that of c-*mos*[18,19]. To exclude the possibility that contamination by DNA of total RNA, especially testis RNA, was responsible for protection of the 925-bp fragment, we performed reconstruction experiments with unlabelled homologous c-*mos* DNA fragments (not shown) or subjected samples to digestion with DNase or RNase before the assay. The hybridization conditions were chosen to favour RNA/DNA hybrid formation (as opposed to DNA/DNA hybrids). RNase but not DNase treatment prevented the detection of the ~925-bp band (not shown). We have variably observed protection of the ~925-bp c-*mos* band in S_1 analyses of total RNA from epididymis, kidney, brain and placenta, but the low amounts detected in these preparations were not analysed further. We did not detect similar bands in total RNA prepared from liver or skin (Fig. 2*a*, lanes 5 and 8), spleen, thymus and intestine (not shown).

We observed that the concentration of c-*mos* RNA in testes, ovaries and embryos was equal to or higher than that in the pm13 control (compare lanes 1, 2 and 3 of Fig. 2*a*), suggesting that we should be able to identify the transcripts by Northern analysis. We detected discrete bands of ~1.7 kb in testis RNA (Fig. 2*b*, lanes 1–6 and 11), 1.4 kb in ovary RNA (Fig. 2*b*, lane 12) and ~1.3 and ~2.3 kb in embryonic poly(A)$^+$ RNA (Fig. 2*b*, lanes 7, 8). In these analyses, the poly(A)$^+$ RNA was prepared from either male (Fig. 2*b*, lane 7) or female (Fig. 2*b*, lane 8) embryos.

Comparison of the concentration of *mos* transcripts in total embryo RNA versus poly(A)$^+$ RNA (Fig. 2*a*, lanes 3 and 4) indicates that the oligo(dT) selection does not result in a significant enrichment of *mos* RNA. However, the same selection procedure[20] does enrich for c-*myc* transcripts (not shown). Even with decreased stringency conditions for poly(A)$^+$ RNA selection, a significant amount of *mos*-specific RNA is not bound to oligo(dT) (Fig. 2*b*, lanes 1 and 2). However, the fraction of *mos* transcripts which are bound in the first oligo(dT) selection (Fig. 2*b*, lane 3) continues to be selected through two additional cycles (lanes 4–6 of Fig. 2*b*); this suggests that the *mos* transcripts may have only short poly(A) tails and may help to explain some of the previous difficulties in detecting *mos* transcripts.

To characterize further the *mos* RNA expressed in testis and embryonic tissue, probes B and C (Fig. 1) were used with S_1 nuclease to determine the extent of protection of sequences downstream and upstream of the c-*mos* open reading frame. With both testis and embryonic RNA, a ~470-bp fragment from probe B was protected (Fig. 3*a*, lanes 1, 2 and 3 respectively). This result indicates that c-*mos* RNA extends 130 bp downstream from the c-*mos* termination codon and ends ~20 bp beyond a consensus poly(A) signal[9]. S_1 mapping of testis RNA with probe C yields two bands of ~400 and 450 bp (Fig. 3*b*, lane 1) corresponding to sites 260 and 310 bp upstream of the conserved *mos* initiation codon[21]. The DNA sequence in this region shows no obvious splice acceptor or promoter sites. However, it is just distal to a region called MUH[22], which is 75% conserved between human and mouse. These analyses demonstrate that the entire *mos* open reading frame plus 260 or 310 bp of upstream sequence and 130 bp of downstream sequence are present in the transcripts. The estimated length of the testis transcript is 1.7 kb by Northern analysis, but only ~1.4 kb can be accounted for by S_1 mapping. Thus, either the

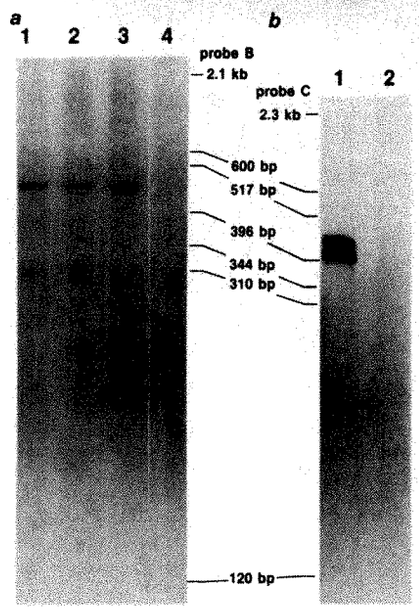


Fig. 3 S_1 nuclease mapping of *mos* transcripts. *a*, S_1 mapping using probe B. Lane 1, 25 μg total testis; lane 2, 2 μg of poly(A)$^+$ testis RNA plus 30 μg of yeast tRNA; lane 3, 3 μg of embryonic poly(A)$^+$ RNA plus 30 μg of yeast tRNA; lane 4, 30 μg of yeast tRNA. The samples were hybridized to probe B at 60 °C overnight. *b*, S_1 mapping using probe C. Lane 1, 25 μg of total testis RNA; lane 2, 30 μg of yeast tRNA. The samples were hybridized to probe C at 52 °C overnight. For analysis of DNA/RNA hybrids and preparation of RNA samples, see Fig. 2 legend. The X-ray film (XAR-5; Kodak) was exposed for 14 days at −70 °C in the presence of an intensifying screen.

transcript size is overestimated by Northern analysis or there is RNA processing 5′ and/or 3′ to the protected regions.

While the embryonic RNA transcripts protect a discrete ~925-bp fragment of probe A (Fig. 2*a*, lanes 3 and 4) and a ~480-bp fragment of probe B (Fig. 3*a*, lane 3), they do not give a detectable signal with probe C (not shown), indicating that they differ from the testis RNA transcripts in the region 5′ to the *mos* ATG. Similarly, S_1 mapping of the ovarian RNA transcripts indicates that they also differ from both the testis and embryo transcripts at the 5′ end (not shown). Collectively, these results suggest that transcripts from the single-copy *mos* locus are expressed in a tissue-specific manner. In addition, these transcripts are either promoted from different sites and/or are spliced differently in testes, ovaries and embryonic tissues.

We have shown that c-*mos* is not efficiently activated by a downstream long terminal repeat (in contrast to v-*mos*[1]); this has been attributed to the presence of an upstream transcription termination sequence termed UMS[21] (M. L. McGeady and G.F.V.W., in preparation) and to the lack of a promoter between UMS and c-*mos* that can function in NIH 3T3 cells[21]. How then can *mos* be expressed? One suggestion[21] is that *mos* expression can be *trans*-activated. The presence of *mos* transcripts in tissues like testes and ovaries may indicate a *trans*-activation mechanism mediated by hormonal control. We also note that an additional 60 codons are proximal to the conserved *mos* ATG in all three *mos* genes that have been sequenced[8,22]. Thus, in addition to tissue-specific regulation of the size of the *mos* transcripts expressed, the presence or absence of these codons (or possibly other exons) could give rise to functionally different protein products in specific tissues.

Finally, the higher levels of *mos* expression detected in gonadal tissues and the differences in transcript size between ovaries and testes suggest that *mos* proto-oncogene expression is regulated in a sex-linked manner and may be associated with expression of primary and/or secondary sex-related phenotypes.

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Note added in proof: We have also detected a 6-kb *mos* transcript in poly(A)$^+$ RNA from embryos and epididymis. In addition, *mos* transcripts are present in rat embryos and testes.

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Errata

Does interocular transfer occur in visual navigation by ants?

R. Wehner & M. Müller
Nature **315**, 228-229 (1985)

The third sentence of the last column on page 229 should read "In honey bees, IOT has been inferred . . ."

Identification and comparison of two sequence elements that confer cell-type specific transcription in yeast

A. M. Miller, V. L. MacKay & K. A. Nasmyth
Nature **314**, 598-603 (1985).

In the eighth line of paragraph two, α/α diploid should read a/α diploid, and in the upper part of Fig. 5*a* and in the third line of the Discussion, $\alpha 1/\alpha 2$ should read $a1/\alpha 2$.

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What's the matter?

Niles Eldredge

The Problems of Evolution. By Mark Ridley.
Oxford University Press: 1985. Pp.159. Hbk £12.50, $19.95; pbk £3.95, $8.95.

PROBLEMS in biology, in sharp distinction to those of philosophy, are solvable — or so writes Mark Ridley in the preface to *The Problems of Evolution*. Ridley has organized his book around a series of ten Great Problems currently (albeit, he says, timelessly) besetting evolutionary biology, problems that curiously decrease in their apparent solvability quotient as one ascends the scale of biological complexity and at the same time approaches the end of the book. Ridley's preoccupation with the answers to questions imparts an eerie quality to his narrative. On the one hand he is often quite sure of having *the* answer to a particular problem (for example the gene is the unit of selection), a sort of certainty I had thought was no longer fashionable in science in general (and particularly in the domain of evolutionary studies, justly known for its authoritative imaginary tales). On the other hand, I must admit to some frustration when Ridley throws up his hands and announces that a problem still defies solution, and that, in fact, our understanding remains at the same low level attained in earlier days. It seems to me that rather more progress has been made towards resolving some of the confusion in evolutionary biology than Ridley seems willing to concede.

Whence this collision between certitude and confusion? Ridley's book accurately reflects the generally unsettled state of evolutionary biology, for which, I think, David Hull is right to lay the blame on a pervasive "common sense" ontology that lies at the very core of the subject. Thus certitude stems from unexamined convictions on the very nature of such entities as genes, organisms, demes, species and taxa of higher categorical rank; and confusion ensues if we get our ontology wrong. Ridley's discussion of what species are — a stunning rerun of the Dobzhansky-Mayr position enunciated nearly 50 years ago — forms the crucial case in point. In this view, species are seen to be simultaneously reproductive communities and fairly coherent groupings of similar organisms, recognizably distinct from other such groups. Ridley is thus forced to repeat the standard claim that species are "real" at any one time (especially well demarcated in sympatry with close relatives), but through time will, of necessity, disappear, evolving themselves out of existence as they transform into descendants. Species exist only in a single time plane, according to this view, a conceptualization that understandably outraged George Gaylord Simpson.

In this received ontology, species are real, individual entities when construed as reproductive communities at any one time; they are also classes of similar organisms. Moreover, the economic adaptations of organisms (the main source of that similarity) are imagined to be in a constant state of overhaul, and so the properties of the members of the class change, and a species evolves itself out of existence. Ridley simply does not report that Ghiselin and Hull over a decade ago pointed out that "things" are either classes or individuals, and that there is no real trick to seeing species, if construed as reproductive communities, as individuals in time as well as in space. Species emerge as spatiotemporally bounded historical entities, regardless of how much, or little, adaptive modification may accrue in the phenotypes of their component organisms. Much of the doubt and confusion permeating the second half of this book stems directly from Ridley's certitude over the basic nature of species, based as it is on a rather garbled ontology.

Indeed, overall the book is an excellent statement of mainstream evolutionary biology as it stands today. If it does not expose the *real* problem in evolutionary theory — the ontological issues — it does paint an accurate picture of the teleological-ridden "who or what benefits" approach that selectionists seem ever more locked into as a source of explanation for all manner of biological phenomena. Nor is Ridley utterly conventional. Within the overall matrix of neo-Darwinian theory, he follows the Williams–Dawkins reductionism that sees the gene as the locus of evolutionary action. And, in an amusing and effective gambit that coincides well with my own prejudices, Ridley discusses systematics by contrasting phenetics and cladistics, claiming the latter is evolutionary and the former not, thereby allowing him to brush aside the vast middle ground of "evolutionary systematics" as an uninteresting muddle of the two extremes. Moreover, while Ridley is clear that cladistics does not depend upon any prior theory of how evolution actually works, in several places he makes a link between nested patterns of resemblance linking up all elements of the biota and the simple notion of descent with modification. Those nested patterns of similarity have always been the strongest evidence that life must have had an evolutionary history, and it is refreshing to see it acknowledged once again, just as a journalist in the United States, in a feat of literary legerdemain, has recently tried to show how cladistics somehow throws doubt on the very idea of evolution.

Ridley's book is not a "fun" read. Indeed, it is difficult to determine to whom it is addressed. The style, superficially zesty ("And the prediction is this .... Here is the final result"), is actually rather flat and utterly matter-of-fact: there seems for Ridley no *joy* in these rather marvellous mysteries. The level is decidedly elementary — as if the intended audience were beginning students or the ever-elusive "intelligent layman" — yet the tone is so unrelentingly serious and the pace so brisk that all but the most dedicated will surely flag.

Perhaps most importantly, however, the book does not get at what I think is *really* the matter with evolutionary theory. But as a quick yet pretty accurate summary of most of the current topics under debate, and as a source of insight about how most evolutionists still approach their topic, it should prove useful indeed. □

Niles Eldredge is at the American Museum of Natural History, New York.

New journals review

On 26 September *Nature* will publish the fifth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1985 issue are that:

(i) the first number appeared, or the journal was retitled, *between June 1983 and May 1984* (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);

(ii) it is published at least three times a year;

(iii) the main language used is English.

Publishers and learned societies are invited to send *four different issues of each suitable periodical, including the first and most recent numbers* (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details for both 1985 and 1986 should be included.



Home in hyperspace

Deborah Singmaster

The Fourth Dimension and How to Get There. *
By Rudy Rucker.
Rider-Hutchinson: 1985. Pp.228. £9.95.

THE dust jacket of the British edition of Rudy Rucker's *The Fourth Dimension* carries a picture that looks like a shot from the final sequence of *Star Wars*. It could be a post card from the fourth dimension, and if it were from the author it would say "Wish you were here!". Rucker admits that he is in love with cosmology and in this exhilarating book he tries to convey his enthusiasm to his readers.

The book is dedicated to "A Square, on his hundredth anniversary" — an apt tribute to another book on the same theme, *Flatland* by the English school master Edwin A. Abbott, published in 1884 and gallantly kept in print in Britain by Basil Blackwell. It deserves to be more widely read than it is. Rucker makes frequent references to *Flatland* throughout his book; he has even written a sequel to the original.

Starting with Plato and the allegory of the shadows on the wall of the cave, Rucker moves by analogy towards a description of a spatial fourth dimension. He introduces the hypercube, "probably the best-known 4-D geometrical problem", and proceeds to the hypersphere, hyperspace tunnels, the shape of space, spacetime and so on. From these literal interpretations of a fourth dimension, he passes to more abstract uses of the term and suggests that we, with our complex interior world of thoughts, feelings, dreams, are "in some very real sense, beings of more than three dimensions".

Rucker has no difficulty in thinking four dimensionally. He quotes Charles Hinton, "the great hyperspace philosopher" and a contemporary of Abbott's, as saying that one of the best methods of preparing oneself for four-dimensional thought is to create a deliberate confusion between left and right, by experimenting with mirror images, for example. One of the most entertaining of the many anecdotes in the book is Rucker's potted biography of Hinton, the son of James Hinton who advocated and practised free love. Charles, who was himself convicted of bigamy, believed that "space is, at the deepest level, to be known by the heart and not the brain". But that way mysticism lies: Rucker is at times unashamedly mystical.

For mathematical greenhorns, Rucker provides simple explanations of major concepts including Einstein's theory of relativity, quantum mechanics and David Hilbert's theory of infinite dimensional spaces. He also discusses less orthodox notions such as synchronicity and the possibility of time travel — he is, after all, apart from being a mathematical logician, a writer of science fiction.

Appropriately, *The Fourth Dimension* is a fourfold book. Besides the text there are witty and helpful diagrams and illustrations by David Povilaitis, the margins contain extensive quotations from writers as diverse as Plato, Ouspensky, Lewis Carroll and Borges, and there are also puzzles for those whom Martin Gardner, in his foreword, describes as "the mathematically competent". (Answers are supplied.)

Rucker's book is not aimed at the trained mathematician or physicist but it should appeal to anyone else who is interested in the nature of the Universe and our part in it. Science undergraduates, even sixth formers, and all science fiction enthusiasts will find it (and *Flatland*) stimulating reading. This is a refreshing and thought-provoking book written by an engaging optimist for whom, unlike Pascal, infinity holds no fears. □

Deborah Singmaster is a freelance writer and broadcaster.

* In the United States *The Fourth Dimension: Toward A Geometry of Higher Reality*, published by Houghton Mifflin. A technical review of the book appeared in *Nature* **312**, 214 (1984).

Distant reactions

Alexander Dalgarno

Interstellar Chemistry.
By W.W. Duley and D.A. Williams.
Academic:1984. Pp.251. $45, £29.

THE discovery in the dense regions of interstellar space of molecules, ranging in complexity from simple diatomic species to extended linear chain polyynes, has provided a unique diagnostic probe into the physical conditions in which star formation occurs. Analysis of the intensities of molecular emission and absorption lines yields estimates of the densities, temperatures, velocities and radiation characteristics of the environment; observations of absorption by rotational levels of the diatomic cyanogen molecule CN have given us a measure of the temperature of the blackbody radiation of the Universe at wavelengths of 2.6mm and 1.3mm; and observations of emission from deuterated molecules have yielded a value for the density of matter in the early Universe, showing that baryonic matter cannot close the Universe.

Molecules are found also in the material ejected by protostellar objects as they begin their evolution to the main sequence, and in the circumstellar shells and stellar winds of massive stars which undergo mass loss throughout their existence. Molecular masers are clear signposts marking phases in the sequence of events that leads to the formation of stars and the dissipation of the remnant material out of which they are made. Vibrationally excited molecular hydrogen is widely observed and provides insight into a diverse range of phenomena, among them galaxies in collision.

Molecules are much more than probes of exotic environments, however; they are also highly effective cooling agents. Molecular emissions carry away thermal energy and bring molecular clouds to the brink of gravitational collapse. Molecules play a controlling role in the ionization balance of collapsing objects and in the dissipation of rotational angular momentum by magnetic braking.

To utilize fully molecular observations in the investigation of astronomical phenomena we must understand the nature of the processes by which molecules are formed and destroyed. Those processes belong to a special kind of chemistry, a chemistry that is now an integral part of astronomy and that is the subject of Duley and Williams's book.

Interstellar chemistry is a combination of gas phase chemistry and surface chemistry. The phenomena under study occur at densities too low for the three-body processes that control terrestrial gas phase chemistry, and the formation of molecular compounds is initiated by radiative associations in which the reactions are stabilized by the emission of photons. The temperatures can be as low as 5K, though in localized regions the passage of a shock heats the gas to several thousand degrees and may dissociate the molecules which then form into new compounds as the heated gas cools and the atoms recombine. The interstellar medium contains grains, refractory particles of dimensions of the order of 10^{-5}cm, on whose surfaces molecules may form to be later released into the gas phase. The gas phase and surface reactions take place together in a medium bombarded by energetic cosmic rays and subjected to X-rays and to ultraviolet photons from embedded stars and exploding supernovae.

Thus a diverse array of basic chemical processes forms the content of interstellar chemistry. Duley and Williams have written a lucid account of these processes in a book in which the background is developed sufficiently to make apparent the relevance of chemistry to astronomy. Here the two disciplines are woven together in a pattern of absorbing interest which should appeal to a broad range of scientists. A limited familiarity with elementary molecular physics is adequate preparation, but at the same time the authoritative tone reflects the fact that both authors are active researchers in this field. Their book will come to be seen as the standard introduction to the subject. □

Alexander Dalgarno is Phillips Professor of Astronomy at Harvard University.

Bone, bones and biomechanics

Charles E. Oxnard

The Mechanical Adaptations of Bones.
By John Currey.
Princeton University Press: 1984. Pp.294. $37.50, £37.20.

FOR earlier generations, D'Arcy Thompson's *On Growth and Form* (published in 1917) and P.D.F. Murray's *Bones* (1934) provided the critical descriptions of what was then known about biomechanics and the mechanical adaption of bone. Nearer our own time, F. Gaynor Evans's *Stress and Strain in Bone* (1957) both provided a summary of the exciting if somewhat confused earlier investigations and marked the beginning of new research based upon the insights of modern engineering. John Currey's book is the natural current successor, together with McNeill Alexander's 1968 and 1984 editions of *Animal Mechanics*, and Wainright, Biggs, Currey and Gosline's *Mechanical Design in Organisms* (1976). Each of these books supplies its own commentary on the general subject, but, although there is inevitably some overlap, they concentrate upon different facets of the field and are aimed at different readerships.

Much of the early work, often dating from the last century and important though it is, confused stress, strain and architecture; the new investigations described by Currey do not. The old studies followed engineering strength-of-materials traditions by presenting biological materials, especially bone, as though they were homogeneous, isotropic and perfectly elastic bodies capable of being described through infinite beam theory. Currey discusses the new approaches which acknowledge that, like many non-organic materials, bone is inhomogeneous, non-isotropic and demonstrates imperfectly elastic, poroelastic and plastic-elastic properties, and each at several different microscopic levels. In particular, these approaches imply that stress in bones, especially irregular bones and the odd-shaped ends of long bones, is better characterized through techniques such as finite element analysis.

Currey recognizes that all bone and all bones are not alike, and gives many examples of how different types of bone and different designs of bones serve differing functions. He discusses, for instance, differences in form and physical properties of bone ranging from the brittleness and stiffness of the auditory elements in whales related to the transmission of sound in water to the curvatures of finger bones used to suspend the bodies of brachiating apes from branches.

In contrast to many other writings on this subject, Currey also describes not only immediate and ontogenetic bony adaptation, but takes into account the evolutionary perspective. He makes a convincing case for the design of bone by natural selection while recognizing phylogenetic constraints; and he considers the all-pervasive action of natural selection in producing optimum structure while emphasizing that all mechanical properties of biological materials are a compromise between conflicting requirements.

Perhaps most importantly of all, however, in this book Currey provides an introduction for biologists to the basic ideas of strength of materials and mechanics. This is a summary that should do much to remove misconceptions that still remain in the minds of some students and even some investigators in this area. Among them are the idea that if one set of stress trajectories is compressive then the other must be tensile (which does not necessarily follow), that a limb under tension implies bone under tension (which it need not) and that mechanical stresses are chiefly borne by bone (they are not: the little known interior complexity of the muscle–tendon unit, and those even less understood and poorly described elements, fascial sheets and ligaments, are all important stress-bearing features especially in the larger land animals).

Finally, although Currey's book unhesitatingly simplifies the ideas of strength of materials in order to aid student understanding, it also reminds us, at almost every step, that real biological situations are much more complex; therein lies the future of these studies. The book is, thus, an excellent source of facts and ideas for students and a resource for their teachers, while at the same time providing a superb set of problems for investigators. □

Charles E. Oxnard is University Professor and Professor of Anatomy and Biology at the University of Southern California. He is author of The Order of Man *(Yale University Press, 1984).*

Light on polymers

Herbert Morawetz

Polymer Photophysics and Photochemistry: An Introduction to the Study of Photoprocesses in Macromolecules.
By James Guillet.
Cambridge University Press: 1985. Pp.391. £45, $79 50.

IN these days of extreme specialization, it is rare to find an author who is equally at home in photophysics and photochemistry. Thirty years ago James Guillet worked under Norrish's direction on a doctoral thesis dealing with the photodegradation of polymeric ketones. Since then, he has not only continued to be a leader in polymer photochemistry, but has made seminal contributions to the study of fluorescence and phosphorescence of polymers, both in solution and in bulk, and has published stimulating accounts of the "antenna effect" in which the energy absorbed by a small number of chromophores is relayed along a polymer chain to excite a group far removed from the absorption site. While Professor Guillet's hope that such a process would lead to a chemical reaction has not been realized so far, it is an exciting possibility which may well be demonstrated in the future.

Although areas in which Professor Guillet has been active are stressed more heavily in this his latest book, it does contain an excellent coverage of the field. The only major area I missed is the extensive Russian contribution to the study of polymers by emission anisotropy, which has been summarized by Anufrieva and Gotlib (*Adv. Polym. Sci.* 40, 1, 1981). The effect of the adsorption of fluorescent dyes on their emission characteristics might also have been mentioned.

While the book is an outstanding introduction to photophysics and photochemistry for polymer scientists, I am less sure that the discussion of the principles of polymer science will be adequate for those unfamiliar with this field. There are a number of errors: the expansion coefficient α (p.33) is not treated as chain length dependent; rotational isomeric state calculations (p.34) depend little on the height of rotational barriers; there are no D and L configurations in stereoregular vinyl polymers; the lamellar thickness of folded polymer chains (p.46) is of the order of 10 nm not 1000 nm; it is not possible to estimate the intrinsic viscosity from measurements at a single concentration without knowledge of the "Huggins constant" (p.74); the spacing of phenyl groups in the helix of isotactic polystyrene is not "of the order of 3Å" but much too large for excimer formation (p.178); and Haas *et al.* and Sisido (p.230) did not study cyclization. It is also a pity that the references only run up to 1981. But these are minor blemishes on a most useful book which is a valuable addition to the polymer literature. □

Herbert Morawetz is a Professor in the Department of Chemistry at the Polytechnic Institute of New York.

New in paperback

- A single-volume edition of Christian de Duve's *A Guided Tour of the Living Cell.* Publisher is W H. Freeman, price is $33 95, £24.95 Sterling price of the hardback is £35.90 per two-volume set, not £39.90 as printed in the review (*Nature* 314, 694; 1985)
- *Radiative Processes in Astrophysics* by George B Rybicki and Alan P Lightman. Publisher is Wiley, price is $26.50, £23 For review see *Nature* 289, 729 (1981).
- *In Search of Mind* by Jerome Bruner Publisher is Harper & Row, price is $6.95, £7 75 For review see *Nature* 308, 779 (1984)

Scattering sources

R.A. Cowley

Theory of Neutron Scattering from Condensed Matter, Vols 1 and 2.
By Stephen W. Lovesey.
Clarendon: 1984. Vol. 1 pp.329. Vol. 2 pp.344. £38, $59 each volume.

NEUTRON scattering now plays an essential role in our understanding of many properties of condensed matter, and is used in the study of atomic structures, interatomic forces, magnetism, liquids and polymers. Stephen Lovesey has made significant contributions on the theoretical side of the subject. With Walter Marshall, in 1971 he published *Theory of Thermal Neutron Scattering*, which has been an essential reference book for neutron scatterers, particularly in the field of magnetism, and which was influential in the development of the use of correlation functions for describing the scattering.

The present two volumes extend the coverage of the field beyond that of the earlier book; Vol. 1 describes elastic nuclear scattering, scattering by lattice vibrations and by liquids, and some chemical applications, while Vol. 2 deals with elastic and inelastic magnetic scattering. Although the second volume is entirely rewritten, most of its contents and the manner of treatment will be familiar to readers of *Theory of Thermal Neutron Scattering*.

In neutron scattering, the Van Hove correlation function is a natural bridge between experiment and theory. The task of the experimentalist is frequently to perform measurements of the function which can then be interpreted in terms of a model for the particular system. Unfortunately there is very little in either of the books about the theory of how to obtain it from measurements of the scattered intensity. There is also nothing on the theory of the experimental resolution or other experimental constraints. The books are a helpful source for the calculation of the Van Hove function for a wide variety of soluble or nearly soluble models, and although I found them difficult to read they will undoubtedly prove valuable for detailed derivations of particular models. It is, however, a pity that the importance of the formulae produced are not illustrated through more examples of experimental results and discussion of when the simple models apply or do not apply to real systems.

Stephen Lovesey has again provided us with a very useful work of reference. These two volumes should not be used as textbooks because of the neglect of experimental aspects of the subject and of applications to real systems. The books, however, will be consulted by neutron scatterers for many years. □

R.A. Cowley is Professor of Physics at the University of Edinburgh.

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Masters of swing

Russell H. Tuttle

The Lesser Apes: Evolutionary and Behavioural Biology.
Edited by Holger Preuschoft *et al.*
Edinburgh University Press/Columbia University Press: 1984. Pp.709. £65, $56.

DESPITE their modest size and colloquial label, the lesser apes (Hylobatidae) can claim a fair share of superlatives. They are the most numerous and variegated, relatively longest limbed, "swingingest", most naturally bipedal, most musical and most socially cohesive of the apes. We do not know whence they came or how they acquired their remarkable adaptive complexes; but few scientists would deny that we are blessed to have them grace the Earth.

Preuschoft and his colleagues have paid them just homage in this well-produced, fact-packed volume, which presents the augmented results of a conference held near Ulm, West Germany, in 1980. It will be a long-lasting source book on *Hylobates*. The organization of the work reflects its major purpose, that is to impress the world with the need to conserve forest habitats and thereby preserve the glorious gibbons of south-eastern Asia. Hence the first of the five sections is devoted to conservation. Its authors are soberly persuasive.

The plights of Javan silvery gibbons, Mentawai Kloss gibbons, South Asian hoolocks, Thai pileated gibbons and Chinese concolor gibbons are particularly acute. Our knowledge about the effects of high-technology warfare on wildlife in Kampuchea, Laos and Vietnam ranges from scanty to nil, but if the destruction of human life is any index the hylobatids probably have only a poor chance of long-term survival there.

It is now virtually certain that monogamy and territoriality are universal among the Hylobatidae, though we cannot yet tell whether new groups are formed by the same mechanisms or whether defence of a section of the range serves comparable adaptive functions in all species. All of them call dramatically (and beautifully) but there is notable interspecific variation in which of the sexes calls, displays athletically, and chases and attacks in reaction to the calls of neighbours, actual intrusions or other factors. The interplay between calling, monogamy, subsistence and territorial defence in *Hylobates* has proved an attractive problem for a host of biologists, and Sections 3 and 4 of *The Lesser Apes* contain a wealth of information on the topic. Given their relevance to salient problems in theoretical biology, anthropologists can no longer be viewed as the proprietors of the hylobatid apes.

Functional morphologists have made great strides in elucidating the mechanisms underpinning hylobatid brachiation (arm-swinging), bipedalism and vertical climbing. As with conservation and ecological and behavioural puzzles, the lesser apes have ecumenically challenged Asian, European and North American anatomists and kinesiologists to explain their peculiar positional behaviour. Section 2 of the book is an excellent report of results from modern, technically and mathematically sophisticated, studies on hylobatid subjects and specimens. Here we learn that because of their elongated forelimbs, the movements of slowly brachiating hylobatids correspond reasonably well with the model of a mathematical pendulum, and that the elongation of the forelimbs economizes energy during rapid (ricochetal) arm-swinging.

It is heartening to see a reiteration (on pp. 94–95) of my 1975 model for the stem hominoids as hylobatid-sized, versatile arboreal beasts that were not especially adapted for dramatic brachiation. But I would have been happier had Herr Hollihn cited Tuttle among the long list of references that inspired him to adopt it. □

Russell H. Tuttle is Professor of Anthropology at the University of Chicago.

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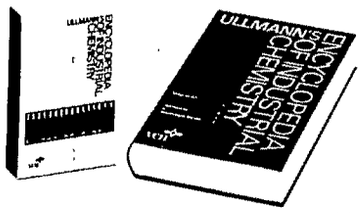
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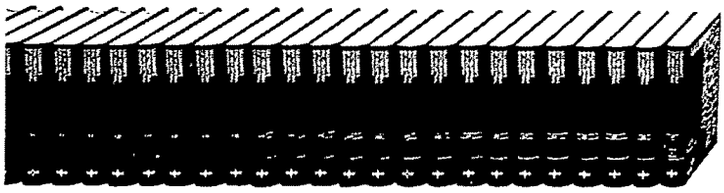
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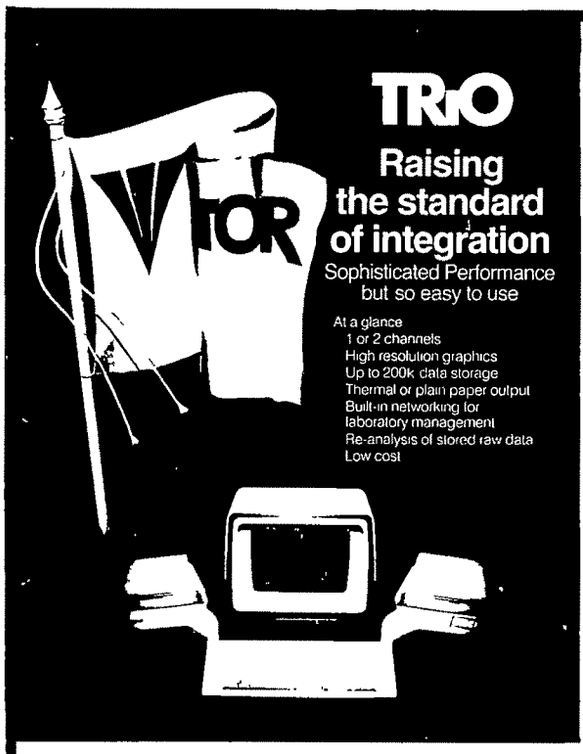
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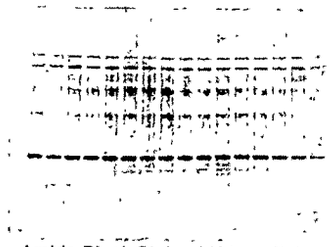
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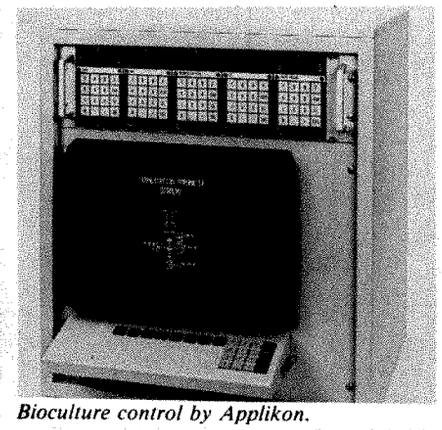
(*) CR Acad. Sci. (Paris), 300, 577, 1985.

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Achema bigger than ever

The Achema-85 exhibition takes place in Frankfurt on 9–15 June. Halls 6-I, -II and -III cover laboratory and analytical techniques, Hall 2 research and innovation.

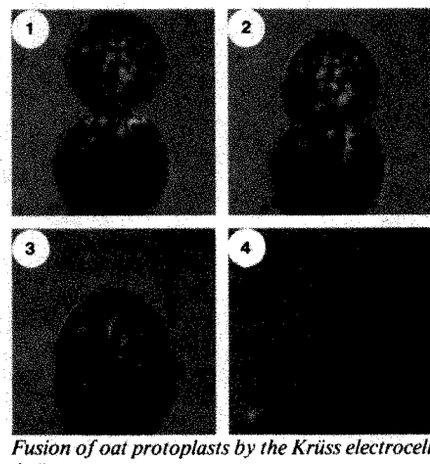
● The theme of Mettler's exhibit at Achema is laboratory efficiency. Mettler is showing a selection of electronic analytical and precision balances, as well as various systems that can be realized with balances or instruments. Of particular note is a compounding system for chemical or pharmaceutical companies where a number of balances are connected directly to a single computer which controls and monitors the balances. Another system demonstrates the use of lab robots with balances and instruments. Also on view are automatic systems for titration and thermal analysis — the DL40RC MemoTitrator, the DL20 CompactTitrator for routine analyses, and the DL18 Karl-Fischer titrator. The TA3000 thermal analysis system is now equipped with a graphic video screen, and many visitors will also be interested in the RC1 reaction calorimeter which measures the heat flow of chemical reactions and physical transformations.
Stands FG5-9, Hall 6-III & AB2-3, Hall 5
Reader Service No. 100.



Bioculture control by Applikon.

● The Applikon Bio-Processor ADI 1020 is an advanced and flexible system for total fermentation control. The system is dedicated to control various parameters during the growth of a bioculture, comprising three control levels. Levels 1 and 2 take care of the measurements, controls and data transmission and level 3 is for data handling. The modular build up of levels 1 and 2 allows the system to be tailored to virtually any biological process. At level 3 there is the option either to use the ADI dedicated and preprogrammed unit, including video and keyboard, or the ADI RS 232 interface to connect to a personal computer.
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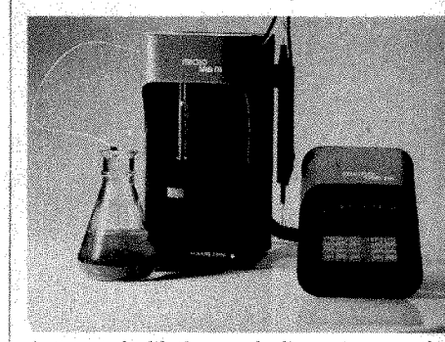


Fusion of oat protoplasts by the Krüss electrocell fusion process.

● Krüss GmbH, of Hamburg, makers of equipment to measure surface tension, has moved into genetic engineering with the introduction of the CFA 400 system for electrically induced cell fusion. The latest development from Krüss is an electroporator making it possible to load biological cells with genetic material. In this method, brief electric pulses induce the reversible breakdown of the cell membrane, the cells are "perforated" for a short time without losing viability. Cells (up to 1 ml) are pipetted into a sterile chamber, mixed with the DNA and exposed to a sequence of electric pulses. The DNA is then taken up by the cells. Subsequently the cells are selected for the desired criteria. During electroporation the cells can be observed under a microscope. As well as DNA, other substances, including drugs, may be loaded in cells in this way, with possible implications for specific therapy. The equipment is controlled by a microprocessor and thus easy to operate. Visitors to the Krüss stand will be able to view the fusion of erythrocytes under the microscope on a video system connected to it.
Stand E-19, Hall 6-I
Reader Service No. 102.

● May & Baker Laboratory Products will be introducing a number of products onto the German market at Achema. On view will be the May & Baker range of HPLC solvents, which includes many solvents that are not available elsewhere. The Pronalys AR range of analytical reagents, Intafaze phase transfer catalysts and Volucon standard concentrates will also be displayed. The Intafaze range includes TDA-1, the first universal crown ether to be made commercially available. TDA-1 is capable of catalysing a wide variety of reactions that would otherwise need expensive solvents.
Stand E22, Hall 6
Reader Service No. 103.

● The Asypor range of membrane liquid filters recently introduced by Domnick Hunter Filters Ltd is claimed to give double the flow rates and double the throughputs achieved with conventional membranes. The Asypor membrane has a unique asymmetrical pore structure. The pore size on the upstream surface is larger than on the downstream surface by a factor of 5 : 1 for optimum performance. Asypor cartridges are available in a range of rated pore sizes from 0.2 to 3 μm with a full range of end cap styles for retrofitting into existing filter housings. The Asypor membrane is also offered in a selection of disks in the standard diameters for process and analytical applications. Asypor disks and cartridges meet FDA requirements and conform to USPXX Class VI. They are integrity testable and have low extractables.
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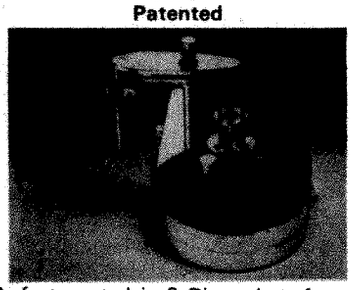


Automated dilution and dispensing — the Hamilton Microlab.

● The new Hamilton Microlab M diluter/dispenser should find application wherever precise liquid handling is required for volumes between 1 μl and 25.5 ml. For instance, for sample distribution, dilution series or in the preparation of samples for further processing in different analytical instruments. The Microlab M can also be linked with a balance and a computer for the automatic preparation of standard solutions. Software packages are already available for various balances. The instrument can be controlled by any computer via an RS 232 C interface. Dialogue with the dispensing unit is conducted in ASCII code via the serial data circuit. The Microlab M is programmed via the controller keyboard (up to 587 program steps, maximum storage capacity 99 methods). The stored methods can be viewed ("roll" function) or printed out. Syringe volume, aspirating and dispensing speeds can now be stored, with aspirating and dispensing speeds individually programmable between 1 and 99 seconds.
Stand 19-20, Hall 6-III
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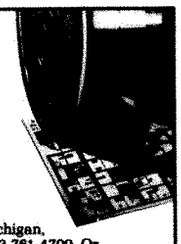
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ACADE DIAGNOSTIC SYSTEMS S.A./N.V., a company specialising in non-isotopic immunoassay using particle counting, is looking for research groups with access to good quality antisera directed towards clinically important assays who would be free to enter into a development contract and/or provide such antisera in commercial quantities.

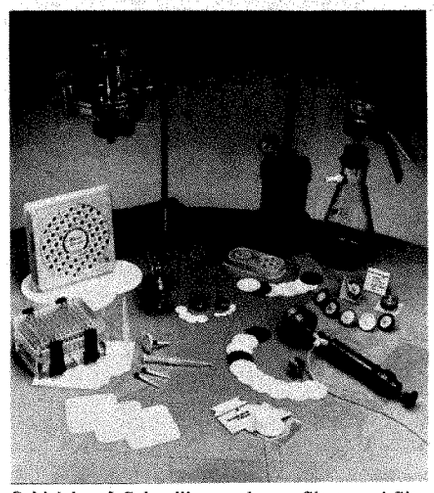
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Schleicher & Schuell's membrane filters and filter holders.

● With the new membrane filters made from Nylon 66, the range of raw materials used in Schleicher & Schuell's membrane filters now includes cellulose nitrate, cellulose acetate, mixed cellulose esters, pure (regenerated) cellulose, as well as polyamide and PTFE. The Nylon 66 membrane filters, suitable for autoclave sterilization, are supplied as circles in diameters from 13 mm up to 293 mm and in pore sizes 0.2 and 0.45 μm. Nytran membranes are positively charged Nylon 66 membranes for the immobilization of nucleic acids and proteins. They interact with negatively charged biopolymers which become selectively bound and immobilized on the membrane. Because of its high binding capacity and good wet strength, Nytran is an excellent medium for electrophoretic transfer methods in molecular biology. Also on the Schleicher & Schuell stand is an extensive range of products for nucleic acid and protein research, including a number developed during the past two years, such as: DEAE and CM membranes for ionic immobilization, the Minifold II filtration manifold, a further development of the Dot-Blot process (Minifold I), the Quick-Blot kit for the selective immobilization of mRNA and Elutip-d, the mini-column which both purifies and concentrates dsDNA.

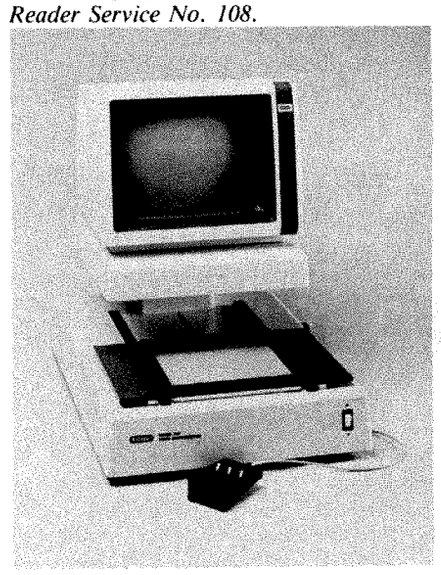
Stand DE3-4, Hall 6.
Reader Service No. 106.

● At Achema Elga Ltd, designers and manufacturers of water purification equipment, will be exhibiting a comprehensive range of laboratory and reverse osmosis products. Of particular interest to those involved in HPLC, atomic absorption or tissue culture, will be the updated Elgastat Spectrum RO unit. This system combines the technologies of reverse osmosis, deionization, media and membrane filtration to produce economical reagent grade water which meets all international standards. Elga will also be launching a completely new product for the production of ultra-pure water for use in medical and research laboratories.

Stand E13, Hall 6-II
Reader Service No. 107.

● Boehringer-Mannheim has introduced a new beta-galactosidase substrate, resorufin-beta-D-galactosidase, which has many advantages as substrate in biological systems. The aqueous solution is yellow (λ_{max} 470 nm). The cleavage product after enzymatic hydrolysis is red-violet (λ_{max} 570 nm), and possesses a light-red fluorescence (λ_{max} 583 nm) at *p*H values higher than 7. The inhibitor programme of Boehringer-Mannheim has been extended with the addition of the protease inhibitors E64 (for thiol proteases), phosphoramidone (for thermolysin), bestatin (for most of the exopeptidases), TLCK (for trypsin) and TPCK (for chymotrypsin), and the new alpha-glucosidase inhibitor bromoconduritol AB. Finally, Boehringer has launched new biochemicals for membrane research. To complement *N*-octylglucoside and *N*-dodecyl-beta-D-maltoside, the new introductions are CHAPS and the peroxide-free tensides Triton X-100, Thesit and isotridecylpolyethylene-glycol ether.

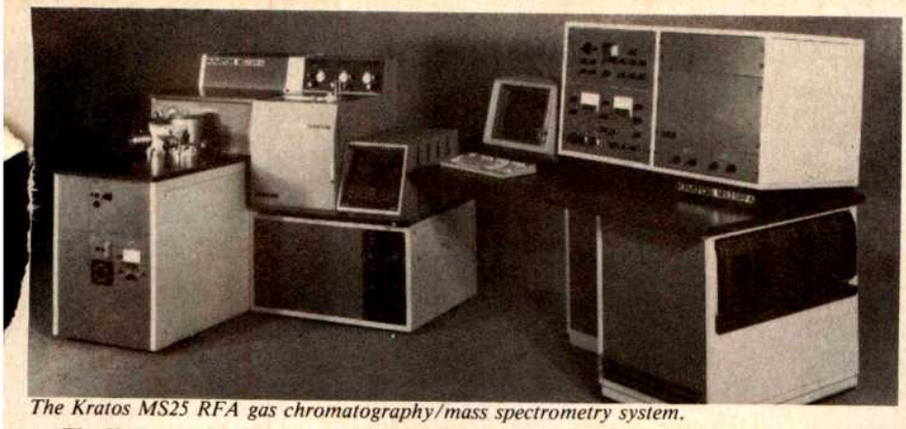
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The Bio-Rad Model 620 video densitometer.

● A number of new instruments will be making their debuts on the Bio-Rad stand. Amongst these are a totally digital video densitometer that provides electronic scanning and instant display of data; a portable HPLC analyser designed specifically for on-the-spot applications; a new HPLC MAPS system for the large-scale purification of monoclonal antibodies and a new moderately priced Digilab Fourier transform infrared spectrometer that provides true research-grade performance. Versatile yet simple to use, the Model 620 video densitometer allows the operator to process, optimize, evaluate and view data on a screen before it is printed onto a chart recorder. All operational procedures are selected from a menu displayed on the screen using a mouse-controlled cursor — there is no keyboard. Up to 30 lanes of data can be displayed and printed, and, if required, the operator can arrange these in a number of different formats.

Stand 19-21, Hall 6-III
Reader Service No. 109.



The Kratos MS25 RFA gas chromatography/mass spectrometry system.

● The Kratos Analytical stand features a complete range of liquid chromatography systems, the new MS 25 RFA GC/MS system (also available as an LC/MS), and a series of components for surface analysis. Shown for the first time in Europe is the Spectroflow 950 programmable fluorescence LC detector, which has full microprocessor control of all instrument functions from a touch-sensitive front panel, RS-232 serial interface, or contact closures. DS 650, a chromatography data system with SmartKey control introduced at the Pittsburg Conference, is also on show, together with a range of gradient formers, UV-Vis detectors, post-column reaction systems and pumps. The MS 25 RFA, another instrument on show for the first time in Europe, incorporates inhomogeneous field magnet technology to provide high mass range and ultra-fast scan speeds without loss of spectrometer resolution or sensitivity. An integral DS90 acquisition and processing computer controls the system in conjunction with the spectrometer's "Autoconsole" and distributed microprocessors.

Stand HJ15-16, Hall 6-III
Reader Service No. 110.

● Rheometrics, manufacturer of high technology instruments for material evaluation, has introduced the Rheometrics dynamic analyser (RDA-700). This versatile analytical instrument measures the viscoelastic behaviour of a broad range of materials (including thermoplastics, thermosets, structural composites, rubber, foods, and adhesives) by determining the sample's response to a sinusoidal shearing action. Since the RDA-700 is easy to use and is sensitive to subtle changes in resins and additives, it is ideal for research projects as well as production control. The RDA-700 is a forced frequency instrument rather than a resonant frequency one. This enables the operator to separate the effects of rate and temperature on material properties, making the instrument a sensitive indicator of molecular structure. Rheometrics manufactures a complete line of rheological and impact testing instrumentation for research and development, design, quality control, and process control.

Stand A8, Hall 9-I
Reader Service No. 111.

● The new range of remote-controlled magnetic stirrers from Dr Hoiss + Partner GmbH can be placed under vessels for normal use but can also be used as submersible magnetic stirrers in fluid baths. One possible application would be in sealed or temperature-controlled chambers, where it is advantageous to control the stirring process externally, without opening the chambers. The magnetic stirrers operate most efficiently when they synchronously stir many vessels in the many stirring positions. Different control units are available, suitable for different kinds of stirring tasks; a small, handy plug-in power supply unit or a bench-top unit. Other features include a start-key which simultaneously centres all the stir-bars on the stirring positions, so that it is possible to freely select stir and pause intervals. Following each pause, the stir-bars are re-centred, and the stirring direction reverses. In addition, it is possible to choose the stirring power and thereby facilitate temperature control in closed systems. The compact magnetic drives are available in two models — a 15-position model to simultaneously stir 15 beakers of 250 ml each, or a model for 6 × 1,000 ml Erlenmeyer flasks.

Stand J13, Hall 6-II.
Reader Service No. 112.



Remote-control stirrers from Hoiss + Partner.

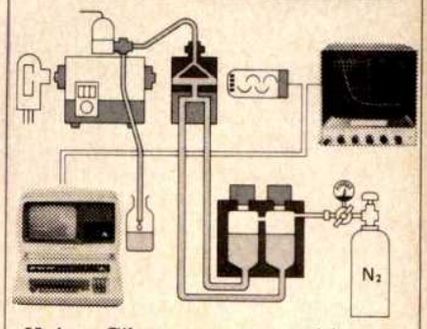
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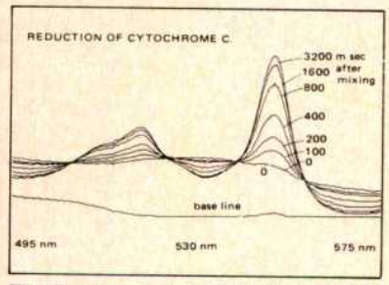
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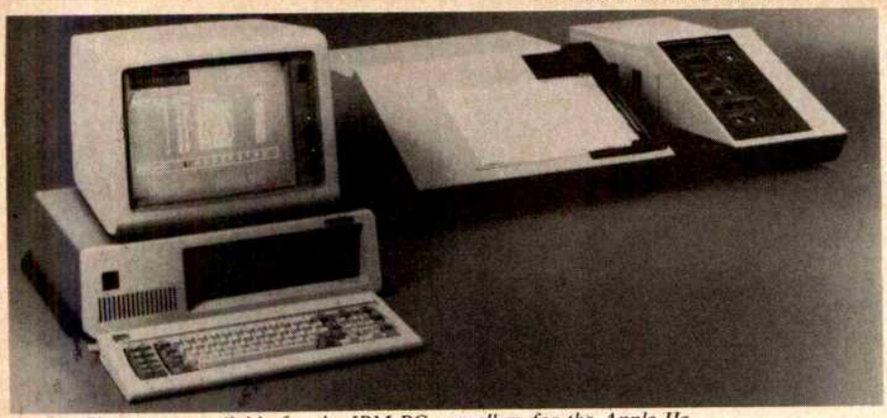
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Reader Service No. 15



Spectrafile — now available for the IBM PC as well as for the Apple IIe.

● Heyden Data systems' stand features a wide range of software for automating laboratory routines using desktop microcomputers, including the IBM PC. Through the cooperation of the instrument manufacturers concerned, Heyden are able to demonstrate the new Sadtler Digital Spectra Libraries on the stand, where Bruker, Digilab and Perkin-Elmer spectroscopy datastations will be operating side by side. The Sadtler collection, formerly in paper volumes, is a comprehensive library of reference spectra. The new digitized collections allow tens of thousands of spectra to be called to the screen. The digitized libraries are also newly available to run on the IBM PC, which can then be used as a stand-alone datastation. Another new development from Heyden display is the IBM PC version of Spectrafile software, available optimized for either IR or UV spectroscopy.

Stand 620/621, Hall 6-III
Reader Service No. 113.



Rapid amino-acid analysis — the Biotronik LC 5001.

● The latest amino-acid analyser from Biotronik, the LC 5001, can analyse physiological fluids in under 2 hours' complete cycle time, and protein hydrolysates in less than 1 hour. Detection levels are 50 picomoles with ninhydrin and 10 picomoles with fluorescence (OPA) detection. Reproducibility is ± 1.5 at 10 nmoles. The instrument uses a microprocessor programming unit which allows 8 different programs to be stored and run in any sequence and combination. Also on the Biotronik stand is the IC 1000 ion chromatograph, now available in two versions. The basic version is for anion determination only and a second version can handle both anions and cations. Full details of Biotronik's user-workshops and applications support services will be available on the stand.

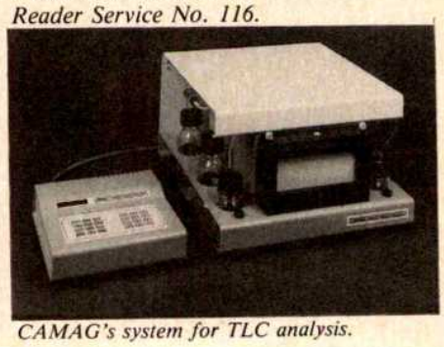
Stand HJ24-25, Hall 6-II
Reader Service No. 114.

● Dyno-Mill, from Willy A. Bachofen AG is the first horizontal, continuous operating agitator ball mill for wet micronization, dispersion and disintegration. In a completely closed system, pumpable suspensions of very low to very high viscosities can be processed. All parts in contact with the product to be ground are easily interchangeable. Models are available with grinding cylinders ranging from 0.3 to 250 litres. A new model in the range, the Dyno-Mill Type KD 20 B, is especially designed for temperature sensitive products and should be suitable for many applications in the pharmaceutical industry and in microbiology. Amongst other items on the stand is the Turbula System Schatz shaker-mixer. This high-performance shaker features three-dimensional overturn motion, with capacities from 2 to 500 litres.

Stand DE-5, Hall 6.
Reader Service No. 115.

● Amongst the new products to be introduced at Achema by CAMAG is a system for the automated multiple development (AMD) of thin-layer chromatograms. With this system reproducibility gradient evaluation can be employed in TLC, making it possible to separate a substantially larger number of fractions than is feasible with conventional development. It also permits separation of mixtures of substances having markedly different polarities. AMD should find applications in environmental analysis, and in all areas where there are complex samples to be analysed. The system, which is fully automatic, consists of a developing unit and developing chamber, gradient mixer, solvent storage bottles, all valves, switching and setting components, and a separate microprocessor control unit.

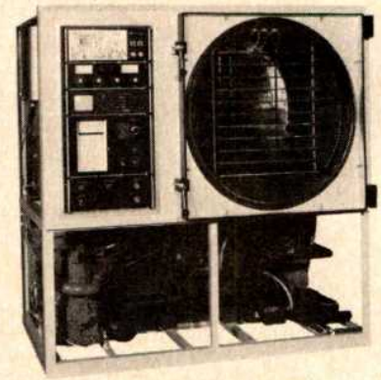
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Reader Service No. 116.



CAMAG's system for TLC analysis.

● The speciality chemicals manufacturer Johnson Matthey Chemicals is exhibiting an extensive range of chemicals at Achema. Five special display modules will be devoted to the company's Honeycat air pollution control units which control the emission of noxious fumes and odours resulting from many manufacturing processes. The four electronics materials modules will be devoted to ultra-high purity raw materials, and metallizing preparations for the electronics industry. The latest addition to the microbonding adhesives range is a conductive silver-glass paste called AuSub (gold sub), the first inorganic adhesive for high reliability die attachment. AuSub has been designed specifically to replace silicon gold eutectics in hermetic packages. Puratronic elements and compounds are a comprehensive range of high purity materials developed to meet the exacting specifications required by the electronics industry. Typical examples are arsenic, gallium and indium for use in III-V semiconductor crystal growth, bismuth germanate and the tungstate systems for compound oxide crystal growth, and boron trioxide and indium for epitaxy applications. The REacton products, comprising all the rare earth elements, are available in a variety of forms and serve as a useful adjunct to the high purity Puratronic series.

Stands D15-20 & E15-20, Hall 5-I
Reader Service No. 117.



The latest Martin Christ freeze-dryer.

● The latest freeze-dryer from Martin Christ GmbH is a unit in which the ice condenser is integral with the drying chamber. During the drying process the water vapour is frozen, taking the shortest path over the ice condenser surfaces which can be cooled to −75°C, installed on right and left of the product shelves. Using this system the drying time can be reduced by 30% compared with conventional systems in which the drying chamber is separate from the ice condenser compartment. The product shelves, up to 4 m^2 in area, are between −50°C and +70°C in a stepless gradient maintained by circulation of a tempering liquid. So thermolabile substances can be both frozen and freeze-dried successfully. The new freeze-dryer is available as either a stand-alone cabinet or for wall installation.

Stand D11, Hall 6-II
Reader Service No. 118.

● Specac's stand at Achema features a wide range of optical components for laser and optical applications. The high performance Fizeau optical test interferometer (FOTI-100) is designed for measuring optical and mechanical flatness and sphericity. It is being demonstrated with its automatic fringe analysis program. The Fizeau interferometer is a versatile topographic tool, consisting of a well-collimated laser beam which is reflected back from both the surface under test and a reference surface. The two reflected wavefronts are superimposed upon a video detector which then displays the resultant fringes on a high-resolution high-linearity video monitor with push-button magnification. The FOTI-100 has a NeNe laser source (wavelength 632.4 μm) and measures flatness to $\lambda/20$ and spherics to $\lambda/10$. It has an aperture of 100 mm but can be fitted with a beam expander to 150 mm or a beam reducer to 33 mm. A simple two-spot alignment system is used and a hard copy of the displayed interferogram on the screen can be obtained using a Polaroid camera. A wide range of accessories for the FOTI-100 are available including 3 and 5 axis interferometric mounts fitted with a self-centering sample holder. These mounts enable firm but gentle holding of sample and precise positioning which are essential in interferometric testing. A linear or non-linear sliding table together with a horizontal test accessory make for simple measurement of fragile and stress free samples.
Reader Service No. 119.



The TRIO system from Trivector Systems International for chromatography data handling.

● Achema sees the European launch of the Trivector Systems computing integrator, TRIO. Hardware and software are designed with the chromatographer in mind. TRIO offers one or two channel, synchronous or asynchronous data collection from any chromatograph through a high accuracy V/F convertor. Incoming chromatograms can be shown in real time on the graphics display. TRIO shows great flexibility in scrolling and magnifying data in real time or post-run mode. The post-run display incorporates baseline and peak identification including graphical comparisons. TRIO complements Trivector's TRILAB, TRISTAR and TRINET to give a wide range of equipment for the data processing needs of the modern analytical laboratory.
Reader Service No. 120.

● The French company Biolafitte is showing a selection of fermentation units at Achema. The emphasis will be on two new automatic *in-situ* sterilizable fermenters, one with a capacity of 20 litres, the other 50 litres. Unlike most units of this type, operation is fully automatic. Integral automation in these units prevents handling errors and reduces the need for an operator to a minimum. The technology used guarantees absolute sterility over a very long period and good stability with time. These Biolafitte fermenters are computer-controlled via a network of multiple microprocessors, and the system can also be connected to the customer's computer system. The computer-control systems, like most other components of Biolafitte reactors, are produced in-house by Biolafitte.
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Reader Service No.07

Graduate path to entrepreneurship

Graduates should be encouraged to join the ranks of the self-employed. Help is available for those who know where to look.

from Richard Pearson

WITH the labour market picking up rapidly from the doldrums of 1981-82, many graduates will be looking again to the large service and revitalized manufacturing employers for jobs. But what about small firms and self-employment?

In the late 1970s the careers services actively sought out opportunities for graduates in small firms. They did this both to widen the range of opportunities open to graduates and to try and help small firms develop by encouraging them to value a graduate recruit. With graduate unemployment rising to, and exceeding, 12 per cent in 1982, and scientists experiencing even higher rates of unemployment, there was a growing urgency to find new opportunities for graduates both in terms of the type of work they might undertake and by finding new employers. As a result graduates often displaced less-qualified personnel. The fact that graduate unemployment did not rise as steeply as overall unemployment levels and has subsequently fallen significantly as the economy has expanded is testimony both to the flexibility of graduates and to the fact that it has been the higher-skilled jobs that have benefited most from economic expansion.

Indeed, of the half-million extra jobs created in the 18-month period to the end of 1984, two-thirds were for the self-employed, with many of the rest being for part-time workers.

To what extent have, or might, graduates follow the path to such self-employment?

It has been estimated that only about 500 graduates leave higher education each year to launch their own businesses. On degree courses in business studies and management, one might expect a reasonable proportion of graduates to start up their own business ventures, but this apparently is not the case. In fact, it is far more likely to find would-be entrepreneurs emerging from courses in three-dimensional design and other courses in the art and design field, after which self-employment is a normal and expected outcome.

Nevertheless, many graduates do see themselves as having the necessary skills for entrepreneurship and have career aspirations which include the possibility of running their own businesses. A study by Durham Business School[1] has shown that as many students would like to work in small businesses as in large businesses, and that one in four of those with clear career plans wanted to be self-employed or run their own businesses. Indeed, one in twelve had already started doing something about it, either making preliminary enquiries, doing basic design work, or developing a prototype or patenting an idea.

As part of the study, 1,000 students were asked to rate themselves against a set of entrepreneurial attributes ranging from the 'desire for power' to 'the ability to work long hours'. The results were largely positive, although they were less sure of their ability to risk-take and, most significantly, to attract finance.

There has been a lengthy debate as to whether entrepreneurs are born or made, and if the latter, then what factors can be brought to bear to encourage and aid more people to follow the route through entrepreneurship into self-employment and starting their own businesses.

A preliminary vacation course on entrepreneurship showed positive results in terms of encouraging students towards self-employment, although a subsequent work-experience programme seemed to offer a dose of reality and reduced the numbers believing they were entrepreneurs. Thus while the report concluded that enterprise attributes need to be encouraged by developing educational programmes of the 'education for enterprise' kind, to increase awareness at the undergraduate level, more specifically, there was a need to develop special training programmes for enterprise or small business management.

To encourage graduates to consider starting their own business and to foster the entrepreneurial spirit, the Scottish Enterprise Foundation, an alliance of business, academic and governmental interests, launched Graduate Enterprise in 1982. Based at the University of Stirling, its aim is to provide a four-month training programme covering topics such as business planning and strategy, marketing and market research, accounts and book-keeping and sources of finance.

Such has been the success of the Stirling scheme that the number of places has steadily increased from an initial 20 to 60 in 1985. Significantly, there are plans to adopt the Stirling idea in a number of English universities — the first, based at Cranfield School of Management and directed jointly with the Manpower Services Commission, will receive its first intake of 40 graduates this summer. This is for six weeks of formal training as part of an 18-month programme of support provided by Cranfield and the scheme's sponsors — Arthur Anderson, the accountants who will provide financial counselling, and BP, who will undertake product development advice and practical help, with National Westminster Bank providing help and advice on financing problems.

As well as the programme of training, counselling and consultancy, the course participants are also eligible for a £4,000 cash package of allowances during the first 18 months, including a basic allowance of £40 per week during training.

With more institutions starting graduate enterprise initiatives, the number of graduates involved is likely to expand significantly. Not all participants will eventually turn out to be successful. If, however, the business success rate of participants is around the national norm, where the success rate for new business helped by an enterprise trust or similar is believed to be around two-thirds, they will have proved their point — that entrepreneurial skills can be developed and business ideas nourished by an appropriate training programme, and this will be of benefit to both the graduates and society.

There are several incentives for self-employment and starting your own business. First is the contraction of alternatives as the number of opportunities in 'big' business and in the public sector decline. Second, a national policy of favourable publicity and special support schemes. These include low-cost advice, premises and finance, support groups such as enterprise trusts, and training programmes. Finally, the academic institutions themselves are being encouraged to follow a more commercially oriented policy.

Those graduates going into self-employment are still to some extent pioneers and few are likely to hit the jackpot immediately. However, such opportunities do exist and with the continuing constraint on traditional job opportunities, students will be wise to widen their horizons in employment terms; if the number of 'traditional' graduate jobs does shrink in the future, the range of opportunities will surely widen. □

1. *Entrepreneurship and Higher Education* (Durham University Business School, 1984).

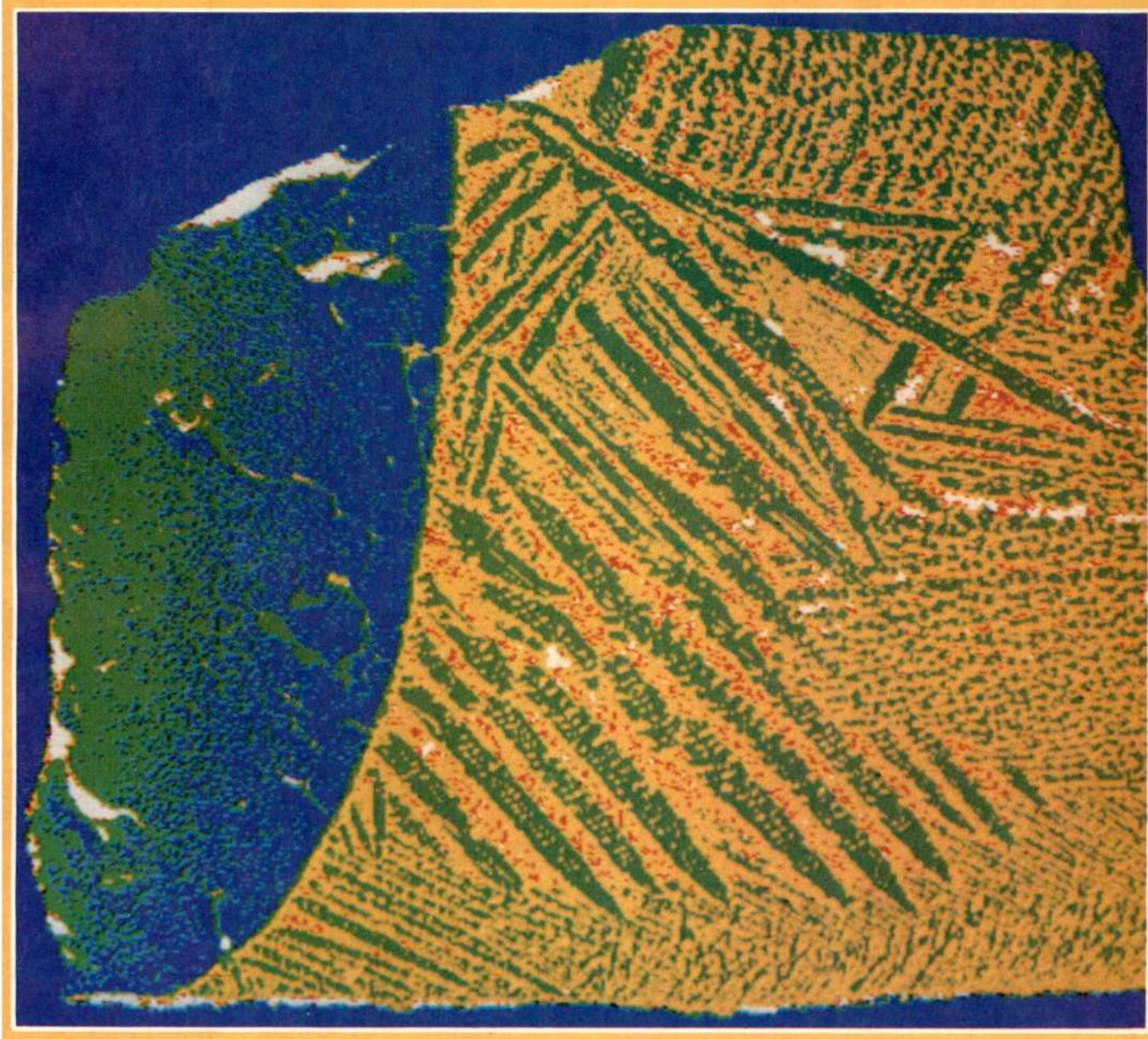
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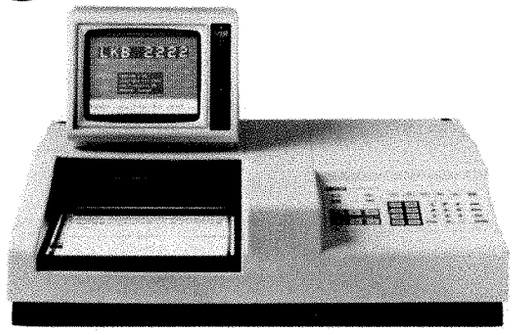
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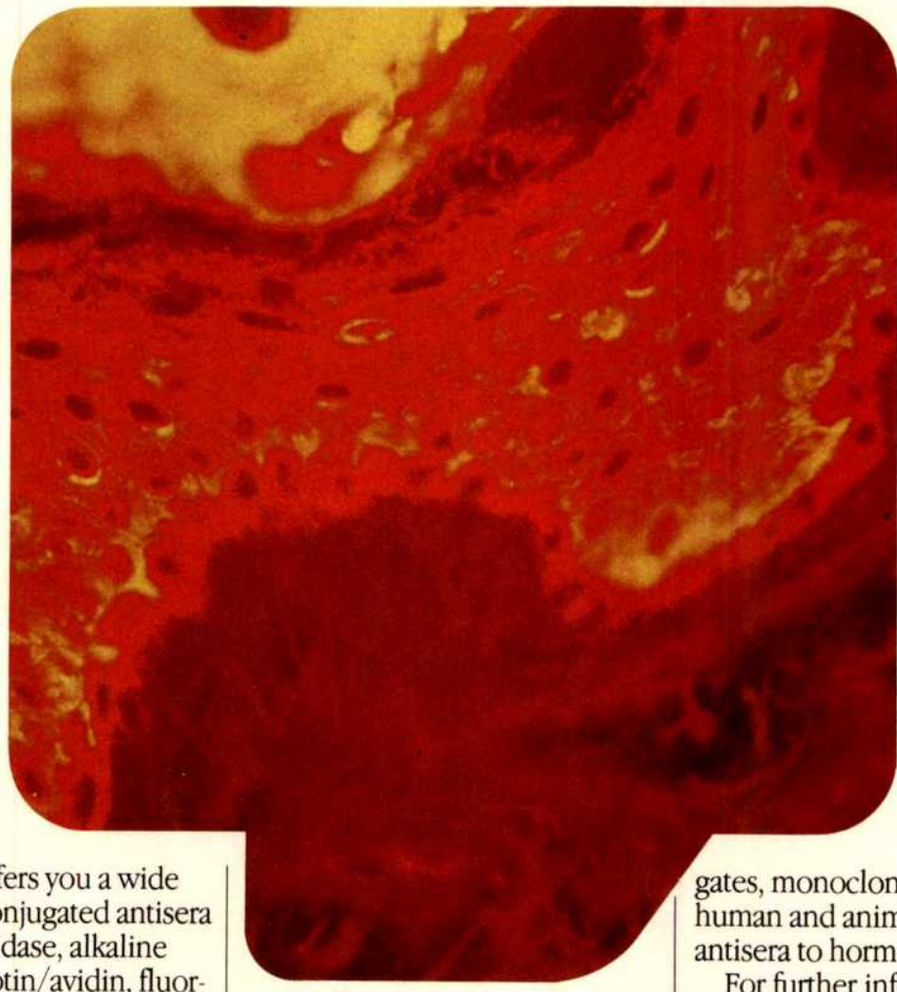
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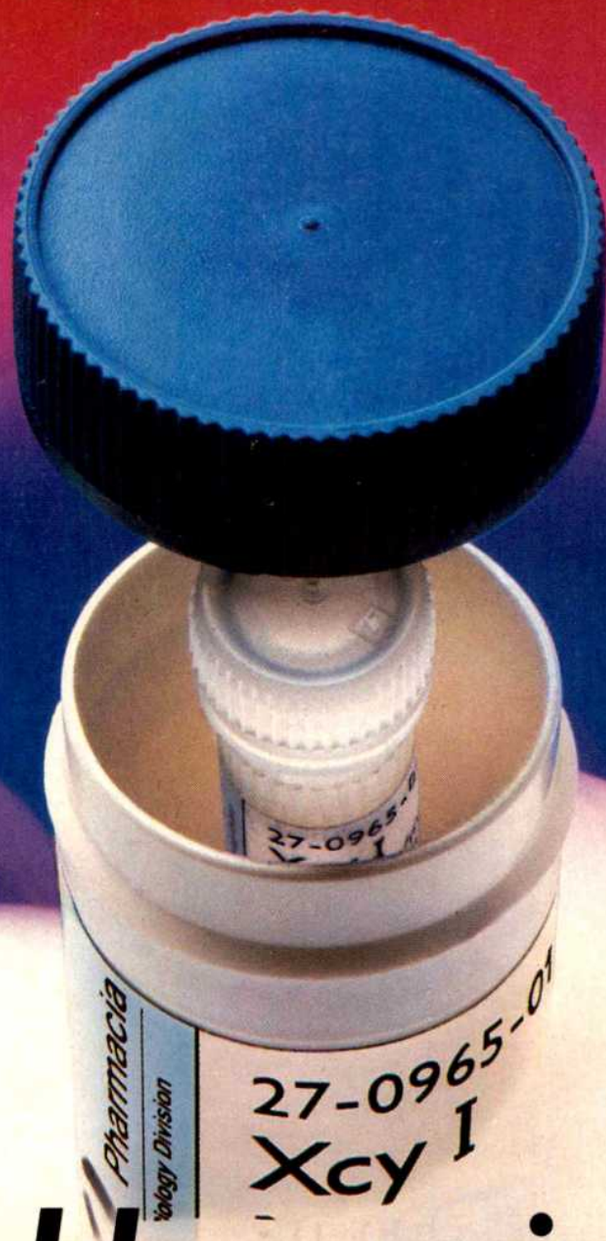
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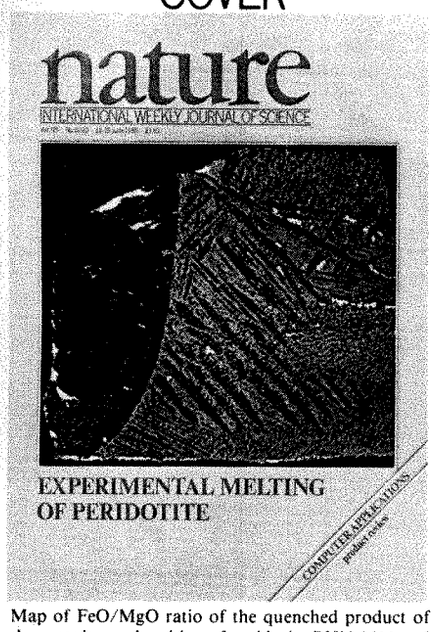
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COVER



Map of FeO/MgO ratio of the quenched product of the experimental melting of peridotite PHN 1611 at 5 GPa and 1,900°C. The komatiitic partial melt on the right shows spinifex texture of olivine and clinopyroxene dendrites with interstitial glass. This is separated from the residual olivine by a smooth curve which may correspond to an isothermal surface within the furnace (see p.566). FeO/MgO colour code: blue <0.04; green <0.1; yellow <0.22; red <0.3; white >0.3.

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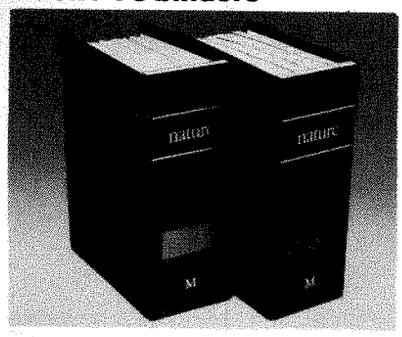
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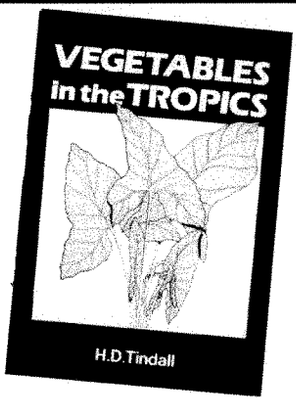
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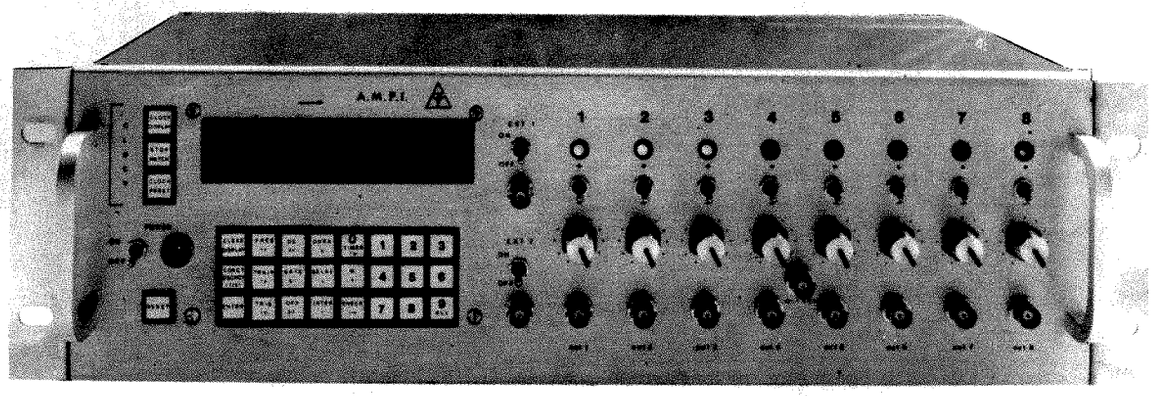
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FLEXIBILITY

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EXAMPLES OF THE FLEXIBILITY OF THE "INDEPENDENT CHANNELS" CONCEPT:

1) WORKING WITH THE OTHER CHANNELS: While some of the channels are running one sequence, the user can still work independently with the other channels in all the modes as described above. *For instance,* he can give test pulses on FREE-RUN through one channel, operate the air pressure manually by another, have a DC output from an additional channel, receive an external trigger which will activate other channels, etc. The user can do all of this without interfering with the main sequence.

2) EXTERNAL GATE INPUT: Master-8 has the External Gate Input option. This option does not usually exist in the **"Single Sequence"** method since the duration of the external input may be variable, while the sequence has to be fixed. *For instance,* a channel can send trigger pulses to an isolation unit at a preset rate during each input pulse from an external device. The duration of the input pulses can be unknown and variable.

3) PARTIAL SEQUENCE: The user can activate only a part of the programmed sequence. *For instance,* suppose the following sequence: channel # 1 activates a tape recorder, channel #2 triggers the oscilloscope, #3 triggers the calibrator, #4 sends a train of pulses to one nerve and #5 to another nerve. The user can trigger manually a partial sequence (for test purposes) like triggering the oscilloscope and stimulating the nerves, or only one of the nerves, without changing the programmed sequence.

4) 'OR' TRIGGERING: The user can trigger internally one channel by any number of other channels (delayed 'OR' triggering). *For instance,* the user can stimulate one nerve after stimulating each of some other nerves (even if one of the stimulating channels was triggered internally and another through the external input at unknown times).

These examples are only a sample of the many flexible variations by which the user can use Master-8.

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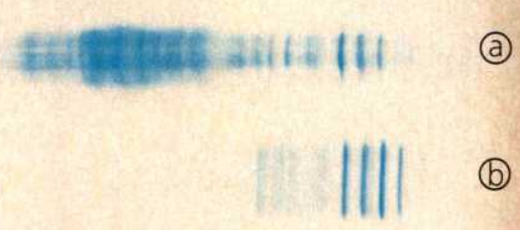
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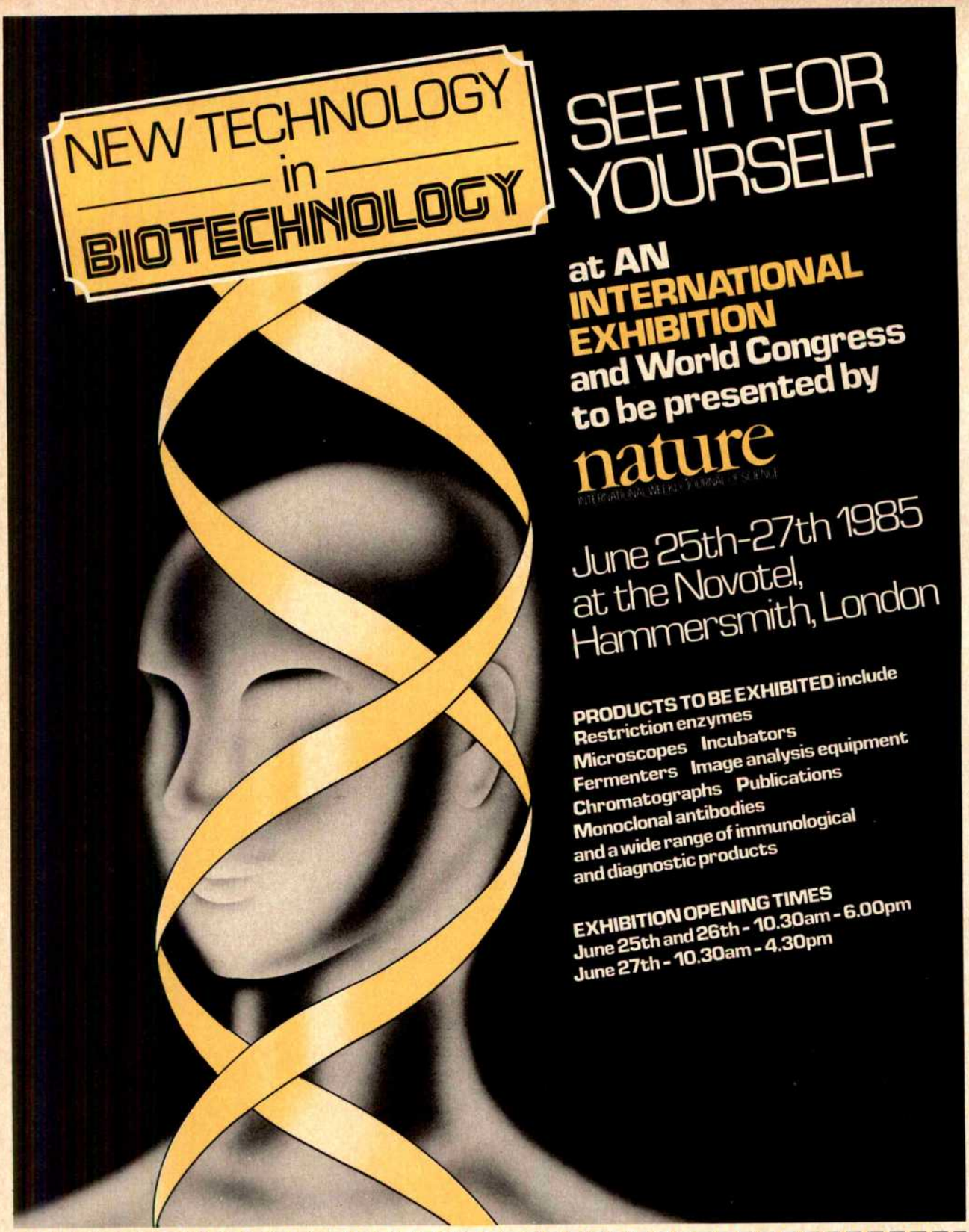


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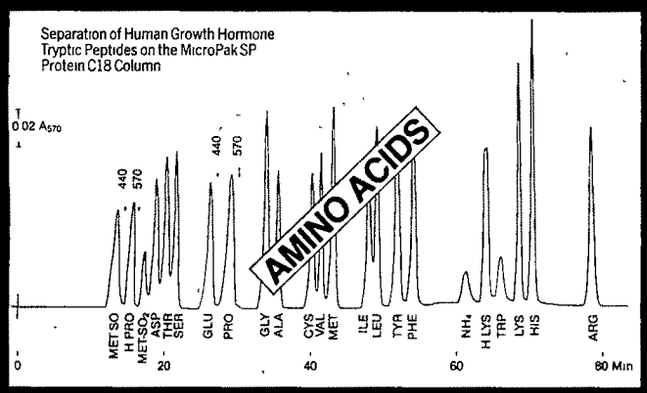
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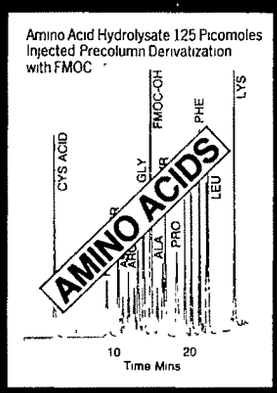
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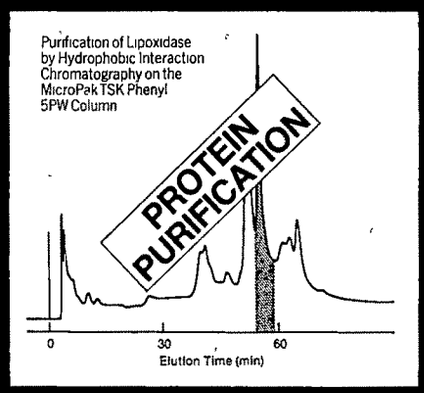
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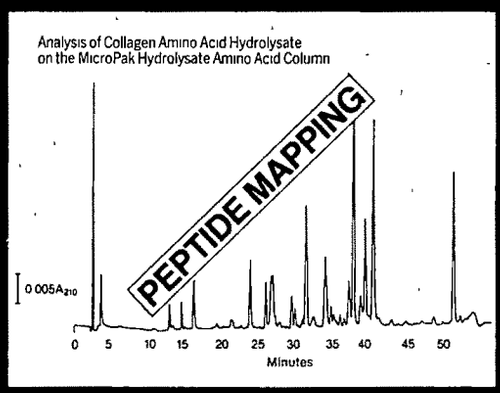
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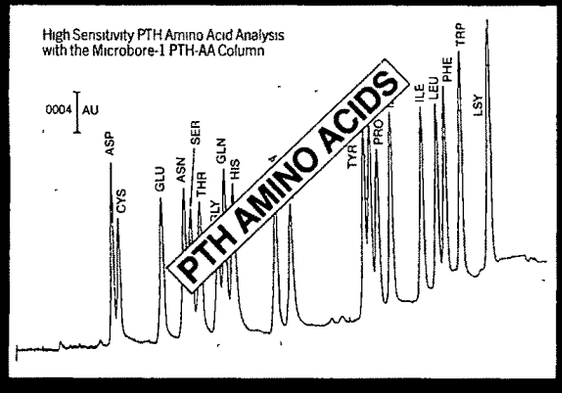
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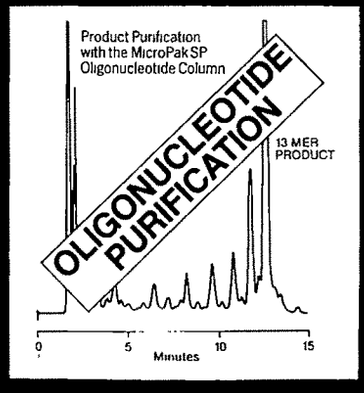












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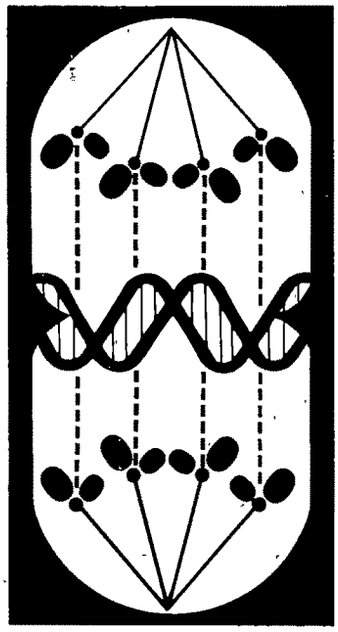
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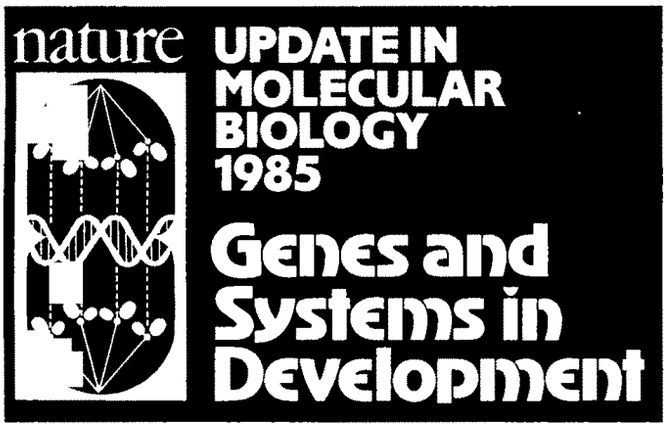
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M. Davis (Stanford)	T-cell receptors
U. Storb (U. of Washington at Seattle)	B cells
J.M. Bishop (U. of California at San Francisco)	Oncogenes
M. Ptashne (Harvard)	Regulatory proteins
. Herskowitz (U. of California at San Francisco)	Yeast development
E. Jaworski Monsanto	Plant transformation

DAY 3: WEDNESDAY, OCTOBER 9

GENES

Speakers	*Topics*
R Palmiter (U. of Washington at Seattle)	Transgenic mice
D. Hogness (Stanford)	*Drosophila* homoeotic genes
M. McGinnis (Yale)	Mammalian homoeotic genes
T. Friedmann (U. of California at San Diego	Human therapy

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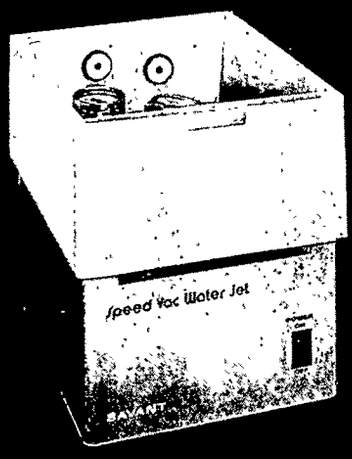
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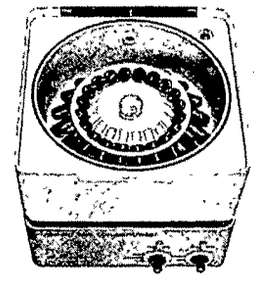
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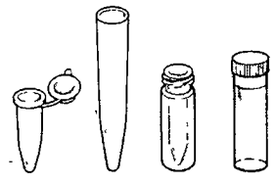
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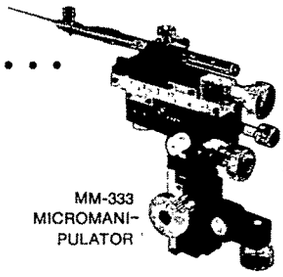
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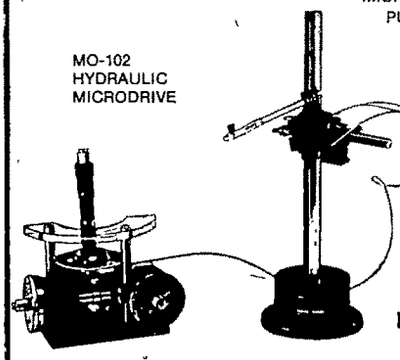
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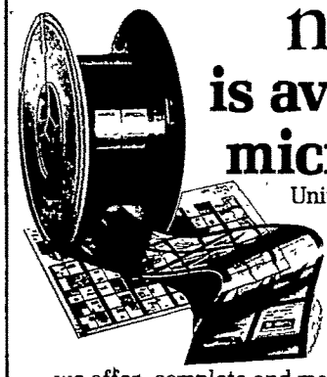
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nature

Parliament's chance to act

This week's debate in the House of Commons about the British government's handling of research and the universities could be a turning point, but the critics must take careful aim.

THE research enterprise and the university system with which it is linked have been badly battered in the past six years, in part because they lost their political friends a long time ago. That is also why it is a long time since the condition of British science was last debated in the House of Commons. (The House of Lords, more leisured but less influential, has been more attentive.) Labour, the chief opposition party, has been crying out for months for a debate about Sir Keith Joseph's part of the enterprise, the universities and the research councils, but has not gone so far as to sacrifice some of its own ration of parliamentary time. Now the government has arranged a debate for Friday, when the House of Commons will be almost empty. Complaints will be aired in a near-vacuum. Let us hope they do not go unheard.

As always, it is important that the complaint should not be overstated. Complainants should at the outset concede a large part of the government's defence. There have been damaging budget cuts, but the Prime Minister has kept her promise, in 1980, to "protect" the direct income of the research councils, the continuing need to reduce public spending notwithstanding. The cuts have fallen instead on the universities in a way that has damaged the underpinning of research, and on the research councils indirectly, by the whittling away of what government departments can afford to spend on commissioned research. The latest cut, by £10 million this year and £20 million next year, in the budget for basic agricultural research, has arisen from the separate dealings between the Treasury and the agriculture ministries, but in a way that suggests the machine is out of control.

Complainants must also concede that there is no reason why research and the universities should have been exempt from the financial pressures to which other government spending has been subject. Most universities are no doubt less efficient than they might be. The research councils are not uniformly paragons of good management (and some of them need urgently to be reorganized). There may even be something in the opinion that because British spending on research and higher education has grown as a result of ill-coordinated decisions to an arbitrary level, it may now be arbitrarily cut back. Put simply, the case against the budget cuts is not that they have been made, but that they have been decided and enforced without much thought for the consequences. Cash limits have become a substitute for thoughtful policy. Paradoxically, the results include not only economies but also diseconomies.

Collapse

The collapse of the dual-support system is the best known diseconomy. Britain has boasted of this device for supporting university research bimodally, by means of project grants from the research councils to researchers whose competence is assured from the recurrent university budget, which provides basic equipment, technician help and other elements of infrastructure. The system has been under strain for at least a decade, as universities have spent their shrinking funds on survival rather than research support. For at least five years, only the strongest universities have been able to support research groups adequately, often by spending precious reserves or by running up debts.

One result is that research council funds intended as research grants must be spent instead on basic research support. Another, more damaging but more common, is that able research groups are frequently ill-provided with research support, even in competitive fields where being second counts for very little. In due course, this imbalance will be put right as the University Grants Committee implements its declared policy of transferring funds within away from less active university departments and even entire universities. But it will be a decade before that policy takes effect. One consequence will be a reduced demand for public research funds, another will be a reduction of the volume of publicly supported research, a third that many creative people will be stranded in places deemed unworthy of public provision of research support. No wonder that people no longer whistle on the way to their laboratories.

The present system is often described as wasteful by its critics, but the remedy now prescribed entails waste not previously imagined. The few thousand researchers from universities and research establishments put out to grass in the past five years have been persuaded to "take" early retirement by financial inducements whose immediate cost (known as that of "restructuring") must be close on £50 million, but whose real cost will be buried in pensions plans for years to come. But the much more serious cost may be that implied by the frequent practice, among those displaced, of working profitably for employers outside Britain. That little irony, with all its implications, deserves a mention on Friday. So, no doubt, will be the diseconomies enforced by the common need to operate educational plant below capacity (because of constraints on student numbers) and to let research equipment lie idle (because operating funds are not to be had). Much of the experience of the past few years is a proof of the diseconomies of contraction.

The British government's rejoinder is that many of these problems would be less serious if only universities would do what they should have been doing all along, and forging stronger relationships with industrial companies. Again, it should be conceded that many (but not all) researchers have been slow to recognize that there may be sources of support other than the government, with the advantages of plurality that would then accrue. But it is unreasonable to expect that such funds will be forthcoming on a scale sufficient to make up for present deficiencies. It would be positively undesirable, and probably an offence against the rules of spending public money, that all hard-pressed institutions should top up their budget deficits in this way. But, in any case, such industrial funds as there may be will probably be distributed on the Matthew principle ("to him that hath ") and will be concentrated among the better off.

Biological manuscripts

Miranda Robertson, Biological Sciences Editor of *Nature*, is now based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees).

Closer industrial liaisons will, however, make for a more effective transfer of science and technology from research laboratories to British industry. That has been a constant part of the government's recent opinions. The argument has some force, but not all industrial liaisons with research are desirable or can even be expected to lead to worthwhile commercial enterprises. There are many fields of research (software development, for example) in which it is more important that universities and public laboratories should remain impartial sources of advice for a whole sector of industry And the ragbag of innovations the universities are likely to produce under the new arrangements for their exploitation will not amount to a new industrial pattern for Britain, however valuable some of them may be. There is nothing wrong with the government's wish that British civil science should be more directly productive (than by the provision of manpower) of prosperity, but the naivety of the assumptions often implied about the nature of major industrial innovation suggest that some enterprising university might profitably design an elementary course in economic history for the British cabinet.

Part of the British government's difficulty in this connection is that it lacks machinery for considering these issues in the round. (The Advisory Committee for Applied Research and Development is a group of part-timers which seems to have gone to sleep.) But there are important issues crying out for remedy beyond the immediate boundaries of the Department of Education and Science. The steady erosion of the research budgets of the other civil ministries (other than the Department of Trade and Industry) suggests the government has abandoned the inconvenient Rothschild principle that all ministries should be competent to pursue research. Is it wise that such a state of affairs should come about by neglect?

The problems at the Ministry of Defence are the opposite: research and development costs not much less than the whole of what the government spends on civil science, and is growing. Yet it is now nearly two years since the Prime Minister and the Secretary of State for Defence promised in public that defence research would be made more accessible to British industry. Ministers are busy people, so that it is understandable that such tasks, full of bureaucratic minefields, should be set aside What puzzles and demoralizes those who work in civil research is the zeal with which change has been ordained in the universities and the research councils

Morale

Morale, not money, is what matters in the end. The dictum that the management of success in research requires the creation of an intellectual climate in which good ideas (and their authors) can prosper is none the less true for being over-used. And although research managers (in universities as well as public laboratories) could have done more to protect creative people from the corrosive insecurity that afflicts them, these same people read the newspapers and learn what government spokesmen have been saying about the value of their work Sir Keith Joseph's own white paper on higher education (see *Nature* 23 May, p 270) is the most striking recent example of the genre By dodging questions whose answers might have resolved uncertainty, by its view of higher education and research as an input/output system and by its repetition of a naive view of where industrial innovation comes from, it can only have made British academic researchers think the time had come to go elsewhere. It seems a pity to hazard the productiveness of a whole community for modest savings at its margin.

The British government's weakness is that few of the changes now decreed, many of them well-intentioned, have been carefully thought through. If the proper calculations had been made, change would have been more deliberate, not necessarily slower. And even at this late stage, much could be done to put things right Making the system more coherent, perhaps by appointing a Minister of Science (part-time) would be the best course Taking careful stock in the hope of learning where the research enterprise is heading would be worthwhile. Replacing the policy of management by cash limits with something more intelligent is essential. And, for the time being, the issue is not one of party politics with the general election in Britain two years or so away, the erosion of morale is too rapid to allow an opposition the conceit that it might be possible to wait until then to earn the kudos of reform, for there may be little left by then. □

The tax man cometh

President Reagan's recent reforms go only part of the way towards fairness.

FORMER President Richard Nixon used to speak of various of his administration's actions as "the greatest (fill in the blank) since the Creation"; President Ronald Reagan may thus be forgiven for calling his tax reform proposal merely "the Second American Revolution"

Amid the hundreds of pages of simplification in Reagan's plan is the retention of the research and development tax credit, a provision that, according to independent economists, has cost the Treasury $1,000 million a year to stimulate $700 million worth of research and development. High technology and biotechnology companies, while grumbling along with other industries about the net 25 per cent increase in corporate taxes embodied in the Reagan plan, are full of praise for the wisdom of keeping the research and development tax credit. Sentiment in Congress is mixed, and since it seems unlikely that the Reagan proposal will be disposed of this year, and since the research and development credit is due to expire this year, it will take a special action by Congress to retain it

The tax credit was passed under a banner of stimulating the United States' supposedly lagging investment in the research and development that tomorrow's products will depend upon. Research and development costs had, of course, always been deductible as an ordinary business expense; the tax credit provides an extra bonus — an outright rebate of taxes equal to 25 per cent of a company's research and development spending that exceeds its average outlay in this area of the preceding three years

Yet, as recent studies have shown, the credit has probably done little to stimulate new research. In fact, the credit has in effect been a reward for those companies that had already decided to invest in research and development To those who know the politics that were at work in passing the enabling legislation four years ago, that is probably no surprise

Of course, the sponsors of the legislation did not come out and say so. But the authors of the legislation will now admit privately that the true purpose of the bill was to equalize what the high-technology companies saw as unfair treatment in the tax code. Heavy manufacturers were reaping a windfall through the investment tax credit (which gives a tax rebate of 6-10 per cent on new purchases of machinery) and accelerated depreciation So lucrative have these provisions been that many leading US manufacturers have paid no taxes for years. The high technology companies, whose products depend more on research and development than machines, did less well Thus was born the research and development tax credit.

If tax simplification is truly the President's goal, then such backhandedness has no place in the tax code. The whole story of special interest politics has been one of obscure provisions masquerading as lofty general principles but in fact carefully crafted to benefit group X (sometimes company X) that gave a nice campaign contribution to the right congressman. And the whole principle of tax simplification is the elimination of special treatment for some in favour of lower rates for all.

The administration is proposing to bite the bullet and eliminate the over-favourable treatment of capital investment: under the Reagan proposal, both the investment credit and accelerated depreciation would be repealed. And a new minimum corporate tax would put a further effective cap on the benefits that the heavy manufacturers can reap from capital investment The cause of simplification and fairness might further have been served by going further with the minimum tax, and eliminating the research and development credit □

US medical research

Hughes tops league of US charities

Washington

THE Hughes Medical Institute became the world's wealthiest charity last week when it sold off Hughes Aircraft Company to General Motors for an estimated $5,000 million. Dr Donald Frederickson, president of the institute and former director of the National Institutes of Health (NIH), said the new endowment should allow the institute to more than double the $100 million it currently spends each year on medical research in genetics, immunology, neurosciences and metabolic control.

The sale also marks a further step in the demystification of the institute, which has been something of an enigma even to the scientists it supports. There is no such thing as applying to the Hughes Institute for a research grant; it only gives money to "affiliate" laboratories, of which there are now eighteen, on medical school campuses around the United States. The institute pays the salaries of the 200 or so scientists who work in these laboratories and leases the buildings from the universities. The most recent campus to join the select ranks is the University of California, San Diego and the Salk Institute. Others include Harvard, Yale, Johns Hopkins and Stanford Universities.

Like its mysterious founder, the institute has tended to operate beyond the public view. It has often been criticized for failing to maintain any open criteria (including peer review) for selecting researchers and projects to support.

That has changed dramatically, however, since Frederickson took charge in 1983, following a court fight in Delaware (where the institute is legally incorporated). In the same year, Delaware's attorney general succeeded in removing most of Hughes's former associates from the board of trustees.

The Hughes Institute's $5,000 million places it far ahead of any other charitable organization or foundation. The Ford Foundation, its closest competitor, has an endowment of $3,500 million The Battelle Memorial Institute, which had previously been the largest independent research institute, has assets of only $250 million.

Frederickson estimates that total public and private support of basic biomedical research totals $1,500-2,000 million in the United States; the Hughes Institute will thus be managing some 10-20 per cent of that total By comparison, the budget of NIH is to be $5,200 million next year, though much of that is for clinical and applied research.

Frederickson expects the eighteen existing affiliate programmes to be expanded, as will a new scheme that he initiated to encourage medical students to consider research careers. Under this scheme, to which the institute has contributed $10 million, students can spend one year working at NIH with senior researchers. The 30 Hughes Research Scholars will live and work in the former Sisters of the Visitation Convent adjacent to the NIH campus; the convent is being renovated with the Hughes Institute funds.

Frederickson said the trustees are in the process of deciding whether the institute should continue business as a "medical research organization" — which, under federal tax laws, means it must conduct the research itself — or instead become a private foundation, which would allow it to make grants and expand its horizons to include medical ethics, scientific communication and science education. Medical research organizations are required by law to spend 3.5 per cent of their assets each year exclusively on "continuous conduct of medical research". For foundations the figure is 5 per cent and they must also pay an excise tax of 1-2 per cent on income. In addition, foundations are subject to restrictions on their business activities.

The sale of the Hughes Aircraft Company has also removed the institute's lingering tax problems. Hughes set up the institute in 1953 as a non-profit organization which, on paper, owned Hughes Aircraft — the piece of his industrial empire that now builds defence electronics but no aircraft. For many years the Internal Revenue Service contended that the institute was being used as a tax shelter by Hughes, noting that very little of the Hughes Aircraft profits actually reached the institute. Before 1976, when Hughes died, the institute had never received more than $4.2 million in any one year from the company. Although it was eventually acknowledged that the institute was legitimately engaged in conducting medical research, other tax problems have remained. **Stephen Budiansky**

Japan in space

Carp to loop the loop?

Tokyo

JAPAN'S first astronaut, who may be a woman, is scheduled to blast off in the US space shuttle in early 1988 with a chicken egg, two carp, some soyabean seeds and 20,000 fruit-fly larvae stored in Japan's section of the Spacelab Module. Not intended for eating, these organisms are vital components in a series of life science experiments designed to shed light on the effects of space travel on man.

Details of the fruit-fly experiment were revealed at a symposium held in Tokyo last week. The fruit fly *Drosophila*, which has been subject to more than its fair share of radiation on Earth, will be used to assess the possible effects on human beings of long-term exposure to space radiation, including X-rays, low-energy protons and neutrons and high-energy cosmic radiation. The larvae will be carried in an incubator aboard the spaceship and returned to Earth once they have grown to become pupae. According to Professor Mitsuo Ikenaga of Kyoto University, exposure to radiation should cause curling and/or multiplication of hairs on the wings of the adult insects, thereby providing information about chromosomal changes induced by the radiation.

In a closely related experiment, soyabean seeds will be cultivated on return to Earth and mutations in the adult plants will be easily detectable from the colour of their leaves. But Taro Fujii, head of the Plant Section of the National Institute of Genetics, fears that the degree of exposure to radiation aboard the shuttle may be insufficient to produce mutations.

In a study of space motion sickness, the behaviour and brain-wave activity of two carp in the weightlessness of space will be examined.

A decade ago aboard Skylab, fish were observed to perform strange looping movements for the first 3-4 days in space which corresponds to the duration of disorientation reported by mission specialists. In Japan's experiment, otoliths, which give the fish its sense of balance, will be removed from one of the carp and the effects of light on the fish monitored — normal carp turn their back toward a light source.

A preliminary experiment has already been carried out by Japan's Institute of Space and Astronautical Science (ISAS) in which one carp and three goldfish in a rocket-shaped capsule were dropped from a balloon at an altitude of 30,000 m. During the 20-second free fall, the carp showed promising behaviour, although the goldfish died during the ascent of the balloon. In another experiment a fertilized chicken egg will be hatched and the effects of weightlessness on the chick's bone cells examined.

The Japanese astronaut responsible for looking after these and other experiments has yet to be selected Of 533 applicants for the job, seven remain after three stages of screening by Japan's National Space Development Agency. These seven, including one woman, Chiaki Naito, will undergo final aptitude trials at the Johnson Space Center in Houston, Texas, beginning on 20 June, and around September three will be selected, of whom one will join the space shuttle flight scheduled for 27 January 1988. **David Swinbanks**

European Community research

The RACE is on at last

Brussels

ALTHOUGH prevented by the Italian delegate from formally ratifying any decisions at their meeting on 4 June, the research ministers of the ten countries of the European Economic Community (EEC) nevertheless made several political decisions. The most tangible is the decision to launch the Community's advanced telecommunications programme, RACE (Research in Advanced Communications in Europe). In addition, there were tentative decisions on an advanced informatics programme, Community participation in the European Synchrotron Radiation Facility (ESRF) and a scheme to provide more access to national facilities.

The Italian blocking tactic came in the form of an insistence by Luigi Granelli, Italian research minister and current president of the Council of Ministers, that any proposal should be dealt with as part of an overall package. By that ploy, Granelli had hoped to prise a formal decision from his nine counterparts finally agreeing to set up the Community's tritium-handling laboratory at Ispra in Italy. A decision was blocked by the United Kingdom and France, both of which have national facilities and object to providing further funds at Community level. It now seems likely, however, that the funds will be found out of the existing budget of the Joint Research Centre at Ispra and that President Bettino Craxi of Italy and President François Mitterrand of France will tie up these and other loose ends at their summit meeting in Florence.

A green light for the definition phase of RACE (see *Nature* 28 March, p.309) was given despite problems in the meeting of the Telecommunications Council on 3 June. This phase will cost six million European Currency Units (ECU) and will last 18 months. It will start in July with the construction of a European reference model for an integrated broad-band telecommunications network that will handle voice, data and video transmissions. As a result of pressure from the United Kingdom and West Germany, the funds proposed by the Commission for the next phase will be reviewed at a later date. The present proposal for the Community to cover half the cost is also to be reconsidered with a view to more industrial participation. On French insistence, the French-chaired European Conference of Post and Telecommunication Authorities will receive a large slice of the definition-phase contract

The ministers took a positive view of the proposed location of ESRF at Grenoble and have asked the European Commission to consider possible participation. This move could help to ease present problems over the siting of ESRF (see *Nature* 14 March, p.125 and 28 March, p.305), although since the facility has been proposed in the context of the European Science Foundation, it does not formally come under the jurisdiction of the European Community But because the problems of the siting of ESRF involve rivalry between member states (Denmark, Italy and France plus West Germany) and because the ministers of the Community long ago agreed to some measure of coordination betwen national policies and a definition of joint interests in science and technology, the research ministers agreed to set up a general information and consultation procedure between the ten member states and the Commission.

This proposal, from the Italian president, seems to have met with general approval, both from the larger countries such as the United Kingdom, whose stakes include the Neutron Spallation Source at the Rutherford Appleton Laboratory, and the small countries that fear being left out of the running for important projects and which want fairer geographical distribution of scientific research sites. West Germany, however, raised the question of just how much access to major research institutes would have to be involved. As a first step, between now and the end of the year, the European Commission is to draw up an inventory of national and intergovernmental scientific laboratories and equipment.

Ministers also considered the Italian-backed Initiative for Research in Informatics applied to Society (IRIS). While the idea has the support of member states, they are not yet prepared to pay for it. Meanwhile, the Commission is to carry out a study of IRIS and to hold a seminar on it in December 1985.

Inevitably the French-backed proposal for concerted research in advanced technologies in Europe (Eureka) also came up for discussion at the research council. But apart from admitting that cooperation will include non-Community countries, officials intend to remain tight-lipped until the Milan summit, at the end of June, where European science and technology will be high on the agenda and at which it is hoped that a series of priorities will be agreed.

Anna Lubinska

US veterans

Leukaemia figures in doubt

Washington

A HIGHER than expected leukaemia mortality rate among military veterans who took part in an atomic test in 1957 seems to be a statistical anomaly, according to a National Academy of Sciences study* that examined mortality rates of veterans who were exposed to similar amounts of radiation in other tests. None of these other groups show a statistically significant increase in leukaemia mortality as compared with the general population.

An earlier study by the Centers for Disease Control had confirmed claims by veterans that an unusually large number of men who took part in the 1957 test code-named Smoky at the Nevada Test Site were dying of leukaemia. But the academy study suggests that chance alone could explain the result.

Approximately 200,000 servicemen participated in atmospheric nuclear tests from 1946 to 1962 at the Nevada site and in the Pacific. The academy study looked at 46,000 who took part in five series of tests in which radiation dosimeter badges were issued to the men.

A review of death certificates and medical records revealed that 10 of the 3,554 men present at the SMOKY test had died of leukaemia by 1982 — 2.5 times more than would have been expected for a similar group in the general population. But the study notes that when leukaemia deaths in the Smiky group are compared with the leukaemia deaths in all five groups, the difference is not statistically significant ($P=0.16$). In fact, the total number of leukaemia deaths (46) in the non-Smoky groups was slightly less than expected (52). Likewise, the number of deaths from all cancers in the Smoky group (67) was less than expected (84). Data from the dosimeter badges implies that there should have been only 0.2 excess leukaemia deaths in the Smoky group. So if chance is not the explanation for the leukaemia deaths, then either the Smoky dosimeter data are several times too low or the generally accepted estimates of radiation-induced leukaemia dose—response curves are wrong.

Although the study notes that it is impossible to prove that chance alone explained the anomaly in the Smoky group, it points out that the more comparisons that are made, the more likely it is that one will seem to be statistically significant by chance. This is illustrated by the analysis of all five groups for eight different kinds of cancer. A small excess of prostate cancers is found in one group; but there is a one-third chance that when eight independent comparisons are made, at least one will produce a *P*-value of 0.5 just by chance.

Veterans' groups have called the report "ridiculous" and complain that it makes no sense to compare the atomic veterans with the general population. Because those selected for military service are healthier than the general population, such comparisons minimize health problems in the veterans **Stephen Budiansky**

**Mortality of Nuclear Weapons Test Participants*, Medical Follow-Up Agency, National Research Council, 2101 Constitution Avenue, Washington DC 20418

Energy

Conservation in vogue

FRENCH nuclear power is increasingly abundant, supplies of natural gas from Siberia and Algeria will soon outstrip demand and the French government has set up a study group to invent new ways of using electricity. So why does France need the Agence Française pour la Maitrise de l'Energie (AFME), which last year spent some FF 2,500 million (£210 million) on supporting energy conservation and renewable energies in community housing, industry and research? Because it pays, a team of financial inspectors has concluded.

The inspectors were provided by finance minister, Pierre Bérégovoy, who is now tearing his hair out over the 1986 (election year) budget, due to go before parliament in November but to the Council of Ministers much sooner. Bérégovoy, who, like most finance ministers, is looking for savings, wondered if an increase in energy taxes could be arrange not only to increase revenue but also to save energy

According to AFME sources who have had a chance to look over the inspectors' work, their conclusion — which is not due to be published — is that the "interventionism" represented by AFME is the best way of achieving energy savings. Moreover, AFME itself is the best way of going about the intervention

AFME director-general M. Bernard Laponche says there has always been "tension" over AFME and its policy of investment aids. The tension arises between the "technical ministries" of industry, energy and research, backed by President Mitterrand himself, and the financial sources. Matters may have been made worse by last year's figures which show French primary energy consumption rising by 2 per cent despite AFME's investments, some 80 per cent of which go towards saving or substituting for oil.

But says the ex-trades unionist president of AFME, M. Michel Rolant, his agency's actions saved 2.2 million tons of oil equivalent (t.o.e.) in 1984 in a total of 191.6 million t.o.e., so things would have been worse without AFME. Moreover, the general export trade improved in France last year because of the cheap franc, so a little AFME juggling shows that the French energy bill went down last year — when measured in terms of months of general exports. In 1984, France worked just two and a half months to pay its energy import bill, the lowest since 1980.

In reality, it is still the cost of oil imports that makes AFME's work politically attractive. In its medium-term programme, AFME aimed to produce energy savings over the decade 1981-90 of 40 million t.o.e., more than a fifth of oil imports, of which some 20 million t o e. were to come from improving home insulation and 10 million from industry; renewables, which contributed 3.4 million t.o.e. in 1981, were planned to bring in 10-14 million t o.e. in 1990, mostly from biomass.

There is still a long way to go to reach those targets (set nearly three years ago) but AFME staff are encouraged by the fact that in 1984, for the first time, energy savings profited the balance of payments by more than AFME aid itself — FF 3,000 million against FF 2,500 million. Nevertheless, AFME aid is rarely 100 per cent, so that actual energy-saving or energy-substituting investments in France last year amounted to some FF 11,000 million. This sum may take a considerable time to pay off.

Robert Walgate

Soviet Union

Phobos mission

THE Soviet Union is planning the first-ever mission to Phobos, the larger and inner moon of Mars, in 1988. The two-probe mission will include a close fly-by of the satellite, at an altitude of 50m, during which a laser gun will be fired to stir up surface dust which will then be captured and analysed.

This will be the first Soviet mission to Mars since 1973. Eighteen months ago, *Aviation Week and Space Technology* suggested that the Soviet Union was planning a mission to Phobos, with an approach to within "a few thousand feet" for 1986.

The actual plans for the mission, at present code-named "Mission-F", seem considerably more sophisticated than the *Aviation Week* speculations, which suggested that the Soviets had abandoned Mars flights because of 14 failures or partial failures out of 15 launches or attempted launches between 1960 and 1973. The Soviets, they said, had realized that they could not compete with the plans for the US orbiter/lander mission. In the opinion of *Aviation Week*, the projected Phobos flight was simply a "poor man's asteroid mission".

Perhaps in answer to such smears, the Soviet space planners are trying to make the Phobos mission as international as possible. Sweden's Kiruna Geophysical Institute has been invited to supply an instrument for measuring the energy and mass of solar wind particles around Mars and Phobos. Poland will supply a device to study plasma-waves, similar to those supplied to the Venus-Halley probes now in flight. Bulgarian and West German scientists are developing a special laser mass spectrometer in conjunction with their Soviet colleagues; the East Germans will supply optical systems for filming and map-making and there are plans for a joint Soviet-French device for studying the chemical composition of the martian atmosphere.

Vera Rich

Chromosome libraries

Ploughshares from swords?

Los Alamos, New Mexico

AN ambitious project to create the first human chromosome-specific gene libraries is expected to have covered the entire genome by the end of the year. The libraries, which are being constructed at Los Alamos and Lawrence Livermore National Laboratories, are already attracting strong commercial and scientific interest. Once the project is complete, the rate of human gene mapping will increase by a factor of ten, according to the project team; batteries of probes for chromosome-specific diseases and chromosome-specific stains are among the other benefits expected.

Twelve commercial companies are among the 500 organizations that have already received chromosome libraries from the National Laboratory Gene Library Project. Investigators who receive libraries (free of charge) are asked to inform the project of whatever characterizations they make. The libraries so far available are made using two different restriction enzymes — *Hin*dIII at Livermore and *Eco*RrI at Los Alamos. The cloned DNA fragments are up to 9 kilobases long. Now it is planned to produce 17-kilobase fragments, which are more likely to contain entire functioning genes and will be of greater use in basic molecular biology. Lawrence Livermore and Los Alamos National Laboratories are primarily weapons laboratories and not the most obvious places to find a cloning project. According to Larry Deaven, principal project investigator at Los Alamos, the laboratories were chosen because of their expertise in chromosome sorting, which arose from studies of the effects of radiation on animal cells

The chromosomes for the libraries are extracted either from human fibroblast cell lines or from hybrid human/hamster cells. After treatment with two fluorescent dyes, different chromosomes are separated automatically under laser light. Chromosomes that are not easily distinguished with two dyes are subjected to a third stain: according to Deaven, the chromosomes that are easy to separate have now all been cloned and "we're left with the hard ones". The efficiency of cloning has improved dramatically since the project started in 1983 whereas several million chromosomes were then needed to start a library, the figure is now down to 100,000.

The library project, at present supported by the Department of Energy with a budget of $400,000 this year, will not always remain at Los Alamos and Livermore As soon as both phases have been successfully completed, stocks will be handed over to the National Institutes of Health, which will also maintain information on the libraries.

Tim Beardsley

UK embryo research

Embryo protection bill resurfaces

IN an unprecedented attempt to use the complex rules of House of Commons procedures, supporters of Mr Enoch Powell's Unborn Children (Protection) Bill failed by only the narrowest of margins to reintroduce it into the legislative timetable last week.

The bill, which would effectively ban all experiments on human embryos, was given a second reading in the House of Commons earlier this year by a large majority of 172 (see *Nature* 313, 618; 1985) But, partly because of lack of official government support, the bill was "talked out" at its third reading on 3 May (see *Nature* 18 April p.573). Normally, a private member's bill would, at this stage, have no chance of further progress, so the government seemed set to proceed sedately with its own comprehensive legislation on reproductive technology, based on the recommendations of the Warnock committee (see *Nature* 312, 389; 1984).

In an extraordinary fight back, however, Mr Andrew Bowden MP agreed to reintroduce Mr Powell's bill by exploiting his position at the head of the queue on private members' motions day, 7 June. He included the motion that the House of Lords should finish its scrutiny of the bill, if passed by the Commons, by 5 July, although such a request cannot be enforced

Opponents of Mr Powell's bill, including the Archbishop of York, re-argued their case in the days leading up to the prosposed debate. Dame Mary Warnock saw a "great tide of moral fundamentalism, sweeping across from America" as a genuine threat to proponents of licensed, controlled research. In fact the question was settled in parliament by procedural means. Mr Dennis Skinner MP found and made use of a rule that a motion to make a writ for a by-election (there is one outstanding) takes precedence over any other business, and ensured that the subsequent debate on the subject took hours rather than the usual few minutes. Only 16 minutes of time remained to debate Mr Powell's bill and this was ruled as inadequate to put the case for a debate, which otherwise might have lasted all weekend

Meanwhile, the Medical Research Council and the Royal College of Obstetricians and Gynaecologists last week pressed ahead with their voluntary licensing authority for *in vitro* fertilization (IVF) and embryology techniques (see *Nature* 4 April, p.397) by publishing the authority's guidelines. These are based on the Warnock committee recommendations but each research programme will be licensed and projects will be considered individually. Programmes involving genetic manipulation or implantation of human embryos in other species will be refused licences. The conditions for receiving a licence include:

- Scientifically sound research on the processes and products of IVF.
- Sound reasons why human, rather than animal, tissue is to be used.
- The aim of research must be clearly defined and relevant to clinical problems.
- Pre-embryos (dividing cells up to determination of the primitive streak) resulting from the research should not be transferred to a uterus except to establish a particular pregnancy.
- Signed consent for the use of ova and sperm to be obtained from both donors.
- Fertilized human ova not to be cultured *in situ* for more than 14 days
- The means of embryo disposal to be specified at the outset.

The voluntary guidelines are particularly appropriate now, as the government has announced that it will not introduce legislation in the next parliamentary session — that is, for at least a year. The popular support for Mr Powell's bill means that the government would be under considerable pressure to include his proposal in any legislation it seeks to introduce.

The tortuous progress of Mr Powell's bill contrasts strikingly with the smooth passage of Mr Norman Fowler's emergency bill to ban commercial surrogacy, which received three readings in the House of Commons between 28 March and 13 May. It is due for a second reading the House of Lords on June 14. The bill bans commercial surrogacy agencies and advertising of and for surrogacy services.

Maxine Clarke

Nuclear winter

Cautious support from SCOPE

INVESTIGATIONS into the environmental consequences of nuclear war by the International Council of Scientific Union's Scientific Committee on Problems of the Environment (SCOPE), whose report will be published in September, essentially confirm the results, and reflect the uncertainties, highlighted by the US National Academy of Sciences (NAS) last year (*Nature* 312, 683; 1985). The likelihood of a nuclear winter — a prolonged period of sub-zero temperatures in mid-northern latitudes following a major exchange of nuclear weapons — is thus given further support. This was the message that emerged at the 16th and final workshop of SCOPE-ENUWAR, held at the University of Essex this week and last week. But SCOPE has taken investigations a step further by considering the possible ecological consequences of the potential climatic effects

Paul Crutzen, of the Max-Planck Institut fur Chemie, a member of the steering committee, emphasized that the SCOPE study differs from its predecessors in that, instead of defining an exact war scenario as a starting point, such as the 6,500 megaton baseline case adopted by NAS, it has considered the destruction of a variety of proportions of the available combustible material and the injection of tonnages of smoke into the atmosphere consistent with a several thousand megaton nuclear war. Taken together with the improved general climatic models which now incorporate, in three dimensions, the interactive feedback between smoke plumes and winds, this should enhance the credibility of suggestions of a nuclear winter, according to S. Schneider of the US National Center for Atmospheric Research.

What has become clear, said Crutzen, is that the proportion of soot produced in urban and forest fires following a massive nuclear attack — the "blackness" of the smoke — is perhaps the most important factor. This in turn is dependent on the type of fires foreseen, with urban and industrial areas being more significant than forest fires. At the end of the day, SCOPE's "baseline case" of an attack on 30 per cent of the combustible material in the developed world produces less smoke than the 6,500 megaton borderline case of NAS, but it is blacker, so the resulting effective optical depth is about the same (4.5 for SCOPE, 5.2 for NAS).

The most important unknowns in this estimate are the nature of the smoke from burning oil stocks, and the physical and chemical processes that could affect particles in individual smoke plumes. Little is known of what happens when an oil pool is set on fire, although experiments with pools up to 50m across suggest that the degree of ventilation is the crucial factor in determining the relative proportions of incompletely burnt hydrocarbons, elemental carbon and gaseous oxides, that is, how dirty the smoke will be.

The NAS and SCOPE studies both highlight the lack of knowledge of processes operating in discrete plumes (up to 50 km across). If mesoscale processes tend to cause particles to agglomerate, this will clearly increase the rate of removal by gravity ("rainout") and may affect the optical properties of smoke plumes. Discussions at the workshop focused on two possibly opposed effects. First, the interaction of the plume with local weather conditions may produce more precipitation than is usually foreseen, which could wash the particles out or cause agglomeration, thereby decreasing the amount of smoke reaching the stratosphere. On the other hand, meso and microscale processes within plumes may increase the lofting of smoke, thus putting more particles into the upper atmosphere.

As Barrie Pittock of CSIRO Atmospheric Research, Australia, stressed, escape of soot into the stratosphere could cause marked climatic effects in the

Southern Hemisphere, especially if the war occurred in July rather than January. In particular, interactive climatic modelling suggests a lowering of tropopause (the boundary between the troposphere and the overlying stratosphere) from its present heights of roughly 9 km in high latitudes and 20 km over the tropics to about 4 km over the North Pole and 10 km in the Southern Hemisphere. This, in turn, would mean that a considerable amount of smoke, perhaps 60 million tonnes, enters the stratosphere where it has two climatic effects. First with an optical thickness of 0.1 to 0.5, the smoke results in concomitant reduction in global insolation of roughly 20 per cent. Second, a July war would cause some smoke to drift into the Southern Hemisphere, with the general consequence of reduced global precipitation. In particular, cooling over the Tibet plateau turns off the southwest monsoon. But it has to be emphasized that there remain significant uncertainties in such calculations because the models do not take account of the potential effects of precipitation.

Although much more research is needed into the implications of these climatic changes for both natural ecosystems and agriculture, the results give cause for concern. The areas likely to suffer the worst effects of nuclear winter, the tundra and boreal forests, are the best equipped to cope, noted T.C. Hutchinson of the University of Toronto, because they are naturally adapted to extremes of climate. But the tropical mangrove and evergreen rain forests have no such protection and are very vulnerable to damage by sub-zero temperatures lasting only a few days or weeks.

The effects on agriculture could be even worse, said M.A. Harwell (of Cornell University). What emerges is that although a July war would have more severe immediate and short-term climatic effects than one fought in January, the precise timing may be important. For example the occurrence of just one or two nights with temperatures below 15°C at a critical period in the growing season could wipe out an entire rice harvest. And perhaps as little as a 3°C fall in temperature would be sufficient almost to eliminate Canadian wheat producion. This loss of production, combined with the likely absence of imports, would be disastrous for most of the surviving world population. For example, on loss of imports alone, even assuming 100 per cent of present production, Japan could only feed half of its present population. And most underdeveloped countries appear to have only about four months supply of stored food. If the climatic effects of a nuclear winter in July lie towards the colder end of the range physicists consider plausible, the ensuing lack of food may have a greater impact on the global population than the immediate effects of the nuclear attacks.

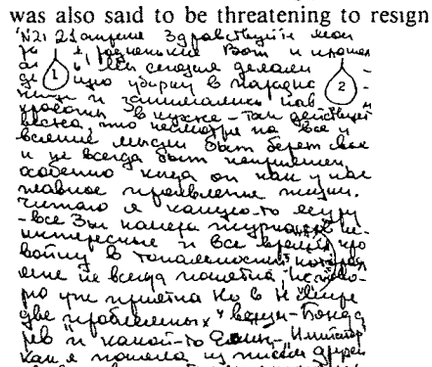
Peter Gambles

Sakharov

Mystery on the cards

RELATIVES of Academician Andrei Sakharov living in the United States are becoming increasingly concerned about his whereabouts and well-being. They know of nobody who has seen either Sakharov or his wife, Elena Bonner, in their Gor'kii exile, since February. During April, they received reports that Sakharov had begun another protest fast, and that he was conveyed to hospital for forced feeding on 21 April. A postcard signed by the couple (see illustration) also apparently dated 21 April, which represents them as commencing their spring-cleaning on that date, now proves to have been tampered with in transmission to conceal an original date of 1 April.

During the past two months, there have been many conflicting reports about the Sakharovs, including rumours that they were to be released to the West under an amnesty to mark the 40th anniversary of the end of the Second World War in Europe, and even that they had already arrived in Zurich. (These rumours may have emanated from Soviet official sources, in order to defuse international tensions before the Ottawa Human Rights "Helsinki Review" meeting.) Sakharov was also said to be threatening to resign



The 1 April or 21 April card

from the Academy of Sciences in protest at the passivity of his fellow academicians regarding his plight and that of his wife, who has been refused permission to travel to the West for eye surgery. The arrival on 25 May of the "21 April" postcard initially led the relatives in Boston to have "serious doubts" about the protest fast, and to be more optimistic.

Closer examination, however, revealed a number of anomalies. Because mail from the Soviet Union frequently fails to arrive, Mrs Bonner numbers all postcards (the couple are apparently not allowed to write letters). The card dated "21 April" was numbered 21, but the family had previously received cards numbered 22 (dated 3 April) and 26 (dated 17 April). The latter took two weeks to arrive, while card number 21 apparently took 34 days. Moreover, on card no 21 Mrs Bonner asked if the family had received the birthday presents that she had sent [marked "4" in illustration] while card 22 (3 April) says: "the day before yesterday we received a card from mother saying that the birthday presents for Sasha and Tanya have arrived".

Furthermore, card 21 contains an odd sentence (with a grammatical error in the verb-form) which seems to imply that the snow had melted (past tense) [3] and the gutters were now running with water (present tense). In card 22 (3 April), Mrs Bonner had reported the snow almost all gone, and according to the *Pravda* weather reports there was no snow in the Gor'kii area during the last ten days of April.

The relatives therefore took the postcard, and the thirteen other cards received from the Sakharovs this year, to Charles Gershin, a certified master graphoanalyst in Massachusetts. Gershin knows no Russian, but the Russian cursive script, unlike printed Cyrillic, is based on the Latin alphabet. Examination under high-power magnification, therefore, made it possible for Gershin to detect interference He found that on card 21:

- A "2" had been inserted before the date [1] which had originally read 1 April.
- The alteration of a single letter in line 2 had changed an original "April has gone" [2] (somewhat premature if written on the 21st) to "April has come".
- Similarly the alteration of one letter in the sentence about the snow changes an original "is melting" to "has melted" (with an unavoidable grammatical error).

The implication, therefore, is that the card number was tampered with in order to give the impression that the Sakharovs were leading their "normal" lives on 21 April, from which the logical conclusion would be that something untoward must have occurred. The unconfirmed reports of Sakharov's alleged fast state that he began it on 16 April, so how would the "tamperers" have had to hand the card mailed on 1 April? One possibility is that, for some reason, the censors had decided to withhold this card anyway, and then, when the story of forced-feeding broke on 15 May, the security authorities quickly looked through their stock of recent cards for anything that could be doctored.

The relatives in Boston are now unwilling to commit themselves on any report about Sakharovs, even whether they are alive or dead. According to Dr Efrem Yankelevich, Mrs Bonner's son-in-law, several friends in Moscow apparently received messages from the Sakharovs on 20 May (the eve of Dr Sakharov's birthday), but these were sent by facsimile, which, he says bitterly, "are even easier to forge than a postcard"

Vera Rich

Velikovsky defended

SIR — Yet another book and book review (*Nature* 25 April, p.692) on Velikovsky! Many people who have been warned off reading him by earlier writings must wonder how long this will go on and why. Are there really people who read Velikovsky, and if so, why would anybody with sense read so poorly recommended an author? Perhaps my experience will provide some indication.

I never dreamed of reading Velikovsky for ten years after his first book, *Worlds in Collision*, because the publisher's ill-considered advertisement suggested it was written in support of religious literalism, and scientific critics, rather rabid sounding but presumably competent, spoke unfavourably of him. I only became curious and started to read him when, as a commuter in need of reading matter, I saw some literary figure quoted to the effect that Velikovsky was much a better writer than his critics. He does indeed read well. His sentences flow, one paragraph leads into another and there is never a moment of boredom at which to break off. The catastrophes are vividly described, the reasons advanced for believing in them interesting.

The reasons may, of course, be quite wrong, but that uncertainty is part of the charm of such reading. If a Velikovskyan catastrophe seems an implausible explanation of the sudden death of the Siberian mammoths, one is at least impelled to wonder again what kind of catastrophe it was. If Velikovsky, in spite of some mildly favourable remarks by Einstein, seems unlikely to have discovered electrostatic forces neglected by the astronomers, one becomes curious to know the basic physics, the degree of unanimity of the astronomers, earlier theories (if any) of electrostatics in astronomy, whether, as Velikovsky claims, the recent space probes tell something relevant and whether Einstein had a habit of encouraging eccentrics.

Critics have made much of Velikovsky's alleged appeal to the ignorant and also to his supposed religious motivation, something never documented and which I do not find in his books. He continues to be read because he appeals to the intellect and the imagination, and to a hunger for good writing.

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Arms talks

SIR — Your leading article (*Nature* 21 March, p.205) about the Geneva arms talks is a sensible argument about strategies for the negotiations. You recommend that expectations not be built too high too soon, and I concur wholeheartedly. These are the most difficult negotiations we have entered into with the Soviets, because there are three subgroups on space and defence, intermediate-range nuclear weapons and strategic weapons.

These negotiations will take years. Interim agreements along the way would be reasonable objectives. Both sides must be willing to pursue confidence-building measures designed to lessen tensions, and to give hope to people living under each flag that a period of lessening fear is possible, even probable. Improving the atmosphere for negotiations by ratifying the two unratified testing treaties might also be helpful. And trying to understand the issues from the other fellow's side of the table is sound advice indeed. To do that, we need to comprehend more about the other fellow, and even more than that, we need to know the fears, hopes and dreams of his countrymen.

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Cuts in wrong place

SIR — I have recently been following the debate over the Natural Environment Research Council (NERC) corporate plan in your pages. I have been interested in the work of some of the NERC institutes, particularly that undertaken by the Institute of Marine Environmental Research (IMER), the Freshwater Biology Association (FBA) and the Institute of Terrestrial Ecology (ITE), all of which are represented in the area in which I am presently but temporarily in residence.

I teach, and have taught, environmental science subjects in colleges and universities in the United States, Canada and New Zealand where I took a similar interest in studies of equivalent organizations. I have found the work of the NERC institutes with which I am familiar to be generally of as high, if not higher, calibre as that in other countries. I was therefore surprised to learn of their present financial predicament. This led me to examine the NERC annual reports which contain the research council's record of diminishing manning levels between 1980 and 1984 (see table). The institutes (plus the British Antarctic Survey, BAS) have suffered a 17.2 per cent drop in manning levels against a 2.6 per cent drop in headquarters (HQ) staff and an actual increase in the NERC Scientific Services (NSS) of 1.5 per cent.

HQ and NSS contribute no commissioned revenue towards the NERC budget whereas the institutes (not including BAS) do offset some of their costs through commissions. As it is also true that NSS is largely administrative in composition, this seems to be a rather good example of the tail wagging the dog. Surely cutbacks should be at least as heavy in the administrative sector (if not greater) as at the business end of the organization? That is certainly a difference between the way things are here in the United Kingdom and in the other countries I have mentioned.

It seems very sad, in light of this, that 1,000 scientific jobs are to disappear when, as seems probable, there should be some slack in administrative staff numbers yet to be taken up.

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NERC staff reductions (from annual report)

	1980-81	1981-82	1982-83	1983-84
HQ	191	191	191	186
NSS*	200	208	190	203
Institutes	2,870	2,750	2,678	2,376

Staff levels (1983-84) as percentage of 1980-81

	% of 1980-81	% Change since 1980-81
HQ	97	−2.6
NSS	101	+1.5
Institutes	83	−17.2

*Excluding ships' crews

Value-free science

SIR — Your correspondent M. Diesendorf (*Nature* 10 January, p.92 and 25 April, p.666) writes as if he believes complexity to be an adequate substitute for validity. If he does so believe he is in plentiful company, but errs nevertheless. Of course LD_{50}s are statistical statements; of course it is sometimes hard to isolate the cause of a particular death; of course juries are sometimes flummoxed. So what? Are "values" supposed to creep in under a sort of smokescreen?

As for the suggestion that the statement "I am going to poison my wife" may be "value-free" because it declares intent, let us recall the wise saying that there are times when one good laugh is worth ten thousand arguments.

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Mass extinctions

SIR — R.C. Hope (*Nature* 18 April, p.574) complains that he has had enough of the wild speculations of recent years about the extinction of the dinosaurs. As a palaeontologist, I heartily agree.

However, Mr Hope thinks that it is the palaeontologists who are to blame. If he examines the credentials of the speculators, he will find that the great majority of them are not palaeontologists. In offering his own speculation (that the mammals destroyed all of the vegetation and ate the eggs of the dinosaurs faster than they could lay them), he falls into the class of people whom he wishes to criticize. No palaeontologist would subscribe to such a simplistic and biologically unrealistic notion.

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Tuning in to neurotransmitters

The slow-acting chemicals that modulate fast synaptic transmission in the nervous system may help to unify the disparate disciplines that make up neurobiology.

FOR too long it has been common to make the analogy between the central nervous system (CNS) and digital computers Both are hard wired, and information is transmitted as digitally coded electrical signals. But in the nervous system communication at the synapses between the neurones is a graded release of chemical neurotransmitters, which have fast, brief effects on very limited targets In the past ten years it has been realized that an enormous number of substances are involved in synaptic communication, subtly modulating the transmission process.

Unlike the fast transmitters, most of the neuromodulators are slow acting, long lasting and often work at long range in very complex ways. A new concept of the nervous system is emerging of hard-wired circuits constantly tuned by chemical signals. This modulation gives the nervous system its flexibility and adaptability to long-term changes, both internal and environmental.

The distinction between the fast transmitters and the neuromodulators was explored on 16–17 May at the symposium that inaugurated the Merck, Sharpe and Dohme Neurosciences Research Centre at Terlings Park, Harlow, United Kingdom. Fast transmitters generally open ion channels in the postsynaptic membrane, thus changing the membrane potential. Onset is rapid and duration brief. As well as acetylcholine at the neuromuscular junction, in the CNS the amino acids glutamate and aspartate seem also to be fast excitatory transmitters, whereas gamma-amino butyric acid and glycine are inhibitory. Modulators, which include the catecholamines noradrenaline, dopamine and serotonin and numerous neuropeptides, act metabolically, like hormones, by activating a chemical second messenger in the postsynaptic cell.

But even this classification is breaking down: it is the postsynaptic receptor that determines the action of a transmitter, not the chemical. Most transmitters have more than one type of receptor; for example acetylcholine has a fast receptor associated with an ion channel and a slow receptor that works through a chemical second messenger. Both can be present on the same cell, so one transmitter can elicit both fast (milliseconds) and slow (seconds or minutes) changes in the membrane potential.

Nor do modulator chemicals all work through the same second messenger. Some alter the activity of adenyl cyclase which in turn changes the level of cyclic AMP; others stimulate the breakdown of phosphatidyl inositides (for review see Berridge, M.J. & Irvine, R.F. *Nature* **312**, 315; 1984). Again the pathway is determined by the receptors and several modulators have two types of receptor, one linked to each pathway.

Receptors for neuromodulators may be on any part of the neurone. Modulators interacting with receptors on presynaptic terminals, seem to modulate transmitter release, possibly by altering calcium flux. Other specific actions of neuromodulators include the suppression or enhancement of various postsynaptic ionic currents, which alter the excitability of the postsynaptic membrane (see *Nature* News and Views, 6 June, p. 454). Some of these effects seem to be mediated by cyclic AMP-dependent activation of protein kinases (S. Waalas, Rockefeller; Nestler, E. & Greengard, P. *Nature* **305**, 583; 1983). Protein phosphorylation could also be involved in the control of synaptic vesicle release; in short-term memory by altering synaptic efficacy; and in regulation of gene expression. The resulting changes in protein synthesis underlie long-term memory, by producing permanent changes at synapses, and in regulating receptor density, which might alter a neurone's sensitivity to transmitters.

Many modulators do not seem to be released at typical synapses but as a cloud that diffuses through the extracellular space to targets that may be over 100 μm away. Here again it seems to be the specific location of receptors that determines which cells respond to such 'parasynaptic' diffuse signals (F.O. Schmitt, Massachusetts Institute of Technology), creating order in place of potential chaos. Unless there is a predictable, structurally defined basis to the CNS, precise responses to such imprecise signals cannot be expected.

Modulators interact not only with fast transmitters but also with each other. Some neuropeptides, such as vasoactive intestinal polypeptide (VIP), work partly by increasing neuronal metabolism and possibly also by increasing local blood flow. Based on evidence for amplification of the action of noradrenaline by VIP, F. Bloom (Scripps, La Jolla) proposed that such interaction could produce very precise spatial control of cortical circuits. The mosaic of VIP-containing cells in the cortex may generate a localized, activity-dependent modulation of the global effects of noradrenaline, so regulating activity in single minicolumns of neurones only 30μm in diameter

Although we are beginning to glimpse the complexity of neuromodulator effects at the cellular level, we know even less about the effect of neuromodulators on behaviour. The most dramatic analysis comes from E. Kravitz and his colleagues (Harvard; Kravitz, E. *et al.* in *Model Neural Networks and Behavior*, ed. Selverston, A. Plenum, New York; 1985) on the control of the reciprocal aggressive and submissive postures in the lobster. In the aggressive posture, all the flexors muscles are contracted; in the submissive, the extensors contract. Both postures can be elicited by injections of monoamine. Serotonin produces the aggressive posture, octopamine the submissive. These modulators act both in the periphery and in the CNS. At the neuromuscular junction both have similar actions producing long-lasting increases in transmitter release and enhancing muscle contractility. In the CNS, however, they have opposing effects on specific groups of motor neurones. The neuronal programmes that result in the two postures are hard wired but the readout of the appropriate programme seems to be facilitated by the particular modulator as it raises or lowers the thresholds of the relevant motor neurones (at least). The cellular mechanisms underlying the serotonin effects in the periphery are already being analysed and the central effects could be pursued to the cellular level. What causes the initial changes in modulator concentrations remains an open question.

It is a serious problem for neurobiological research that the subject is studied on a number of different levels, ranging from the molecular constituents of membranes to whole animal behaviour, and it is difficult to access one level from another. The lobster studies are a good example of the power of neuropharmacology to provide a bridge between these levels. In order to understand how neuronal circuits work, it is now necessary to appreciate the way a chemical messenger can cause short or long-term changes in receptors or channels, which in turn can influence a neurone's excitability, change its metabolism, modify transmitter release or affect the synthesis of new proteins, including receptors.

We are only just beginning to understand these processes, but it seems that the ever-increasing list of neuroactive substances and the complexity of their actions may be the unifying factor so far lacking in neurobiology **Jennifer Altman**

Taxonomy

How many unknown species are yet to be discovered?

from Jared M. Diamond

IN THE nineteenth century, when much of the globe was still biologically unexplored, the discovery of new species was the most exciting and productive area of biology. Today, the study of molecules is the trend, and searches for new species are outmoded. The most vocal searchers, the cryptozoologists, are ridiculed because of their fixation on finding mythical large species — preferably primates, carnivores or long-extinct taxa such as dinosaurs[1].

No one doubts, and few people care, that thousands of small terrestrial invertebrates, cold-blooded vertebrates and plants await description, especially in the tropics. These groups have even yielded major surprises, such as the discovery of the world's largest lizard (the three-metre-long Komodo dragon) in Indonesia in 1912, or of the dawn redwood tree (*Metasequoia*) in China in the 1940s. Nor does anyone question that novelties occur in the abysses of oceans: for example, recent finds of a new family of large-mouthed shark, living crossopterygian fish and a primitive class of molluscs (monoplacophorans) previously thought to have become extinct about 400 million years ago.

Some biologists, undeterred by being unfashionable, continue to scour the Earth for new birds and mammals — but are there any more species to be found? The answer is yes: new birds and mammals turn up every year and occasionally even a species thought extinct is rediscovered. Although not satisfying the cryptozoologists' desire for monsters, these finds have proved important in several branches of biology[1,2]. Tables 1 and 2, assembled from the recent taxonomic literature[3-12], summarize the new finds by region, date and taxon.

Since 1900, 134 accepted new genera of living mammals have been discovered[3-6], mostly in the tropics (Table 1), to yield a current total of around 1,050. From its nineteenth century peak the discovery rate dropped steeply to an asymptote of about 1 genus per year, maintained since 1940 (Table 1). No new genus has been found in the United States since 1916 (the last being the doubtfully distinct bat *Idionycteris*), and none in Europe since 1922 (the vole *Dinaromys* in Yugoslavia).

The 20 mammalian orders are represented unequally in the new finds (Table 2). Eleven orders, all of them poor in species, have contributed no new genera, whereas four (rodents, bats, shrews and marsupials) have produced 90 per cent of the novelties. Re-expressing the results as the ratio of new genera to total number of species to take account of differences in richness of species among orders, these last four orders are still the most abundant, joined by rabbits and whales (new genera/total number of species is > 2.8 per cent). Among the species-rich orders, primates and carnivores have yielded no new genera since 1907 and 1919, respectively. Thus, large terrestrial mammals seem to be almost completely known at the genus level; the recent new species are all to be found among small terrestrial mammals and whales. In fact, no new species of a large terrestrial mammal has turned up since the kouprey, a primitive, rinderpest-resistant species of wild ox, discovered in Indochina in 1937 (ref. 13).

Small does not mean unimportant. Recent finds include two mammals so distinct that they had to be placed in new families. One of these, the bumblebee bat (*Craseonycteris*), found in Thailand in 1974, is the smallest known warm-blooded animal (see figure)[13]. With a weight of only 2 grams and a length of 3 cm, it challenges physiologists to understand how it reconciles small size with heat conservation. The other new family is the dormouse *Selevinia*, found in Kazakhstan deserts, which has the peculiarity of moulting its epidermis as well as its hair[5].

Several genera of supposedly extinct late Pleistocene mammals have suprised zoologists by turning up alive. The Australian mountain pygmy possum (*Burramys*), described in 1896 from fossils 20,000 years old, was caught rummaging behind the garbage pail in the kitchen of Melbourne University's ski lodge in 1966. The 'ice-age peccary' *Catagonus* is now the largest of living peccaries, having been found running in herds through the Paraguayan Chaco[14]. Bulmer's fruit bat *Aproteles* was described from 10,000-year-old fossils, then discovered alive in a New Guinea cave, and finally re-exterminated or driven out by hunters, all within the same year[15].

Among birds only nine new genera (but 134 new species) have been found since 1934 (refs 7-12). As with mammals, the discovery rate plummeted in the first decades of the twentieth century, but has remained steady at around three species per year since 1941 (Table 1). Most of the novelties have come from the neotropics and Africa, with bursts of discoveries occurring when the mountains of New Guinea and the Philippines were explored between 1938 and 1962. In recent years South America, particularly Peru, has been responsible for about half the novelties. Neither Europe nor the United States has yielded a new species in a long time. Disproportionately few of the new species belong to big, conspicuous and wide-ranging orders of birds such as hawks (new species since 1934/total species = 1/287), parrots (1/346), ducks (0/148), charadriiforms (waders and gulls, 0/334) and ciconiiforms (herons and ibis, 0/122). Disproportionately many belong to groups that tend to be cryptic, geographically localized or small, such as owls (4/146), petrels (3/66), tinamous (2/47) and babblers (10/255). Eight of the nine newly described genera are songbirds (passerines) with small geographical ranges.

Although most of the new bird species are similar to previously known species, several are very unexpected in distribution,

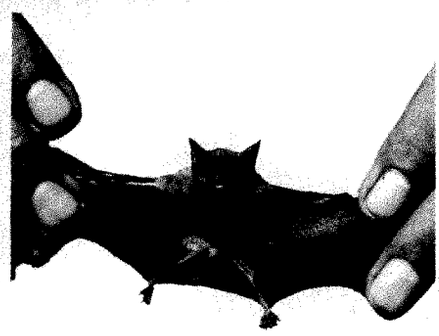
Table 1 New genera of living mammals described from 1900 to 1983 and new species of living birds described from 1934 to 1975

Region	Mammals	Birds
Neotropics	31	56
Africa	27	35
Oriental	20	8
Philippines	7	8
Celebes	6	0
New Guinea	19	9
Australia	4	3
Pacific islands	1	10
Palaearctic	14	2
North America	2	0
Oceanic	3	3
Date		
1900-09	42	
1910-19	23	
1920-29	24	
1930-39	12	
1934-39		27
1940-49	7	27
1950-59	8	32
1960-69	9	31
1970-79	8	
1970-75		17
1980-82	1	

Table 2 Newly described genera of living mammals in each order

Order	New genera/total number of species
Monotremata	0/3
Marsupialia	11/260
Xenartha	0/29
Insectivora	11/391
Scandentia	0/16
Dermoptera	0/2
Chiroptera	26/917
Primates	2/180
Carnivora	2/269
Cetacea	4/78
Sirenia	0/5
Proboscidea	0/2
Perissodactyla	0/18
Hyracoidea	0/7
Tubulidentata	0/1
Artiodactyla	3/185
Pholidota	0/7
Rodentia	72/1,752
Lagomorpha	3/65
Macroscelidea	0/19

The number of new genera are those described between 1900 and 1983, the total number of species are those found up to 1983.



The bumblebee bat *Craseonycteris thonglongyai*, the world's smallest mammal, was discovered in 1974 (photograph courtesy of J. McNeely).

morphology or behaviour. Peacocks were thought to be confined to southeast Asia until the discovery of the African peacock (*Afropavo*) in 1936. The tiny Philippine babbler *Micromacronus*, although in size no rival of the bumblebee bat, is still one of the smallest songbirds. The New Guinea bowerbird *Archboldia* proved to have a uniquely sadomasochistic display: the male crawls prostrate on the bower floor while the female whips her wings over him[16].

In contrast to the case for mammals, there seems to be no bird species described as a fossil and then found alive. But several species thought to be extinct have been rediscovered after a few decades or, in one case (the Bermuda petrel), after three centuries. The most important such rediscovery was of New Zealand's kakapo, the largest and only flightless parrot in the world, and the only flightless bird with a lek mating system[17]. Other notable discoveries were those of the takahe, a flightless grazing gallinule in New Zealand's alpine grassland, and New Guinea's yellow-fronted bowerbird, whose ornate plumage and spartan bower lend support to the transfer theory of bower evolution[18].

In summary, finds of new birds and mammals will please neither cryptozoologists nor those who consider biological exploration an anachronistic waste of time. New species are continuing to turn up at a slow but steady rate, and many areas of biology are being enriched by the discoveries. But, ominously, the rate of extinction of birds and mammals is rising steeply, and will soon overtake the rate of discovery as the destruction of continental tropical rain forests proceeds. Few of the endangered tropical species will have been subjected to much field study before they vanish. Of the very few species thought extinct and subsequently rediscovered, almost all are now endangered. Man seems to find it much easier to bury species alive than to resurrect them permanently from the dead. □

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Mathematics

The power of positive thinking

from Ian Stewart

IN 1900 David Hilbert delivered a famous address to the International Congress of Mathematicians in Paris in which he discussed 23 open problems whose solution he considered to be of outstanding importance. C.N. Delzell has now improved the known results of the 17th of Hilbert's problems: the representation of positive functions as sums of squares. This lies at the heart of the algebraic theory of inequalities. For example, the inequality $x^2 + y^2 + z^2 \geq xy + yz + zx$ follows easily from the observation that $2(x^2 + y^2 + z^2 - xy - yz - zx) = (x-y)^2 + (y-z)^2 + (z-x)^2$. Hilbert's 17th problem effectively asks whether a similar trick is capable of proving any inequality involving only rational functions. A rational function is the quotient $f(x,y,z...)/g(x,y,z,...)$ of two polynomials. If a rational function is positive for all real values of its variables, must it be the sum of a finite number of squares of rational functions? Hilbert could prove it for functions of one or two variables, but not in general.

The first solution of Hilbert's 17th problem in the affirmative was obtained by E. Artin (*Abh. Math. Sem., Hamburg* **5**, 100; 1927). In the 1950s the solution was improved by A. Robinson, H. Henkin and G. Kreisel, who used ideas from model theory (the study of structures satisfying axiomatic conditions) and mathematical logic. Recently, Delzell (*Inventiones Mathematicae* **76**, 365; 1984) has refined the solution further, showing that it can be made continuous and constructive.

To understand Delzell's work, consider the simpler case of the Four Squares Theorem. J.-L. Lagrange proved in 1770 that every positive rational number is a sum of four rational squares. For instance, $5/3 = 1^2 + (2/3)^2 + (1/3)^2 + (1/3)^2$. The proof was constructive, in the sense that it provided a definite process for finding the four squares required; but there was no obvious pattern to the final results. Small changes in the rational number concerned could lead to large changes in the summands. H. Heilbronn, however, proved the startling result that a uniform analytical solution exists (*J. London Math. Soc.* **39**,72; 1964), in the following sense. There are four specific complex-analytical functions p, q, r and s, such that for all complex z the identity

$$z = p^2(z) + q^2(z) + r^2(z) + s^2(z)$$

holds. Further, whenever z is rational and positive, then each of $p(z)$, $q(z)$, $r(z)$ and $s(z)$ is rational. Thus, the same analytical formula provides a representation as a sum of squares for any positive rational z.

It is natural to enquire whether Hilbert's 17th problem has a similar type of solution. Because of the generality in which the problem is currently posed, the notion of an analytical solution must be replaced by that of a continuous solution: basically a fixed sum of squares of continuous functions. In 1982, Delzell produced some negative evidence showing that any representation of fourth-degree polynomials in three variables, as a sum of squares of second-degree polynomials, must be discontinuous.

This negative evidence, however, has a loophole. Nothing in the problem specifies that positive polynomials must be represented as sums of squares of polynomials. Indeed, if the problem were to be stated under this restriction, it would be false. T.S. Motzkin (in *Inequalities* ed. Shisha, O., 205, Academic, New York; 1967) has proved that $1 + x^2y^4 + x^4y^2 - 3x^2y^2$ is always positive, but cannot be written as a sum of squares of polynomials. (But it is a sum of squares of rational functions, thanks to some fortuitous cancellations of terms). And Delzell's current work exploits this loophole to obtain a constructive, continuous solution.

To state Delzell's theorem precisely would involve too much jargon, but its spirit is this: there exist specific continuous functions $p_1, p_2, \ldots$ and $r_1, r_2, \ldots$ such that every positive polynomial f can be written as a finite sum of terms $p_i(c,d,\ldots,x,y,\ldots)\, r_i^2(c,d,\ldots,x,y,\ldots)$. Here, p_i are positive numbers, r_i are rational functions, the terms $c,d,\ldots$ are the coefficients of f and $x,y\ldots$ are its variables. The restriction to polynomial f is made to obtain a simple statement of the theorem. The result holds if the coefficients of f are real numbers or, more generally, lie in a 'real closed field'. The case of a general ordered field of coefficients remains open.

A good mathematical problem doesn't just die, nor does it merely fade away into oblivion, but remains a source of inspiration. Hilbert's 17th problem continues to be a part of living mathematics. □

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Astronomical clocks

Probing the early Universe with the millisecond pulsar

from Bernard Carr

COULD the millisecond pulsar known as PSR1937+21 provide information about processes that occurred within the first millisecond of the Big Bang? That is one of the intriguing questions raised by the results reported by M.M. Davis, J.H. Taylor, J.M. Weisberg and D.C. Backer on page 547 of this issue.

The millisecond pulsar was discovered in 1982 and immediately gained prominence as the pulsar with the shortest known period; according to theory, its signal must be produced by a neutron star rotating a thousand times a second. What promises to make PSR1937+21 such a useful cosmological probe, however, is that its rotation rate remains remarkably constant: apart from a steady and well-understood increase due to its energy emission, the rate has remained stable to one part in 10^{13} over two years. The pulsar thus provides an extraordinarily accurate clock, of relevance to cosmology in that many processes in the early Universe are expected to produce a background of gravitational waves, which should induce a tiny jitter in the clock's regularity.

To demonstrate the stability of the millisecond pulsar required considerable effort. The radio signal of the pulsar was timed initially with a rubidium clock at Arecibo Observatory but a multitude of corrections had to be applied before its regularity could be inferred. The rubidium measurements had first to be checked for any drifts relative to the master clock in Washington and then corrections had to be made for such effects as the relativistic delay induced by the gravitational field of the Sun, the changing Doppler shift associated with the Earth's motion through the Solar System and the dispersion produced by interplanetary and interstellar plasma. Most of these effects can be estimated with high accuracy and, after they were allowed for and a best fit made for the location, period and spin-down rate of the pulsar, the residual noise is only about one μs over the observation period of two years. This is comparable with the accuracy of the best man-made atomic clocks. The residual noise may in part derive from the fact that the corrections mentioned above are imperfect. If so, improved estimates of these corrections could eventually reduce the residual noise to only 0.1 μs. For the present discussion, the most interesting source of noise would be gravitational waves. If the waves have a dimensionless amplitude h and a period P (assumed to be less than the light-travel time to the pulsar), then they will change the arrival time of the pulses by an amount hP, an effect first pointed out by S. Detweiler (*Astrophys. J.* **234**, 1100; 1979). For a continuous background of waves, this will induce a residual of no more than $h\tau$ over a time τ, because the pulsar is most sensitive to waves with $P > \tau$. (The effects of waves with $P > \tau$ would be largely absorbed into the fit to P and its derivative.) If the pulsar clock has an accuracy of Δt, it can be used to look for waves with period τ and amplitude $h = \Delta t/\tau$.

For PSR1937+21, the limiting amplitude is presently down to 10^{-14}, which means that one may already exclude a density of gravitational waves comparable with that in the microwave background ($\Omega_r \simeq 10^{-4}$ when expressed in units of critical density). However, the limiting density scales as $(\Delta t)^2\tau^{-4}$ and so the sensitivity of the millisecond pulsar as a gravitational wave detector is likely to improve dramatically with time. For example, if the timing residual can be reduced to 0.1 μs, and if this stability can be maintained over 10 yr, the pulsar could eventually search for a background of waves with density as low as $10^{-4}\,\Omega_r$.

The sensitivity of the millisecond pulsar is optimized for waves whose period is comparable with the observation time (a few years). Unfortunately, the most prolific sources of gravitational waves at the present epoch (supernovae and black holes) are likely to produce waves with periods much shorter than this. The one exception might be a population of binary black holes; these would produce radiation through quadrupole losses and could, in principle, be detectable if they were sufficiently numerous (J.R. Bond and B.J. Carr, *Mon. Not. R. astr. Soc.* **207**, 585; 1984). However, the most likely source of gravitational radiation in the required waveband would be processes that occurred in the early Universe.

This can be understood as follows. Cosmic processes which occured at a time t after the Big Bang tend to produce radiation with a wavelength of the order of the horizon size at that time (ct); when one allows for redshift effects, this corresponds to a present period of about $(t_0 t)^{1/2} \simeq (t/10^{-4}\,\mathrm{s})^{1/2}$ yr, where $t_0 \simeq 10^{10}$ yr is the age of the present-day Universe. Thus, the millisecond pulsar can be used to look for waves generated at around 10^{-4} s, providing their amplitude is large enough. It turns out that the condition on the amplitude can be satisfied only if the waves are generated later than a time Δt after the Big Bang. The timing residual of PSR1937+21 thus directly determines how far back we can probe the history of the Universe.

The period around 10^{-4} s is rather significant as it corresponds to the time of the cosmological phase transition at which free quarks transform into nucleons. Recently, E. Witten (*Phys. Rev.* D**30**, 272; 1984) has suggested a rather interesting way in which this phase transition could have generated gravitational waves. If the transition is first order and proceeds by the formation of bubbles which expand explosively, one would expect a gravitational wave background to be generated when the bubbles collide. It is not clear whether the amplitude of the waves would be large enough to be detected by the millisecond pulsar, but it is conceivable. Another consequence of this scenario is that the fraction of the Universe outside the bubbles would be compressed into invisible quark 'nuggets', which might be candidates for the dark matter in galactic halos.

Various other effects in the early Universe could produce a gravitational wave background. For example, if the Universe undergoes an inflationary phase (a period of exponential expansion) at some epoch, characterized by a Hubble rate H, then one expects quantum effects to generate waves with density $(H/M_p)^2\Omega_r$ over a wide period range; here M_p is the Planck mass. This effect was first pointed out by A.A. Starobinsky (*JETP Lett.* **30**, 682; 1979). Such an inflationary stage may occur at around 10^{-35} s after the Big Bang, according to the Grand Unified theories, as a result of the Universe getting trapped in a false vacuum state; it may also occur because of quantum gravity effects. In this situation, the waves detectable by the millisecond pulsar would not be generated at 10^{-4} s, but they would fall within the horizon at 10^{-4} s.

Another possibility is that the Universe could undergo spontaneous symmetry breaking at some epoch, resulting in the formation of cosmic strings. These strings can be thought of as topological defects which are trapped in the symmetrical state and, if some of them form closed loops, they should generate gravitational waves through oscillations. The spectrum of the waves expected is rather complicated (C.J. Hogan and M.J. Rees, *Nature* **311**, 109; 1984) but in the period range relevant to the millisecond pulsar it would be flat with a density of the order of $(T/M_p)\Omega_r$, where T is the temperature of the symmetry breaking. It has been proposed that sufficiently large loops could serve as seeds for galaxy formation (A. Vilenkin, *Phys. Rev.* D**24**, 2082; 1982); this suggestion is already close to being tested by using the millisecond pulsar to look for the associated gravity waves. Although there are other ways of detecting gravitational waves, such as laser interferometry, none is sensitive to waves with a period of a year. Therefore, given time, PSR1937+21 could serve as a unique cosmological probe. □

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Experimental petrology

Putting the squeeze on rocks

from E.G. Nisbet

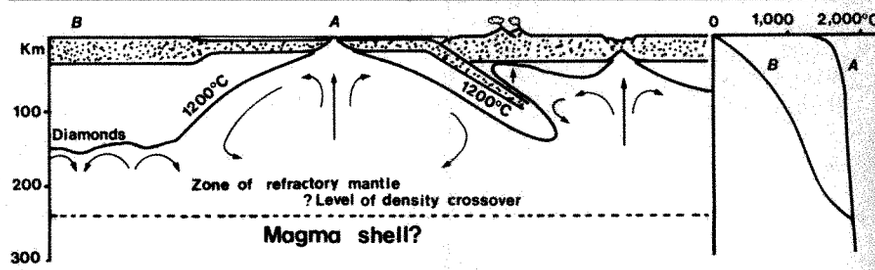
CONTINENTS, oceans and the very air we breathe are the cumulative product of aeons of activity deep in the Earth's mantle. Although there are indirect means of investigation available, it would obviously be advantageous to be able to squash rocks in the laboratory to the high pressures and temperatures to which they are subjected in their natural environment. Unfortunately there are technical problems associated with this high-pressure route to the mantle. This has meant that questions such as the origin of komatiites and the controversial suggestions of an Archaean 'magma ocean' have remained unanswered. But recent improvements, especially in Japan[1], have enabled experimenters to attain pressures of up to 30 GPa (as a rule of thumb 1 GPa is equivalent to 30 km of rock) and simulaneously maintain temperatures well over 2,000°C. On page 566 of this issue, E. Takahashi and C.M. Scarfe[2] report results at pressures up to 14 GPa that have interesting consequences for models of the Archaean mantle and thus for the evolution of the Earth.

One of the most interesting problems is how komatiites originated. These lavas, commonly of Archaean age, are highly magnesian (up to 30 per cent MgO) and thus had high temperatures of eruption (over 1,650°C). Crude closed-system mass-balance calculations would suggest that they represent high degrees of partial melting of mantle; a more likely alternative is that they are the product of early melting in an open system at great depth. This suggests that, since the mantle must have been very hot, it was also fairly refractory. Until recently, most experimental work has been with apparatus capable of reaching pressures of 4-5 GPa (40-50 kbar) and 1,700-1,800°C. Both D.H. Green[3] and M.J. Bickle[4], for instance, have shown that natural komatiite magmas must indeed have been very hot. In addition, there have been various suggestions that at great depth the crystalline phases, such as olivine, are lighter than the melt[5-7]. If such a density crossover existed, it may be that the Archaean mantle contained an 'ocean' of dense komatiitic magma at a pressure of about 8 GPa overlain by a thermal boundary layer of floating olivine crystals[5].

These exotic models have provoked a flurry of experimental work using various techniques. At Lamont-Doherty Geological Observatory, E.M. Agee and D. Walker are carrying out experiments at 5-6 GPa and high temperatures, while at California Institute of Technology, shock measurements give strong circumstantial support to the proposed density crossover[8]. Using the 5,000 ton press at Okayama University, Takahashi and Scarfe[2] have melted peridotite under pressures of up to 14 GPa. Their results show clearly that, as predicted[9], early melts close to the peridotite solidus at 5-7 GPa and roughly 1,800°C are komatiitic with over 30 per cent MgO by weight. Equally nice is the confirmation that the slopes of the solidus and liquidus[5-7,10] are roughly as expected, though there is considerable controversy about the extent to which they converge. At high pressure, solidus and liquidus are probably sub-parallel and not greatly different from adiabat of the liquid[10].

The consequences of all this for our models of the Archaean asthenosphere and lithosphere are considerable. Magma ocean models are strongly supported by circumstantial evidence but are as yet quite unproven; however the results reported by Takahashi and Scarfe place severe constraints on thermal and structural models of the asthenosphere. Not the least of these is the interesting contrast between the high temperatures implied by the experiments with komatiite and the strong evidence from diamonds for much cooler temperatures at depths of approximately 150 km beneath ancient continental nuclei. Diamonds have been produced in some numbers from Witwatersrand gold mines (McIver, personal communication) and are generally found in 'pipes' cutting through ancient continental crust. Inclusions in South African diamonds have been dated at roughly 3,300 Myr[11] (although the intrusive pipes are much younger). This would imply a thick continental lithosphere, at least under part of southern Africa, as far back as the Archaean. Taken together with the stability field for diamond, this in turn requires that part of the mid-Archaean subcontinental mantle must have been at a temperature of 900-1,300°C at 4-5.5 GPa[12]. How can this be reconciled with the indications from Takahashi and Scarfe[2] of 1800°C at 5-7 GPa?

The most likely answer is that, rather than being uniform, the thermal structure of the mantle was strongly regionalized[13], with much hotter conditions under oceanic areas than under continents. It may be that under the early continents the lithosphere slowly thickened as the continent aged and cooled, eventually allowing diamonds to form and become frozen into the lithosphere. In contrast, the lithosphere under the oceans would have been much thinner. Regionalized models of this sort with relatively low sub-continental temperatures (1,200°C at 130 km depth) have previously been proposed[5] to account for variations in the nature of erupted lavas. Perhaps under old (3,800-4,000 Myr) continents, the thermal base of the lithosphere was at about 150 km by the mid-Archaean (~3,000 Myr ago), while under zones of rifting and under oceanic areas the base was much shallower. Komatiitic liquids may have ascended in various tectonic settings, ranging from rifting basins within continents to mid-oceanic ridges. Their generation may have ranged from low degrees of open-system partial melting of refractory asthenosphere at pressures of about 5 GPa, to tapping a putative magma ocean. Away from recently active zones of hot asthenosphere, the sub-continental lithosphere must have slowly cooled and thickened.



Cartoon illustrating aspects of some fo the more richly speculative ideas about Archaean tectonics (see refs 5 — 7, 13, 14). Geotherms for locations *A* and *B*.

What next? The Japanese experiments are on the verge of tackling some of the most interesting problems in petrology. No doubt we shall soon know much more about the high-pressure shape of the solidus and liquidus curves and the density relationships at depth. Perhaps soon we will be rid of the uncomfortable notion[5] that a magma shell existed in the Archaean asthenosphere: if it is disposed of we will be left with a better understanding of the mantle's history. If it survives, komatiites may have much to teach us about the chemical differentiation of the planet and the history of the lithosphere and exosphere. □

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Oncogenic intelligence

Oncogene activation by fusion of chromosomes in leukaemia

from Jerry M. Adams

AMONG the many chromosomal abnormalities associated with human neoplasms, the most frequent is the unusually small Philadelphia chromosome (Ph[1]), named after the city in which it was discovered[1,2]. The Ph[1] chromosome is present within all leukaemic cells in at least 90 per cent of patients with chronic myeloid leukaemia (CML), an invariably fatal disease characterized by insidious proliferation of the white blood cells[2], and results from a translocation (exchange of arms) between chromosomes 9 and 22. It has long been suspected that this event activates a cellular oncogene which then stimulates uncontrolled proliferation of the myeloid stem cell and its progeny. Increasingly over the past three years[3-9], it is the cellular proto-oncogene *abl* (c-*abl*), identified by its relationship to the oncogene (v-*abl*) of the Abelson leukaemia virus of mice, that has been implicated in CML, but how the *abl* gene might be altered by the translocation has remained obscure. Now, reports by Shtivelman *et al.* on page 550 of this issue[10] and by Heisterkamp *et al.* soon to be published in *Nature*[11], establish that the translocation fuses c-*abl* to another gene in such a way that a novel hybrid polypeptide can be made.

As illustrated on the left side of the figure, the Ph[1] chromosome is generated by a reciprocal exchange between chromosome 9, which bears the c-*abl* gene, and chromosome 22. The exchange always occurs at the same bands (q34 on chromosome 9 and q11 on chromosome 22) and yields an extended chromosome 9, 9q[+], and Ph[1], the shortened chromosome 22.

The c-*abl* gene was first associated with CML when the 9;22 translocation was shown to shift *abl* to the Ph[1] chromosome[3]. More importantly, the fusion region between chromosomes 9 and 22 was later cloned from one CML cell line and shown to involve the linkage of a region of chromosome 22 to sequences near the 5′ end of *abl*. The region on chromosome 22 was subsequently denoted *bcr* (for breakpoint cluster region) because all chromosome 22 breakpoints associated with the 9;22 translocation fell within a 5.8-kilobase-pair (kb) segment[5]. Although breakpoints near the c-*abl* gene were rarely detected (for reasons that will become clear below), nevertheless a novel *abl* messenger RNA of about 8 kb was formed in CML cells as well as, or instead of, the normal 6-kb and 7-kb *abl* messengers. It seemed likely that the new mRNA was derived from the Ph[1] chromosome[6,7] and an *abl* polypeptide with an altered amino(N)-terminal segment, presumably translated from the new *abl* messenger, was also identified[8,9].

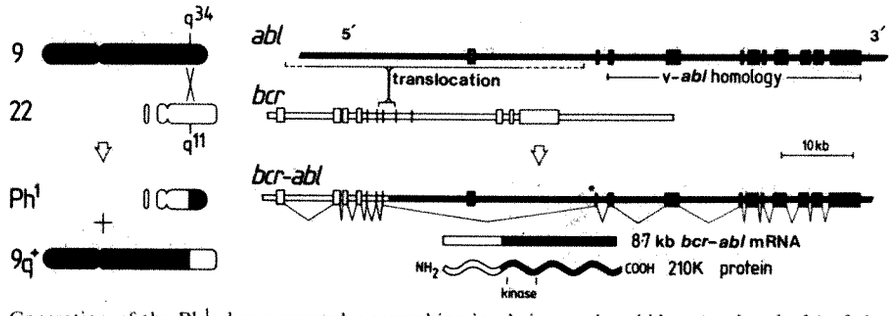
These results raised the intriguing possibility that the translocation fuses c-*abl* to a *bcr* gene, as now clearly established by Shtivelman *et al.*[10] and Heisterkamp *et al.*[11]. The emerging picture of the 9;22 translocation is outlined in the figure. The newly identified *bcr* gene[11] is oriented on chromosome 22 in the same direction as c-*abl* on chromosome 9. The breakpoints on chromosome 22 fall within a 5.8-kb region near the middle of the *bcr* gene, spanning two small exons (coding segments)[11]. On the other hand, the chromosome 9 breakpoints may be scattered over a much wider region, as yet poorly defined but often more than 50 kb 5′ to the c-*abl* gene[4,11].

With such a gap between the *bcr* and *abl* sequences, how can a fused *bcr-abl* messenger RNA, and hence polypeptide, be made? It is assumed that a giant primary transcript is made from the entire *bcr-abl* locus and Shtivelman *et al.* plausibly suggest that, by means of the subsequent RNA splicing, the last *bcr* exon before the fusion point always becomes joined to the second *abl* exon (marked with an asterisk in the figure) in the messenger RNA. In this scheme, the first *abl* exon (hatched) will be lost during RNA splicing because it lacks the 'splice acceptor' signal required for splicing to the *bcr* exon. Consequently, in each CML patient there will be a novel mRNA of about 8.7 kb that includes precisely the same *abl* mRNA segment, despite the large variation in chromosome 9 breakpoints. In support of this model, cells from two CML patients with different chromosome 9 breakpoints generate *bcr-abl* mRNAs in which the *abl* segment begins at precisely the same nucleotide[10].

Cells from all CML patients so far examined contain an *abl* polypeptide of relative molecular mass of approximately 210,000 (210K), whereas normal cells contain only a 145K *abl* polypeptide[8,9]; the N-terminal segment of the normal *abl* polypeptide, probably comprising at least 25 amino-acid residues, has been replaced by some 600-700 residues from the N-terminus of the *bcr* product. Since all the *bcr* breakpoints seem to fall after the second or third of several very small *bcr* exons, the predicted *bcr-abl* polypeptides may differ only by the presence or absence of the third small *bcr* exon, which encodes 24 amino-acid residues[11]. It is intriguing to note that the v-*abl* polypeptide encoded by the Abelson virus is also an N-terminal substitution of c-*abl*; 236 amino acids of the viral *gag* polypeptide replace about 113 residues of the normal cellular *abl* product. Hence N-terminal substitution may be critical to convert c-*abl* to a true oncogene.

How the *bcr-abl* gene may promote myeloid cell proliferation is unclear, but one interesting functional difference between it and c-*abl* has already emerged. In common with the v-*abl* gene product, the 210K polypeptide from CML cells has tyrosine kinase activity; that is, it catalyses the transfer of a phosphate from ATP to tyrosine residues in certain proteins[8,9]. At least as assessed by autophosphorylation, the product of normal c-*abl* lacks kinase activity[8,9], although it will probably be found when the appropriate substrates are identified, since c-*abl* bears a tyrosine kinase domain. Thus, it seems likely that the substrate specificity of c-*abl* is altered by the replacement of its N-terminus by *bcr* sequences. Perhaps that modification also explains why the *bcr-abl* gene is most effective in transforming myeloid cells whereas v-*abl* preferentially transforms early B lymphoid cells[12].

Receptors for several growth factors, including insulin and epidermal growth factor, are tyrosine kinases, so it is possible that the normal c-*abl* product is a com-



Generation of the Ph[1] chromosome by recombination between the *abl* locus at band q34 of the chromosome 9 and the *bcr* locus at q11 of chromosome 22. The reciprocal 9;22 translocation is depicted on the left and the corresponding gene structures on the right, with boxes and lines denoting the approximate position of large and small exons, respectively. In the *abl* locus, the position and size of the 5′ exons is not yet well fixed, nor have the breakpoints been located in most instances, although most seem to be a considerable distance 5′ to the *abl* gene. For *bcr*, not all exons have yet been defined but all breakpoints fall within the bracketed region. In the *bcr-abl* locus, a nuclear precursor RNA (thin lines) is presumed to be spliced in such a way that sequences of the most 5′ *abl* exon (hatched) are excised during generation of the illustrated 8.7 kb hybrid *bcr-abl* mRNA, which almost certainly encodes the 210K polypeptide that contains the *abl* tyrosine kinase domain at the indicated region. The molecular counterpart of the 9q[+] chromosome is not shown.

ponent of a growth factor receptor. On the Ph[1] chromosome, the expression of the fused *bcr-abl* gene is presumably regulated by the control elements of the *bcr* gene. Since this chromosomal abnormality features prominently only in CML, which is a neoplastic proliferation of haematopoietic stem cells, it is tempting to speculate that the normal *bcr* gene is expressed primarily in that cell lineage. If the *bcr* product proves to be a cell-surface protein, one intriguing possibility would be that *bcr-abl* represents a hybrid receptor.

While the function of *bcr-abl* is a matter of speculation, there now seems little reason to doubt that its formation by the 9;22 translocation is a critical step in the development of CML. Thus the work on the Ph[1] chromosome, when considered together with the large body of work on *myc* oncogene translocations in lymphoid tumours, makes a strong case that many of the karyotypic alterations found in neoplastic cells represent the activation of specific cellular oncogenes. □

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Neurobiology

Orchestration at the synapse

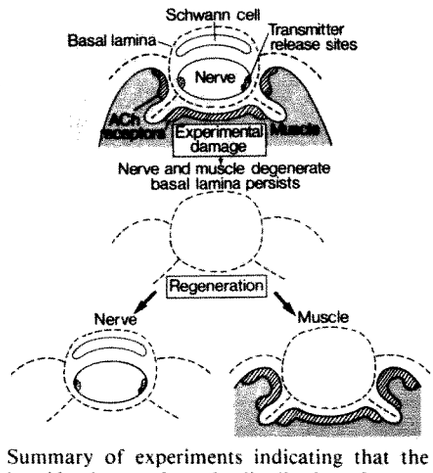
from Clarke R. Slater

CHEMICAL transmission between neurones or from neurone to muscle takes place at junctions termed synapses. Structural specializations at these junctions ensure that receptor proteins in the post-synaptic membrane are aligned opposite the sites in the presynaptic membrane where transmitter is released. How this alignment comes about and is maintained is important for understanding the hardware of the nervous system and its development. Recent work from U.J. McMahan and his colleagues on the neuromuscular junction has shown that the basal lamina is important for determining the distribution of synaptic components on both sides of the synapse[1,2]. In two papers starting on page 571 of this issue, McMahan *et al.* now show that the synaptic basal lamina contains a component chemically very similar to one that causes clustering of molecules that are essential for the functions of the neuromuscular junction[3,4].

The basal lamina is an extracellular layer of material composed largely of a form of the durable connective tissue protein, collagen. A basal lamina sheath surrounds each skeletal muscle fibre and each motor nerve fibre. At the neuromuscular junction, nerve and muscle share a common layer of basal lamina, which has been known for some time to differ chemically from other basal laminae in that it contains a high concentration of acetylcholinesterase (AChE), the enzyme that breaks down acetylcholine (ACh), the transmitter chemical at the neuromuscular junction.

The idea that the basal lamina might influence the distribution of other synaptic components emerged from a series of experiments in which the cells of the neuromuscular junction were destroyed and then either the nerve or the muscle cell was allowed to grow back within the framework provided by the persisting basal lamina sheaths (see figure). When the regenerating nerve made contact with the original synaptic basal lamina, detectable by its persisting AChE activity, it stopped growing and formed the structural features characterizing transmitter release sites[1]. When the muscle cell made contact with the synaptic basal lamina, a high density of receptors for ACh formed in its surface membrane at the site of contact[2]. As no cellular components of the original neuromuscular junction persisted to induce the local specialization of the regenerating cells, it seems that this information could only have come from the surviving basal lamina.

Since those experiments were reported, several groups have been trying to identify molecules that might mediate the effect of synaptic basal lamina on the distribution of ACh receptors. One approach has led to the isolation of a variety of tissue extracts that cause the aggregation of ACh receptors on cultured muscle cells. In other studies, antibodies that specifically label synaptic basal lamina have been raised and used to isolate and characterize the antigens. McMahan *et al.* have effectively combined these two approaches. Starting with a preparation of basal lamina from the electric organ — essentially a stack of giant neuromuscular junctions — of the fish *Torpedo*, they obtained a soluble extract that increased the clustering of ACh receptors on cultured chick muscle cells when present at a concentration of not more than 10^{-10} M. An antiserum against this extract bound specifically to the synaptic basal lamina of frog neuromuscular junction and blocked the ability of the extract to induce clusters of ACH receptors[5].



Summary of experiments indicating that the basal lamina regulates the distribution of synaptic components (adapted from ref.2).

In the first of their present papers, McMahan *et al.* describe monoclonal antibodies, raised against the same extract, that have effects similar to those of their original antiserum[3]. In the second paper[4], they report that the extract causes the formation of clusters of AChE activity, as well as clusters of ACh receptors. It is very likely that both clusters occur at the same sites. Because of the specificity and homogeneity of monoclonal antibodies, it can be concluded that a component of the basal lamina at the neuromuscular junction is closely related to, if not identical with, molecules that can induce the clustering of the two most essential molecular components of the synaptic surface of skeletal muscle cells. By studying the effect of these antibodies on the developing neuromuscular junction, it should be possible to define the role that the basal lamina plays in synapse formation.

The antibodies will also be useful in exploring whether the cluster-inducing molecules are present in the central nervous system and how they are produced during synapse formation. Recently, Kelly and his colleagues described a glycoprotein component of the basal lamina of the electric organ[6]. This molecule is also present in the spinal cord of *Torpedo*, where it is located specifically in the electromotor neurones and is transported in their axons to the periphery. This raises the possibility that, during development, growing motor nerves add components to the basal lamina of the muscle fibre that cause aggregation of the molecules constituting an effective synapse. Experimental tests of such hypotheses should soon be possible. □

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Palaeontology

Cosesaurus — the last proavian?

from Andrew R. Milner

SYSTEMATIC palaeontology sometimes seems to resemble a scene from Dante's Purgatory. As fast as some palaeontologists tidy fossils into manageable groups, others describe new specimens which cannot possibly fit into any orthodox framework. Some supposedly bizarre or highly significant fossils do maintain their status on investigation but it has been, and still is, the fate of many an oddity to prove on revision to be more mundane than its original author(s) believed. One such fossil, *Cosesaurus aviceps*, now seems to have joined the ranks of the demoted.

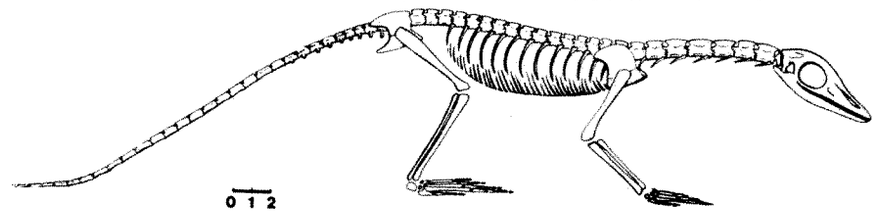
Cosesaurus was first described a decade ago as a proavian from the Middle Triassic of Spain[1]. It is a small fossil reptile, 14 cm long, represented only by a single negative impression of a skeleton having a large-eyed skull with a relatively large brain case and beak-like jaws, a lizard-like body with a long tail and hindlimbs slightly longer than the forelimbs. It was originally described as possessing a furcula (the 'wishbone', or fused clavicles), a bird-like pelvis and traces of feather impressions; this evidence, coupled with the bird-like skull, led one of its discoverers to propose it as a closer relative of true birds than *Archaeopteryx*[2]. *Cosesaurus* was even used to support the hypothesis of a Palaeozoic origin for the birds, quite distinct from dinosaurs or even archosaurs[3]. It seemed in many respects to be the much-sought arboreal 'proavis' rendered tangible at last. But *Cosesaurus* never gained many adherents as a protobird and, in recent years, the relationship of *Archaeopteryx* and the birds to the dinosaurs has been thoroughly supported by cladistic analyses[4,5]. This has left *Cosesaurus* in an increasingly anomalous position.

A recent reassessment by Sanz and López-Martínez seems to have found a more modest position for *Cosesaurus* in the phylogenetic framework of the sauropsid reptiles[6]. They dismiss the supposed furcula and feathers as overinterpretation of a poor specimen, and argue that the remaining suite of derived characters support a position within the Prolacertiformes, a group of Permo-Triassic diapsid reptiles, once thought to be early lepidosaurs, but more recently argued to be primitive relatives of the archosaurs[7,8]. Sanz and López-Martínez also note *Cosesaurus's* specific resemblance to the much larger *Macrocnemus*, a prolacertiform from the contemporary and ecologically equivalent Monte San Giorgio in Switzerland site (see figure). They suggest that other characteristics, including the superficially bird-like head, are juvenile: if *Cosesaurus* is related to *Macrocnemus*, it must be either a hatchling or a dwarf species, the former being more likely in the absence of other evidence.

This identification agrees well with the presence of the nothosaur genera *Lariosaurus* and *Nothosaurus* in the same beds at both localities and implies that the Spanish site has simply produced a small sample of the Monte San Giorgio-Muschelkalk vertebrate association — a typical Middle Triassic Tethys coastal fauna. The general resemblance between the skull of *Cosesaurus*, a juvenile archosauromorph, and that of a bird is an interesting reminder of how progenetic dwarfing may have reshaped the typical archosaur skull to result in the avian skull.

Another strange reptile recently and more cautiously described from the Upper Triassic of Italy[9] is less likely to suffer such a reduction in status. *Megalancosaurus preonensis* is also tiny and has a superficially bird-like head, long cervical vertebrae, a very long forelimb with five short digits, each bearing a deep blade-like claw, slender ribs and an archosaur-type pelvis. Only a preliminary description has yet been published and only a tentative position within the thecodont-grade archosaurs has been proposed.

Some recent analyses of archosaur relationships place the pterosaurs and dinosaurs close together within the archosaurs[10] and, judging by such characteristics as are visible, *Megalancosaurus* seems to belong close to, or within, the pterosaur-dinosaur clade. Its small size, peculiar claws and slender forelimbs suggested to its describers that it was arboreal and, indeed, its forelimb is distinctly reminiscent of that of the Colugo or flying lemur, the patagial gliding mammal of south-east Asian forests. Could it be that this fossil represents a conservative patagial gliding relative of the pterosaurs?



Reconstruction of *Macrocnemus bassanii* from two specimens from the Monte San Giorgio site, Switzerland, studied by B. Pryer. The limb bones are shown in their longest view, without an anatomical perspective. Scale in cm. Drawing by N. López-Martínez (from ref. 6).

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Geochemistry

Uses for synthetic fluid inclusions in quartz crystals

from Edwin Roedder

FLUID inclusions are small droplets of the fluids from which crystals grew and are found in many minerals, both natural and synthetic. In natural gem minerals, fluid inclusions diminish their clarity, and hence their value; in synthetic crystals they detract from the useful mechanical, electrical or optical properties for which the crystals are grown. Why then should anyone want purposely to form microscopic inclusions of fluid in otherwise optically clear quartz crystals? Yet S.M. Sterner and R.J. Bodnar[1] have recently developed a procedure to do just that, and have shown that the resulting inclusions are valuable in a surprisingly wide range of geochemical and mineralogical studies that may help us to understand better the natural processes that may have taken place millions of years ago.

Sterner and Bodnar start with solid cylinders of clear quartz, cut from a fluid-inclusion-free natural crystal. These are heated and then cracked by quenching in water. The cracked but still intact cylinders are dried and sealed into thin-walled noble-metal capsules with the starting materials appropriate to generate the desired fluid composition under the experimental conditions. The collapsible capsules are then subjected to external pressures of 0.5–7 kbar, while being heated to 200–850°C, for periods of between 3 hours and 87 days, before being quenched.

During the time at elevated temperature and pressure, the fractures in the quartz crystal are healed by dissolution and recrystallization of the crack surfaces, thereby decreasing the total surface energy

(see Fig.1). In so doing, small portions of the capsule fluid are trapped within the new growth — samples of the fluid, sealed in single crystal quartz 'bottles'. Thus, these new synthetic inclusions are analogous to the 'secondary' inclusions found in most natural quartz crystals (Fig.2). (Secondary inclusions are those that are formed by the healing of fractures in a pre-existing crystal; the fractures are generally caused by external stress.) Possible applications include the study of inclusion-trapping mechanisms and rates, post-trapping changes and even the determination of phase equilibria and pressure, volume and temperature data on otherwise experimentally very difficult high-temperature, high-pressure hydrothermal systems.

The full range of uses for these synthetic fluid inclusions has yet to be explored but applications already discussed by Sterner and Bodnar[1] have some fascinating ramifications. Fluid inclusions, both natural and synthetic, undergo various phase changes on cooling after becoming enclosed in their crystal host. Henry Clifton Sorby (the 'father of fluid inclusions') showed in a study 127 years ago[2] that these phase changes could be observed and reversed in the laboratory while heating the host crystal under the microscope. The most common phase change observed occurs because liquids shrink at a much higher rate than crystals. Thus, if a hot liquid is sealed into a relatively rigid, fixed-volume crystal 'bottle' and then cooled, differential shrinkage of the liquid relative to the crystal will cause the formation of a vapour bubble in the liquid. Sorby observed the point when this bubble disappeared in a crystal on a microscope heating stage and used it as a measure of the temperature at which the cavity had originally been sealed, perhaps millions or billions of years earlier. Each tiny inclusion (and ordinary white quartz may contain 10^9 inclusions per cm^3) is, in effect, a built-in thermometer, recording the temperature of a past event.

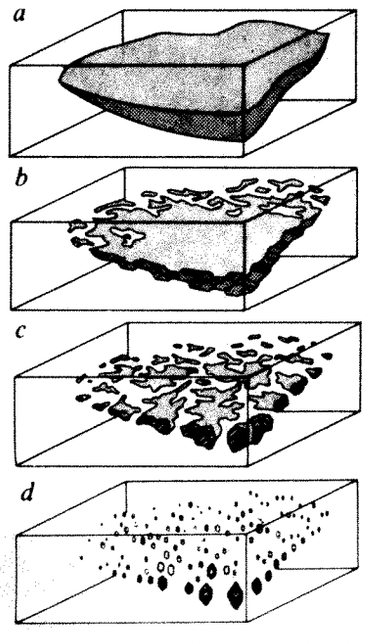


Fig. 1. Stages in the healing or 'necking down' of a crack in a quartz crystal resulting in secondary negative crystal inclusions (from ref.3).

Sorby's proposal to use fluid inclusions in this manner — only one of his many brilliant ideas — was, and still is, the subject of considerable discussion. In part this debate arose because the geothermometric data from fluid inclusions contradicted some of the preconceived notions of the day, and in part becaue of the difficulty in assuring that the geological conditions under which an inclusion formed actually fulfilled the several relatively simple but necessary assumptions behind the method. Previous studies of fluid inclusions accidentally trapped in synthetic industrial quartz crystals, grown under approximately known conditions, have verified the general validity of the concept of inclusion geothermometry. Several minor problems have, however, interfered with the widespread use of the method, not the least of these being, surprisingly, that of accurately calibrating the microscope heating stages[3]. The most common application of synthetic inclusions made with the new method may be as a device for calibrating instruments.

In the years since Sorby's work, fluid inclusions have provided much geological information in addition to temperature. Measurements of phase changes visible under the microscope at temperatures ranging from roughly −190°C upwards, and with appropriate conditions and caveats, can provide data on the density, pressure and the major solute and solvent compositions of the fluids at the time of trapping. Most of these data simply cannot be obtained from any other source. In the search for metallic ore deposits, it would obviously be desirable to understand the chemical processes that resulted in transport of the ore metal to the site of the ore body, and its precipitation there. This requires an understanding of the chemistry of the ore-forming fluid; with a few rare exceptions, the only samples we have of these fluids are in microscopic fluid inclusions.

The past few decades have seen a tremendous increase in studies of the composition of such inclusions. Many of these have involved the use of new methods of ultramicrochemical analysis, such as laser-activated micro-Raman spectroscopy and laser-ionized mass spectrometry. Such methods have attempted to overcome the difficult problems of extraction and chemical (or isotope) analysis of major and trace constituents of these inclusions, without serious loss or contamination. In addition to the exceedingly small total sample size (frequently less than 10^{-9}g) usually available, the problem of finding suitable analytical standards has been particularly limiting. The new synthetic inclusion technique developed by Sterner and Bodnar permits the preparation of optimum standards for both microscopy and chemical analysis — inclusions of the same size range, enclosed in the same host mineral and containing fluids of known density and composition.

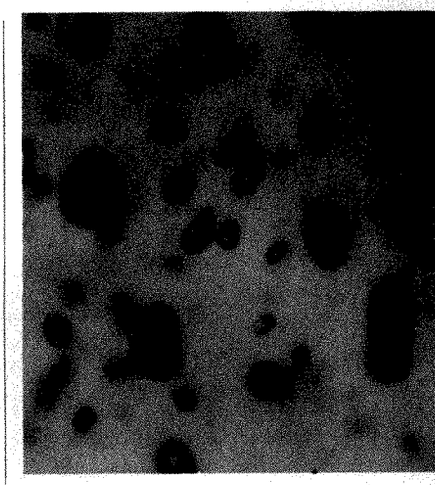


Fig. 2. Synthetic fluid inclusions in quartz. Those with small bubbles (of CH_4) initially trapped liquid and are now dominated by a carbonate; those with large bubbles trapped vapour and are largely CO_2: ×156 (photomicrograph courtesy of S.M. Sterner and R.J. Bodnar).

Still another application, with a particularly exciting potential, is the use of these synthetic inclusions in studies of the physical chemistry of otherwise difficult chemical systems. N.P. Ermakov[4] pointed out 35 years ago that fluid inclusions in transparent minerals are unique miniature 'visual autoclaves', permitting visual observations of high-temperature and high-pressure phase assemblages and critical phenomena. Bodnar and Sterner[5], have explored the use of synthetic inclusions in pressure-volume-temperature studies of hydrothermal systems and Bodnar *et al.*[6] have gone on to show how valuable these synthetic inclusions can be in the determination of the phase-equilibrium properties of the system H_2O-NaCl to 1,000°C and 1,500 bar. Such data are necessary for an understanding of several important groups of ore deposits that have formed from hot, extremely saline fluids and are also useful in understanding the mechanism and energetics of volcanic eruptions such as that at Mount St Helens in May 1980. Conventional experimentation is very difficult because these fluids are exceedingly corrosive. The use of even a few trial runs with synthetic inclusions to locate the critical fluid parameters could reduce the effort involved in larger-scale experiments, or the inclusion technique itself could even be used for the entire phase-equilibrium study. □

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Oncogenes homologous to steroid receptors?

SIR — Why do tumours that initially respond to hormone therapy lose their hormone responsiveness after a time and then grow autonomously? This problem is of great clinical importance, for instance in breast cancer where tumours that diminish in size after endocrine treatment almost invariably recur and then tend to progressively become nonresponsive. The discovery that many breast cancers consist of subclones of oestrogen receptor (ER)-positive and ER-negative cells[1,2] suggests that tumour progression is the survival of the 'fittest' and most malignant ER-negative subtype. But where do the hormone-independent cells come from in the first place? Are they always present or are they derived from hormone-dependent cells? And how do these manage to survive and proliferate if they do not have the oestrogen receptor to give the proliferation signal?

A general opinion is that the cells become hormone-independent because they have *lost* the receptor. But such cells should be unable to respond to their growth stimulus and so be eliminated from the tumour population. A more plausible alternative is that they have undergone a change that leads to their getting a growth signal even in the absence of ER. Steroid receptors interact with nuclear DNA at enhancer regions that regulate the transcriptional activity of structural genes[3-6]. Perhaps a mutation in the enhancer leads to its activation of genes without the need for receptor to bind to it. However, it is unlikely that the loss of ER and a mutation in the enhancer region would occur simultaneously. We suggest another explanation, namely the appearance of aberrant receptor-like molecules that bind to DNA and give a proliferation signal even in the absence of oestrogen. Unlike traditional ligand-binding studies, the recently developed monoclonal antibodies to steroid receptors have made it possible to seek aberrant receptors which fail to bind steroids in hormone-independent cells. Thus immunoreactive glucocorticoid receptors have been detected in receptor deficient (r^-), nuclear-transfer deficient (nt^-) and nuclear-transfer increased (nt^i) mouse lymphoma variant cells[7], and this has been confirmed by the finding that mRNA encoded by the receptor gene in these variants is changed quantitatively and qualitatively[8]. In r^- cells, one allele is altered so that no product is detectable with either steroid or antibody, while the other allele produces an immunoreactive polypeptide devoid of hormone-binding activity[7]. In nt^- and nt^i lymphoma cell variants, the glucocorticoid receptors react abnormally with DNA[9,10].

What are the structural changes associated with this abnormal functioning of receptors? In nt^i cells part of the receptor domain is missing which binds neither steroid nor DNA, but which may modulate nuclear association and DNA binding[7,11]. The complexes of this aberrant receptor plus glucocorticoid might still bind to the biologically relevant sites on DNA, but without this leading to transcriptional regulation[7]. Thus, the available evidence suggests that the loss of steroid responsiveness of some tumour cells may be due to alterations in the steroid receptor gene(s) leading to altered receptor molecules which may act in a 'deregulated' manner and induce cellular proliferation even in the absence of hormone. The mutated (possibly truncated) receptors may or may not retain the hormone binding properties. A biological precedent for this possibility has been described: the v-*erb-B* oncogene product is homologous to the non-hormone-binding domain of the epidermal growth factor (EGF) receptor and cells expressing the gene do not require EGF for continued growth[12]. This raises the question: are there oncogenes that are homologous to parts of the steroid receptor genes and confer loss of hormone dependence upon cells that contain them? If so, is this because the aberrant receptors produced by these oncogenes have the ability to bind to DNA without the aid of steroid hormones, and so give a continuous growth signal to the cell? In this area of research progress is rapid, so we should soon know the answer to these important questions.

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The infectious nature of prions

SIR — In a recent discussion of prions, Colin Masters emphasized that a protein-only composition of prions requires protein-directed protein synthesis for its replication, "a situation which could be without precedent in biological science"[1]. While protein-directed protein synthesis may be without precedent, protein-directed protein-folding has a precedent and could explain the infectious nature of prions.

Geneticists are well aware of this as a mechanism of gene effect in a number of dominantly inherited diseases. For instance, it is possible for an abnormal collagen to cause a severe phenotypic abnormality[2], while a non-expressing mutant for collagen is recessive[3].

This is most easily interpreted if the mutant collagen gene directs the synthesis of abnormal collagen chains which affect structure in a negative way through mixed trimers with the normal collagen chain which are conformationally abnormal. One can also see the effects of the conformation of one protein chain on another in haemoglobins (for instance, the increased affinity of a normal alpha-chain for oxygen secondary to a mutant beta-chain such as occurs with haemoglobin Ypsilanti[4]).

These protein interaction examples involve inherited genes, but the interaction is not transmitted, that is the original information is in the genome but the negative effects of an altered protein conformation are limited to the zygote. However, it is possible for such altered conformational information to be transmitted from generation to generation. The best example I know of this is that of reversed cortical pattern in *Paramecium*. Beisson and Sonneborn showed some years ago that a reversed patch of cortex could be transmitted to descendant *Paramecium* through its usual binary fission[5]. Thus, the cortex grows by the addition of macromolecules to a pre-existing cortex, receiving positional information from the pre-existing cortex. When the positional information is altered in some way, in this case by microsurgery, the abnormal information can be transmitted.

It is conceivable that the prion could be a normal protein in an abnormal configuration which, upon transmission to a new host, leads the normally synthesized protein to start folding in the new abnormal configuration, thus multiplying the number of prions in the abnormal conformation. In order to account for the increase in the number of prions, one must assume that the prion is not permanently affixed to the aggregate of 'new' and 'old' prions and that the new configuration of this normally-present protein is highly stable. Of course, such a hypothesis does not help us understand how prions cross cell membranes and the other aspects of infectivity which must be taken into account to explain their transmission between individuals.

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High-precision timing observations of the millisecond pulsar PSR1937+21

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Long-term studies of pulse arrival times for the pulsar PSR1937+21 yield estimates of the pulsar's period, spin-down rate and celestial coordinates with unprecedented accuracies. Over periods exceeding six months, this pulsar is at least comparable in stability to the best man-made atomic clocks. Our results place a limit on the energy density of low-frequency gravitational waves in the Universe.

SHORTLY after the discovery[1] of PSR1937+21, we began a series of high-precision measurements of the arrival times of its pulses, observations being made at intervals ranging from daily to once every several weeks since November 1982. Data collected in the first two weeks were sufficient to determine the period derivative of the pulsar[2]. Here, we present an analysis of a much longer span of data obtained between 28 November 1982 and 12 October 1984. The results confirm our earlier measurements of the pulsar position, period and spin-down rate, improving their accuracies by several orders of magnitude.

The microwave beam that produces the pulses from PSR1937+21 has a constant angular width of ~15° and is rigidly attached to a spinning neutron star with rotational kinetic energy of almost 10^{52} erg. The measured rate of energy loss, presumably resulting from low-frequency electromagnetic radiation and particle acceleration in the pulsar magnetosphere, corresponds to a rotational quality factor, $Q > 3 \times 10^{19}$. After a constant slow-down rate has been removed from the timing data, we find that the measured fractional stability of the pulsar is better than one part in 10^{13} over timescales of ≥ 6 months. This remarkable long-term stability is equal to or exceeds that of the best existing atomic clocks.

There are many potentially interesting applications of a series of regular comparisons between a distant, natural standard of dynamical time and Earth-bound atomic clocks. For example, we already can place cosmologically interesting limits on a stochastic background of gravitational radiation in the Universe, by extending to higher precision the methods used recently with slower pulsars[3,4]. In addition, we can use the data to achieve sub-milliarc second astrometric accuracies relative to a coordinate system based on the motion of planets in the Solar System. Consequences of the astrometric results of this experiment will be published elsewhere[5]. Over the next few years there is good reason to expect that timing data on PSR1937+21 will help to elucidate knowledge of the Earth's motion in the Solar System, provide substantially tighter limits on the energy density of low-frequency gravitational radiation in the Universe and possibly even provide a useful new long-term standard of dynamical time.

Arrival time observations

Our observations have been made at 1,408 MHz, using the Arecibo 305-m telescope and the Princeton pulsar-timing equipment[6] developed for observations of the binary pulsar PSR1913+16. Incoming signals with both senses of circular polarization are amplified, converted to 30 MHz and introduced to a $2 \times 32 \times 0.25$ MHz filter-bank receiver. The detected outputs from the two orthogonally polarized channels at the same frequency are added, and the 32 total-power sums are sampled at ~25-μs intervals and progressively summed in a digital delay line serving as a de-disperser[7]. Output from the de-disperser is converted back to analogue form and fed to a signal averager which samples the signal every 1/64 of a period and produces an integrated pulse profile for each 2 min of observation. The sampling is accurately synchronized to the pulsar period and corrected for Doppler shifts induced by the Earth's motion. Near the middle of each 2-min integration the time corresponding to the sample stored in the first 'bin' of the accumulating waveform is automatically recorded; at the end of each integration, the timing information is saved on magnetic tape together with the observed pulsar waveform.

Approximately 60 integrations can be recorded during the time the pulsar remains in view on a given day. After the observations are complete, the phase of each 2-min integrated profile is determined by cross-correlation with a 'standard profile' formed by averaging a large number of profiles. To reduce the volume of data, we then average the observed phases in groups of 15 consecutive integrations. The mean phase is converted to seconds and added to the time recorded for the first bin at the midpoint of the eighth integration, thereby yielding an effective pulse arrival time for each 30 min of data.

Our basic observational data now consist of 196 30-min observations of pulse arrival time at the Arecibo telescope, measured in a locally kept approximation to coordinated universal time (UT). During these observations, time at Arecibo Observatory was maintained by a clock that counts the cycles of a 5-MHz signal from a Hewlett-Packard model 5641 rubidium clock. The offset between this clock and UT (kept by the United States Naval Observatory, USNO) is measured once a day by the use of Loran-C timing signals broadcast from Jupiter, Florida. (The free-running rubidium clock typically accumulates phase drifts of a few tenths of a microsecond per day.) We apply retroactive corrections to epochs of the Loran-C signals, as published by USNO, to remove drifts of the master clock in Florida relative to the more accurate master clock kept by USNO in Washington. In addition, epoch comparisons with other time standards are made occasionally by means of very long baseline interferometry observations[8] and by occasional checks with travelling clocks from USNO. We believe that our corrected timescale at Arecibo Observatory has been consistent with UT (USNO) to within 1 μs at most times.

Data analysis

Our goal in analysing the data of this experiment is to compare the pulsar 'clock' with a uniform standard of dynamical time. To this end, we convert our measured times to UT (USNO) and then to International Atomic Time (TAI) by adding a constant offset plus appropriate leap seconds. The topocentric TAI arrival times are converted to times at the Solar System barycentre by applying geometry based on interpolations of an accurate ephemeris of the Earth's motion. We also adjust the arrival times to correct for (1) dispersive delays in interstellar and

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interplanetary space; (2) relativistic propagation delay caused by the pulsar signal moving through the gravitational field of the Sun; (3) gravitational redshift in the rates of terrestrial clocks caused by the changing gravitational potential at Earth; and (4) varying time dilation caused by changes in the Earth's velocity. Our procedure is basically that described by Manchester and Taylor[9] and used routinely for slower pulsars[10,11]. However, the precision desired in the present experiment requires an improved model for the conversion of proper time at Washington, TAI (USNO), to coordinate time at the Solar System barycentre, or barycentric dynamical time (TDB).

Corrections applied to the observed pulse arrival times are summarized in the equation

$$t_b = t_{obs} + (\mathbf{r}\cdot\hat{\mathbf{n}})/c + \Delta t_r - \Delta t_g - D/f^2 \quad (1)$$

where t_b is the infinite-frequency coordinate time of pulse arrival at the Solar System barycentre, t_{obs} is the measured TAI of pulse arrival at the observatory, $\mathbf{r}$ is the vector displacement of the telescope from the barycentre, $\hat{\mathbf{n}}$ is a unit vector pointing from the barycentre to the pulsar, c is the speed of light, Δt_r is the relativistic correction from proper time (TAI) to coordinate time (TDB), Δt_g is the gravitational propagation delay, D is the dispersion constant and f the observing frequency, transformed to the barycentric reference frame. Note that for the convenience of terrestrial observers, our unit of time, even for quantities measured in the barycentric frame, is the A.1 second as measured by a fictitious clock moving in a circular orbit 1 AU from the Sun. (This timescale is not presently defined by any international agreement. A precise definition must also specify an interval over which the gravitational influences of the planets have been averaged, for the proper times-to-coordinate times tabulated in the PEP740-R ephemeris (J. F. Chandler *et al.*, personal communication), this interval was JED 2430000-2451545.)

In practice, we obtain the required values of $\mathbf{r}$ and Δt_r by interpolation of the Center for Astrophysics PEP740-R ephemeris, using 10-point interpolation formulae. Calculation of Δt_r by numerical integration of the planetary ephemerides is necessary to achieve the desired accuracy of 0.1 μs. (The closed-form approximation derived by Moyer[12] seems to contain errors of a few μs.) After applying all known corrections of ≥ 0.1 μs amplitude to obtain values of t_{obs} in TAI units, and then calculating t_b with the aid of equation (1), we obtain a series of barycentric arrival times t_i, $i = 0, 1, \ldots, 195$, where we have dropped the original subscript on t_b. The expected pulsar phase (in turns) corresponding to the ith arrival time is given by

$$\phi(t_i) = \phi_0 + \nu_0(t_i - t_0) + \tfrac{1}{2}\dot{\nu}(t_i - t_0)^2 \quad (2)$$

where ϕ_0, ν_0 and ν are the pulsar phase, frequency and frequency derivative, respectively at time t_0. The arrival time 'residual' (in s) is then given by

$$R(t_i) = \frac{\phi(t_i) - n(t_i)}{\nu_0} \quad (3)$$

where $n(t_i)$ is the integer closest to $\phi(t_i)$. Using standard procedures we minimize the sum-of-the-squares of all residuals, solving for corrections to ϕ_0, ν_0, $\dot{\nu}$ and the two position and two proper motion terms implicit in the unit vector $\hat{\mathbf{n}}$ in equation (1) The results of such a fit are shown in Table 1, where by convention we list the pulsar period and derivative, instead of frequency and derivative. Post-fit residuals from the solution are plotted in Fig. 1.

It is clear from Fig. 1 that the residuals are dominated by systematic effects, rather than by random measurement errors. For example, residuals from data obtained in a single day tend to be highly correlated and weaker correlations over timescales of several months are also evident. Possible causes of systematic errors that we cannot yet eliminate at the ~1 μs level include clock errors (perhaps related to the transfer of time from Washington to Arecibo), imperfect removal of dispersion from the scintillating pulsar signal, ephemeris inaccuracies and small changes in propagation time caused by interstellar gas clouds drifting across the line-of-sight[13]. Since October 1984 we have been making observations designed to identify and reduce these possible sources of error. (Time is now transferred to Arecibo Observatory by signals from satellites in the Global Positioning System, with accuracies in the tens-of-nanoseconds range. In addition, we have built a new multichannel signal averager that exactly follows the inverse frequency-squared dispersion law.)

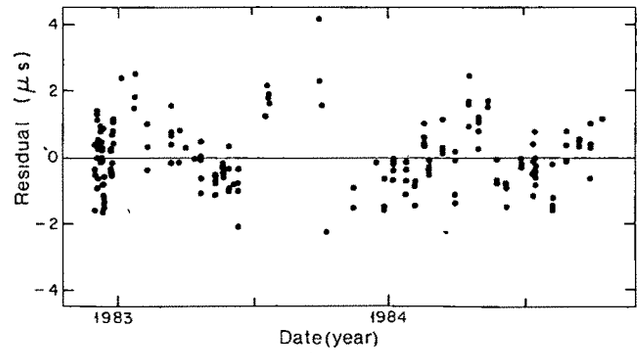


Fig. 1 Post-fit timing residuals for PSR1937+21, after a solution for period, period derivative, position and proper motion. Each circle represents a 30-min observation; the r.m.s. residual is 0.9 μs.

Table 1 Measured parameters of PSR1937+21

Period	$P = 0.0015578064488724 \pm 2$ s
Period derivative	$\dot{P} = (1.05110 \pm 0.00008) \times 10^{-19}$ s s^{-1}
Right ascension (1950.0)	$\alpha = 19$ h 37 min 28.74600 s ± 0.00003 s
Declination (1950.0)	$\delta = 21$ h 28 min 01.4606 s ± 0.0010 s
Proper motion in α	$\mu_\alpha = -0.3 \pm 0.4$ marc s yr^{-1}
Proper motion in δ	$\mu_\delta = -1.4 \pm 1.0$ marc s yr^{-1}
Epoch (at barycentre)	$t_0 = 2445303.2731658$ JED
Assumed dispersion	$D = 2.94788 \times 10^{17}$ Hz

Quoted positions are in the coordinate system of ephemeris PEP740-R.

For the 1982–84 data, however, we must conclude that the root-mean-square residual, ~0.9 μs, is dominated not by instabilities in the pulsar or by receiver noise but by systematic (possibly instrumental) effects. For this reason, we have adopted a conservative attitude toward the formal standard deviations of the parameter estimates. The uncertainties quoted in Table 1 are four times the formal standard errors from the least-squares fit, and are approximately equal to those which would be obtained if we had available one measurement of ~2 μs accuracy for each observing day. We hope to be able to relax this cautious procedure in the near future and to improve further the accuracies of the fitted parameters.

Pulsar parameters

The pulsar period and derivative listed in Table 1 are consistent with, but much more accurate than, our previously published values[2]. They confirm that the characteristic age of this pulsar is rather large, $P/2\dot{P} \approx 2 \times 10^8$ yr, and that if the slow-down torque is provided by magnetic dipole radiation, the surface magnetic field must be unusually small, $3.2 \times 10^{19} (P\dot{P})^{1/2} \approx 5 \times 10^8\, G$ (where G is the Newtonian constant) (see, for example, Chapter 9 of ref. 9). Attempts to solve for a second derivative of period yield only an upper limit, $|\ddot{P}| < 10^{-29}$ s^{-1}. This limit is not surprising because the value expected for magnetic dipole braking is $\ddot{P} = -\dot{P}^2/P \approx -7 \times 10^{-36}$ s^{-1}.

The measurement of the pulsar position in the sky described here is also consistent with previous work, but the precision of our new result is considerably higher. The uncertainties quoted in Table 1 are relative to the coordinate system implicitly defined by the PEP740-R ephemeris. The orientation of the ephemeris coordinate system relative to, say, the FK4 system, is *a priori* uncertain by ~0.2″ (J. F. Chandler *et al.*, personal communication). Recently, it has been shown that the reference frame of the PEP311 ephemeris, which is in close alignment with PEP740-R, is offset in the direction of PSR1937+21 by ~0.2″ with respect to both the Jet Propulsion Laboratory planetary ephemerides

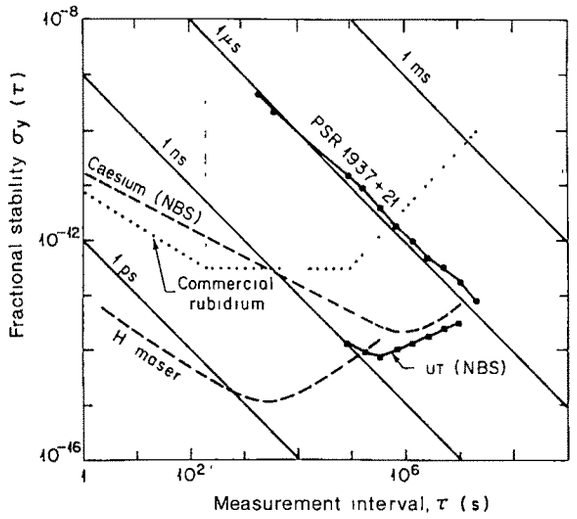


Fig. 2 Measured frequency stability $\sigma_y(\tau)$, for PSR1937+21, over intervals, τ, from 30 min to 256 days. For comparison we also plot stability curves representative of several types of atomic clocks. The curve labelled 'rubidium' refers to typical commercially available standards; those labelled 'caesium (NBS)' and UT(NBS) are based on Fig 3 of ref. 28 and the 'H maser' curve is adapted from Fig. 5 of ref. 29

and the FK4 system[14,15]. These astrometric details are of considerable interest, and will be discussed elsewhere[5].

Until we have removed all ~1-μs systematic errors from the data and acquired a better understanding of possible ephemeris errors at the same level, our solution for proper motion should be treated as an upper limit of $|\mu| \leq 3$ marc s yr^{-1}. At a distance[16] of 5 kpc, this limit corresponds to a transverse velocity ≤ 70 km s^{-1}, which is puzzling as differential galactic rotation is expected to create an apparent transverse velocity of ~100 km s^{-1}. It is possible, of course, that the pulsar's peculiar velocity happens to cancel a large portion of the transverse component of differential galactic rotation.

It is reasonable to enquire about the magnitudes of effects not included in our timing model which might influence the pulse arrival times significantly and bias some of the parameter estimates. In Table 2 we list several such possibilities, in addition to the effects that are explicitly included in the model. Our model will need further improvements if the data are to be improved by another order of magnitude.

We are aware of several other effects that may contribute significantly to the measured pulse arrival times, such as secular changes in the Doppler shifts caused by differential galactic rotation and possible changes in G. However, these effects will be stable over very long timescales, so their effect is simply to bias our estimate of the pulsar spindown rate by ~1% without contributing significantly to the post-fit arrival-time residuals.

Stability of the pulsar clock

The predictability of man-made clocks is customarily measured in terms of the fractional frequency stability $\sigma_y(\tau)$ measured over various time intervals τ. The square of $\sigma_y(\tau)$, termed the 'Allan variance,' is defined by

$$\sigma_y^2(\tau) = \frac{1}{2}\left\langle \left(\frac{R(t+\tau) - 2R(t) + R(t-\tau)}{\tau} \right)^2 \right\rangle \quad (4)$$

where $R(t)$ is the post-fit residual at time t and the angled brackets signify an average taken over all available triplets of residuals spaced by $\pm\tau$. We have computed Allan variances from the post-fit residuals to the PSR1937+21 timing data, and the results are plotted in Fig. 2. For comparison we have also plotted curves that correspond to the stabilities of typical rubidium and caesium clocks and hydrogen masers and for the bank of caesium clocks maintained by the United States National Bureau of Standards. We note that although $\dot{P}/P$ for PSR1937+21 and other fast pulsars far exceeds the fractional drift rates of atomic clocks[17], the physical process that produces $\dot{P}$ is sufficiently well understood to predict that higher-order derivatives will be much smaller for pulsars than for atomic clocks. Thus, the timescale at which the stability curve for PSR1937+21 turns upward will probably be much longer than that for the man-made clocks.

Figure 2 makes it clear that over timescales of the order of one year, radioastronomical observations of PSR1937+21 can provide a standard of time that is competitive with any other available standard. Obviously the practical attractiveness of this new basis for barycentric dynamical time will be greatly enhanced if several more millisecond pulsars are found and their timing behaviours monitored and compared.

Table 2 Contributions to observed pulse-arrival times

Cause	Model magnitude (μs)	Estimated error (μs)
Pulsar		
Spin	$3\times10^{13}\,t$	—
Spindown	$3\times10^{4}\,t^2$	—
Timing noise	—	$0.01\,t^{3/2}$
Propagation at 1.4 GHz		
Interstellar medium	1.5×10^{5}	$0.5\,t$
Interplanetary medium	0.4	<0.4
Ionosphere	—	<0.04
Troposphere	—	<0.01
Clocks and corrections		
UT(AO)-UT(JUP)	1	0.5
UT(JUP)-UT(USNO)	1	0.2
UT(USNO)-TAI(USNO)	50	0.0
Errors in TAI(USNG)	—	$0.3\,t$
Reduction to barycentre		
Observatory to centre of Earth	2×10^{4}	0.01
Earth to barycentre	4×10^{8}	$0.6\,t$
TAI-TDB	1,600	0.1
Gravitational propagation delay	20	<0.1
Parallax of pulsar	—	0.06

For each of the named contributions to pulse arrival time, the left-hand column gives an indication of the magnitude of the effect currently included in the model and the right-hand column gives our estimate of the errors caused by having ignored it or having modelled it imperfectly. Times, t, are measured in years. AO stands for Arecibo Observatory and JUP for the Loran station at Jupiter, Florida.

Gravitational radiation

According to some cosmological models, a substantial fraction of the energy density, ρ_c, required to close the Universe might be expected in the form of very low-frequency gravitational radiation[18-21]. Specific mechanisms that have been proposed include the transition of the Universe from a de Sitter inflationary phase to a radiation-dominated era[22], oscillating cosmic strings[23,24], a quantum chromodynamic phase transition in the early Universe[25] and other exotica. Clearly, the eventual measurement of a cosmic background of gravitational radiation and the determination of its spectrum could play an important role in understanding the early Universe.

At some level, the apparent stability of any distant clock sending its 'ticks' to Earth by electromagnetic signals must be limited by perturbations in the spacetime metric between the clock and Earth. This fact is the basis for using pulsar timing data or spacecraft-tracking data to place limits on the energy density of a cosmic background of gravitational radiation[26]. The basic argument is that a plane gravitational wave of dimensionless amplitude h and period τ carries energy density

$$\rho = \frac{1}{8\pi G}\left(\frac{2\pi}{\tau}\right)^2 h^2 \quad (5)$$

Such a wave would produce pulsar timing perturbations of size $\delta t \approx h\tau$ which, if large enough, would dominate the residuals in the timing data. The longest period τ that will contribute significantly to the residuals is roughly half the time span of the observations. Thus, our two years of timing data with r.m.s. residual $\delta t = 0.9\ \mu s$ would seem to place an upper limit of $h \lesssim \delta t/\tau = 3\times10^{-14}$, or $\rho \lesssim 2\times10^{-35}\ g\ cm^{-3}$, for a stochastic background of gravitational waves of period ~1 year. This limit is substantially below the cosmological critical density $\rho_c = 3H_0^2/(8\pi G) = 2\times10^{-29}\ g\ cm^{-3}$ for a Hubble constant $H_0 = 100\ km\ s^{-1}\ Mpc^{-1}$.

A more rigorous treatment of the limits that can be placed on a cosmic background of gravitational radiation from pulsar-timing data has been given by Blandford *et al.*[27]. Their method makes quantitative allowance for the fraction of timing noise power that is absorbed into fits for the parameters of Table 1, and more accurately assesses the varying sensitivity of the method to gravitational waves of different frequencies. Their result can be summarized in the equation

$$\Omega_g(f) = 7.4\times10^{-5}\,(s-1)\,(\beta/N)^{(s-1)}\,H_{100}^2\,\delta t_{\mu s}^2 \qquad (6)$$

where $\Omega_g(f)$ is the ratio of gravitational wave energy density (per natural logarithm frequency interval at frequency $f \approx \beta/N$) to the cosmological critical density ρ_c, s is the power law spectral slope expected for the timing residuals due to gravitational waves, N is the length of the data span in years, β is a parameter characterizing the effective low-frequency cutoff in the sensitivity to gravitational waves, H_{100} is the Hubble constant in units of $100\ km\ s^{-1}\ Mpc^{-1}$ and $\delta t_{\mu s}$ is the post-fit r.m.s. timing residual in microseconds. For cosmologically interesting mechanisms of generating gravitational waves, s is expected to lie in the range 5–7, and for $N = 2$ Blandford *et al.*[27] find that $\beta = 2.05$. Thus, for $H_{100} = 1$ we can now place the very firm limit $\Omega_g < 5\times10^{-4}$ for a stochastic background of gravitational waves in the frequency range ~1–3 cycles $year^{-1}$. This limit is not yet sufficient to constrain models which use primordial cosmic strings as an aid to galaxy formation[24]. However, the limit on Ω_g at the lowest accessible frequencies varies approximately as $\delta t_{\mu s}^2/N^4$, so that if: (1) the apparent pulsar stability remains high; (2) instrumental improvements can reduce the contributions of random noise and systematic errors to δt; and (3) the duration of observations N can be significantly extended, another two or even three orders of magnitude are potentially within reach.

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Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukaemia

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Human chronic myelogenous leukaemia is characterized by a reciprocal translocation between chromosomes 9 and 22 resulting in an abbreviated form of chromosome 22 and the transfer of the abl *cellular oncogene from chromosome 9 into the* bcr *gene of chromosome 22. Characterization of an 8-kilobase RNA specific to chronic myelogenous leukaemia shows it to be a fused transcript of the two genes. The fused protein that would be produced is probably involved in the malignant process.*

CHRONIC myelogenous leukaemia (CML) is characterized by the proliferation and accumulation of myeloid cells and their precursors[1] and is clinically divided into two phases. The initial chronic phase lasts for three to four years and the primary abnormality is a marked increase in the stem-cell compartment committed to myelopoiesis. This is followed by the acute phase (blast crisis) of 3–6 months' duration and characterized by a decrease in the differentiation ability of the cells and the loss of response to regulatory factors. In some patients there is a transitional accelerated phase.

Most (90–95%) CML patients carry in their myeloid cells a unique chromosomal aberration—a shortened chromosome 22 termed the Philadelphia (Ph^1) chromosome[2–6]. The origin of Ph^1 was identified as a reciprocal translocation between chromosomes 22 and 9, referred to as t(9; 22) (ref. 7). The localization of the cellular oncogenes *abl* and *sis* close to or at the breakpoints on chromosomes 9 and 22, respectively[8–10], and the finding that the oncogenes are included in the exchanged segments of these chromosomes[11,12] suggest that one or both are involved in development of CML. Subsequent studies failed to detect *sis* transcription in CML[13], indicating that this oncogene does not play a role in the disease.

Mapping of the breakpoints on chromosome 9 indicated variable distances 5′ (upstream) of the first known exon of *abl*. In one individual the breakpoint was 14 kilobases (kb) 5′ of the oncogene, and in others it occurred further upstream and has

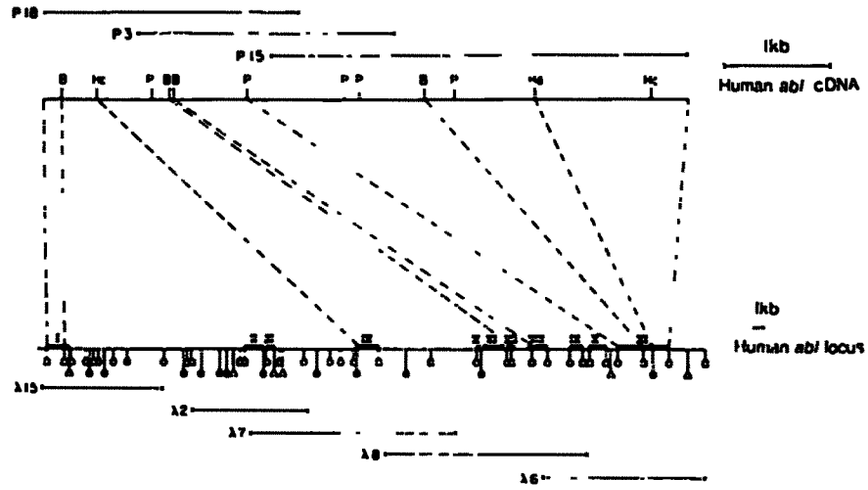


Fig. 1 Physical maps of normal human *abl* cDNA and locus. Restriction enzymes sites on cDNA are of *Bgl*II (B), *Hin*cII (Hc), *Pst*I (P), *Hin*dIII (Hd) Sites on locus are *Bam*HI (Δ), *Bgl*II (□), *Hin*dIII (●), *Eco*RI (○) Exons within the genomic DNA were determined by hybridization of radiolabelled cDNA fragments to fragments of the cloned genomic DNA Exons are depicted as dark boxes delineated between restriction sites bordering the hybridizable fragments With the exception of exon XI the size of the boxes does not reflect the length of the corresponding exons
Methods. Analysis of cDNA was performed on the three overlapping phage clones P18, P3 and P15. A cDNA library was constructed[29] with poly(A) RNA isolated from the SV80 cell line[28] An 8-μg sample was treated with 1 mM methylmercury for 10 min at room temperature, neutralized with 10 mM mercaptoethanol and alcohol-precipitated. The cDNA was made according to published procedures[29-31] the first strand was synthesized with reverse transcriptase using oligo(dT) as a primer and the second strand synthesized with a combination of the enzymes PolI, RNase H and DNA ligase. Following 'filling in' in Klenow fragment[32], the cDNA was treated with *Eco*RI methylase and ligated to *Eco*RI linkers Excess linkers were removed by selective precipitation for 20 min at room temperature in 2 M ammonium acetate with an equal volume of isopropanol, followed by agarose electrophoresis, electroelution and absorption and elution from Elutip d-Columns. From a total of 200 ng cDNA obtained, 50 ng were used and cloned in λgt10 vector[20] to yield ~200,000 recombinant plaques Following amplification, the library was screened with a v *abl*-specific probe[33] and nine positive plaques were detected, purified and analysed The locus was cloned by partially digesting normal genomic DNA with *Mbo*I, selecting fragments of 10-20 kb and cloning it into the *Bam*HI site of λL47 vector[23] Phages containing *abl* sequences were detected using the cDNA as a probe Sites in the 2 kb gap between λ15 and λ2 are from ref 14. Southern blot analysis of human DNA showed no hybridization to *abl* cDNA probe of fragments spanning the gap

not yet been accurately mapped[14]. The situation on chromosome 22 is different; the breakpoints in diffeent CMLs occurred in a restricted region of ~5.8 kb. This region was termed the breakpoint cluster region or *bcr* (ref. 15), and its consistent rearrangement in CML patients strongly implicates it in the pathogenesis of CML.

Studies of *abl* transcription provided the first strong evidence for involvement of the oncogene in CML A novel 8-kb *abl* RNA larger than the normal species of 6 and 7 kb has been identified in the leukaemic cells of essentially all CML patients and cell lines containing Ph^1 (refs 13, 16, 17). This RNA was found both in bone marrow and peripheral blood of patients in chronic phase or blast crisis. Finally, analysis of the Ph^1-positive K562 cell line identified a new *abl* protein of relative molecular mass (M_r) 210,000, larger than the normal human species of M_r 145,000 (refs 18, 19). Unlike the normal species, the larger protein has a tyrosine kinase activity, which is a common feature of retroviral transforming proteins. By cloning and characterizing complementary DNA of the normal and CML-specific *abl* RNA, we show here the latter is a hybrid molecule containing *bcr* and *abl* sequences. Nucleotide sequencing analysis indicates that the protein translated from the fused RNA has *bcr* information at its amino terminus and that it retains most but not all of the normal *abl* protein sequence

Cloning of normal *abl* cDNA

The direct approach to study the relationship between normal human 6- and 7-kb *abl* transcripts and the altered CML cell-specific 8-kb species is to obtain cDNA copies of the transcripts and compare them by restriction enzyme mapping. To obtain normal *abl* cDNA, RNA from the simian virus 80 (SV80) human fibroblast cell line was used to construct a cDNA library in the λgt10 vector[20]. The library was screened with a radiolabelled probe specific for v-*abl*, the Abelson virus homologue of the mouse *abl* gene[21] Nine positive plaques (out of 200,000 screened) were detected, then purified and analysed. The physical map of cDNA based on three overlapping clones is shown in Fig. 1 (upper part) The cDNA is 6 kb long and ends with a stretch of poly(A) at the 3' end (B. Roe *et al.*, in preparation). It extends 520 nucleotides 5' of the beginning of v-*abl* (ref. 22) positioned eight nucleotides left of the *Hin*cII site present in clone P18. A long open reading frame begins at the 5' end of the cDNA and extends into the region homologous to v-*abl* An ATG identified 150 nucleotides downstream of the cDNA 5' terminus (Fig. 3; B. Roe *et al.*, in preparation) might serve as a translational initiation codon. It is not yet known whether normal human *abl* RNA contains additional sequences at its 5' end which are not included in our cDNA

The cDNA was used as a probe to clone the *abl* locus. A partial *Mbo*I digest was prepared from white blood cells of a normal individual and, after size fractionation into 10-20-kb fragments, was cloned into the *Bam*HI site of λL47 vector[23]. A region of >50 kb was cloned in five overlapping phages and mapped with restriction enzymes (Fig. 1, bottom). The relationship between *abl* cDNA and locus was determined by hybridizing segments of the former to fragments of the latter (Fig. 1). Hence it was possible to identify 11 exons in the locus, 4 of which (I, II, IV, VI) were not detected or distinguished from other exons from a previous analysis where v-*abl* was used as a probe[14]. Sequences spanning the 3' two-thirds of the cDNA (delineated between a *Pst*I site and the 3' terminus) are apparently all included in a single exon, XI. The distribution of exons in the mouse *abl* locus[24] has common features with the situation described here for the human *abl* locus.

Cloning and sequencing of *abl* cDNA

To clone cDNA derived from the CML-specific 8-kb *abl* RNA, we used RNA extracted from the cell lines K562 and EM2. In both lines the predominant *abl* RNA is the 8-kb species (ref 13, Fig. 4). cDNA libraries were constructed in λgt 10 vector and screened with a radiolabelled probe prepared from the 0.9-kb *Bgl*II fragment located at the 5' region of normal *abl* cDNA (Fig. 1) About 30 positive plaques of 1×10^6 screened were detected in each library; all were purified and examined by digestion with restriction enzymes.

The physical maps of two clones from each library were compared with normal *abl* cDNA (Fig. 2). Clone E6 has an identical map compared with the corresponding region on the normal cDNA, whereas clones E3, K28 & K30 share most restriction enzyme sites with the normal *abl* cDNA, but vary from the latter at the 5' region. Thus, a constellation of sites for *Ava*I, *Bgl*II and *Pvu*II enzymes present at the left end of the normal cDNA is missing from the three clones and is replaced in E3 and K28 with *Pst*I and *Hin*dIII sites.

To analyse further the variance between normal *abl* cDNA and K562 and EM2 cDNAs, we sequenced the 5' regions of

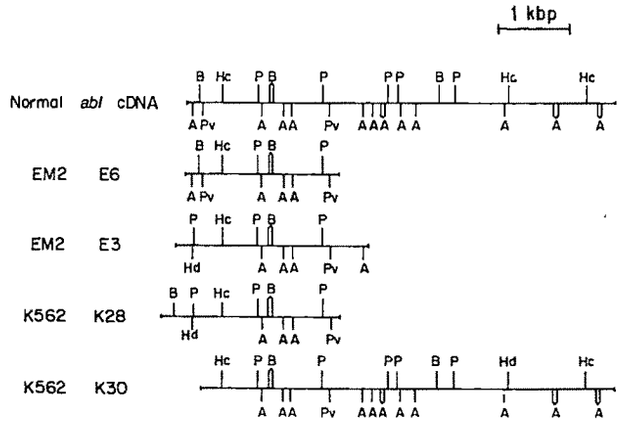


Fig. 2 Comparison between maps of normal *abl* cDNA and of *abl* cDNAs synthesized on RNA from the Ph[1]-positive K562 and EM2 cell lines. Restriction enzymes used included *Ava*I (A), *Bgl*II (B), *Pvu*II (Pv), *Hin*cII (Hc), *Pst*I (P) and *Hin*dIII (Hd).

these species (in clones P18, K28 and E3). The nucleotide sequences are depicted in Fig. 3 along with the sequence[24] of a mouse *abl* exon for comparison. The normal *abl* cDNA sequence is composed of an open reading frame extending into the region homologous to v-*abl*. K562 and EM2 have an identical sequence that diverges from the corresponding normal species in nucleotide 1. The sequence 3′ to this point is shared by the three cDNAs, whereas 5′ to this position the normal cDNA sequence is substituted in K562 and EM2 cDNAs with a new sequence. The inserted new DNA fragment is composed of an open reading frame that joins in phase the rest of *abl* open reading frame.

Characterization of new sequence

The finding of a new DNA linked to *abl* cDNA and substituting a portion of the coding region of the latter in CML cells suggested that the CML-specific 8-kb *abl* RNA originates from this linkage. To test this directly we subcloned into the puc13 plasmid the 0.45-kb *Eco*RI/*Pst*I fragment located at the 5′ end of clone K28 (this fragment is composed entirely of non-*abl* sequences), radiolabelled it and examined its hybridization to RNAs from non-CML and CML cell lines. As a control, the blot was first hybridized to the v-*abl* probe and was found to show the normal 6- and 7-kb *abl* RNAs in all lines and the 8-kb *abl* RNA in K562 cells (Fig. 4*A*). The *Eco*RI/PstI fragment reacted with the 8-kb species but not with normal *abl* RNAs (Fig. 4*B*). Two additional transcripts of ~4.5 and ~6.7 kb were apparent in all lines, in particular in SMS-SB. We conclude that cDNA clones E3, K28 and K30 originate from the 8-kb altered *abl* transcript and that the non-*abl* sequence contained in this RNA is also expressed in other human cell lines in the form of two distinct transcripts of 4.5 and 6.7 kb.

To study further the nature of the new sequence we used the *Eco*RI/*Pst*I-radiolabelled fragment to probe Southern blots of normal DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII, and we detected single fragments of 17, 12 and 11 kb, respectively (not shown). The 17-kb *Eco*RI fragment was cloned into λ Charon 4A vector[25] and mapped with restriction enzymes (Fig. 5*A*). Examination of the map of our clone revealed that it is very similar to the physical map of the breakpoint cluster region (*bcr*) reported previously by others[15]. As the hallmark of this region[15], located on chromosome 22, is that it is cleaved in all Ph[1]-positive CMLs (so that sequences 5′ to the breakpoint remain on chromosome 22 and sequences 3′ to this site translocate to chromosome 9), we tested whether our *Eco*RI/*Pst*I DNA probe would detect such breaks. DNAs from leukaemic cells of eight CML patients were cleaved with *Sst*I enzyme, electrophoresed on agarose gel, blotted onto nitrocellulose filter and hybridized to our probe. Six of eight samples showed a new fragment not detected in the normal control. Part of this analysis is shown in Fig. 5*B*. We conclude that the new sequence linked

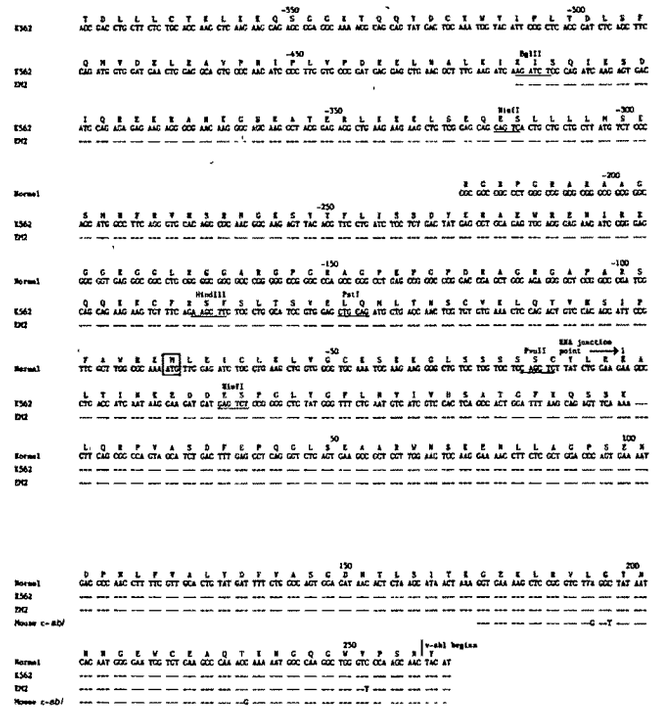


Fig. 3 Nucleotide sequence of the region of divergence between normal, K562 and EM2 *abl* cDNAs. Regions delineated between the 5′ terminus and the *Hin*cII site in clones p18 (normal), E3 (EM2) and K28 ((K562) were sequenced on both strands by the Maxam and Gilbert technique[34]. The sequence numbers are from the point of divergence between the normal *abl* cDNA and the two other species. Dashes represent the same nucleotides as in the line above; single letters above the sequence correspond to amino acids translated from the sequence; boxed ATG at position −79 on the normal cDNA could be the initiation codon for normal *abl* protein. Alternatively, initiation is in as yet uncloned sequence 5′ to the beginning of clone p18. A portion of mouse *abl* sequence[24] is shown for comparison.

to *abl* cDNA (RNA) in K562 and EM2 lines is derived from the *bcr* and that CML-specific 8-kb RNA is a fused transcript of *abl* and *bcr*.

After cloning *abl* and *bcr* loci, we were able to map the sequences at the RNA junction point to their coding regions in the loci. This was done by isolating the *Hin*fI/*Hin*cII fragment from the K28 clone and using it as a radiolabelled probe. The fragment spans the cDNA linkage point and contains 65 and 258 base pairs of *bcr* and *abl* information, respectively (Fig. 6). The probe was annealed to a *Bgl*II + *Hin*dIII digest of the DNA of phage λ2 containing exons II and III of the *abl* locus (Fig. 1). A 2-kb fragment containing exon II and a 1.1-kb fragment containing exon III reacted with the probe (Fig. 6*b*). This probe did not hybridize (Fig. 6*a*) to DNA of phage λ15 that encloses *abl* exon I. These results show that *abl* RNA at the junction point is transcribed from exon II and that sequences of exon I are not included in the 8-kb transcript. Hybridization of the same probe to different segments of *bcr* showed a reaction (Fig. 6*c*) with a 1.3-kb *Bam*HI/*Hin*dIII DNA at 15–16.3 kb on the *bcr* map (Fig. 5*a*). Thus, a sequence transcribed from this area forms the RNA junction point.

Conclusions

The results described here help to explain previous observations that implicated the *abl* oncogene and *bcr* in the pathogenesis of CML. The CML-specific 8-kb RNA is a hybrid transcript of *abl* and *bcr* genes. As a result of the t(9; 22) translocation both genes are juxtapositioned on Ph[1], conceivably in a head-to-tail configuration with the *bcr* closer to the centrometer[15]. The 8-kb transcript presumably initiates in the *bcr* and extends into *abl* sequences by a splicing mechanism. The great variance in the distance between the chromosomal breakpoints and *abl*[14]

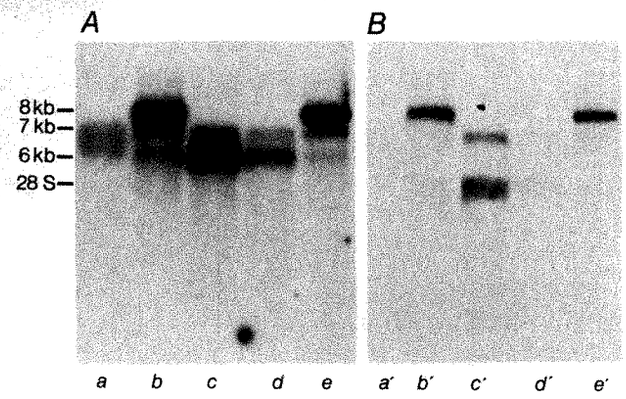


Fig. 4 Hybridization to human RNA of the sequence linked to *abl* RNA in K562 and EM2 cell lines. The blot was first hybridized to v-*abl* probe (*A*), then 'stripped' by washing in methyl mercury hydroxide and rehybridized (*B*) to the 0.45-kb *Eco*RI/*Pst*I fragment from the 5′ terminus of clone 28. 10-μg aliquots of poly(A) RNA from the cell lines K562 (*b*, *e*), HL-60 (*a*), SMS-SB (*c*) and Molt 4 (*d*) were electrophoresed, blotted onto nitrocellulose filter and hybridized as described[13].

(although the breakpoints are presumed always to be positioned 5′ of the main body of the gene) is not reflected in the size of the hybrid transcript which seems to be identical or very similar in different CMLs[13]. The RNA linkage point in respect to *abl* sequences is either at the border between exon I and II or within exon II of *abl*. This means that although the region containing exon I is included in the Ph[1] of many CMLs[15], this exon is spliced out and not included in the fused transcript of K562 and EM2 RNA and possibly in all CMLs. The *bcr* RNA at the junction point is included in an exon coded by the region at 15–16.3 kb on the *bcr* map (Fig. 5). Thus, if the RNA junction point is identical in all CMLs, this exon always has to be included in the Ph[1] and the chromosomal breakpoints must map to the right-hand side of the *Bam*HI site at 15 kb on the *bcr* map. As the breakpoints in some CMLs[15] were tentatively mapped to positions left of this *Bam*HI site, the question of whether all CMLs have identical RNA junction points must await further investigation. Nevertheless, our results suggest that CMLs will have long primary *abl*/*bcr* fused transcripts differing in size according to the distance between *abl* and the breakpoint on chromosome 9 in a particular CML. These transcripts will be spliced into identical or similar sized mature RNAs. The splice between the exon at 15 kb on the *bcr* map and *abl* exon II could involve dozens of kilobases in some CMLs. In these cases the hybrid gene on the Ph[1] would resemble in its large size the bithorax complex of *Drosophila*[26].

The 8-kb RNA in CML cells will contain ~5.7 kb *abl* information. The rest of the sequence is presumably derived from the 4.5- or 6.7-kb normal *bcr* transcripts identified here. Comparison of the *bcr* sequence at the junction point (Fig. 3) with a cDNA sequence of the normal *bcr* RNA[27] indicates that it coincides at a position ~1,500 nucleotides from the 3′ end of the latter. This suggests that the fusion partner in 8-kb RNA will be the 4.5-kb *bcr* RNA and that its 5′ 3 kb are joined to the 3′ 5.7 kb of *abl* to form a 8.7-kb species.

Comparison of the signal intensity of the 8-kb altered RNA and 4.5-kb normal *bcr* transcript in hybridizations using the *bcr* probe (Fig. 4) show the former to be considerably stronger (with the exception of the SMS-SB line). If, as suggested here, both transcripts initiate at the same chromosomal region, it seems that *bcr* RNA initiated on the Ph[1] (and giving rise to the 8-kb transcript) is more abundant than the 4.5- (and 6.7) kb species initiated on normal chromosome 22. Therefore, it is possible that an additional consequence of the t(9; 22) aberration is the enhanced transcription of the *bcr* positioned on Ph[1].

Fusion of the reading frames of both genes in phase will result in the translation of the 8-kb RNA into a hybrid protein. The amino terminus of the normal *abl* protein (26 amino acids if initiation of the normal protein is at the ATG in position −79

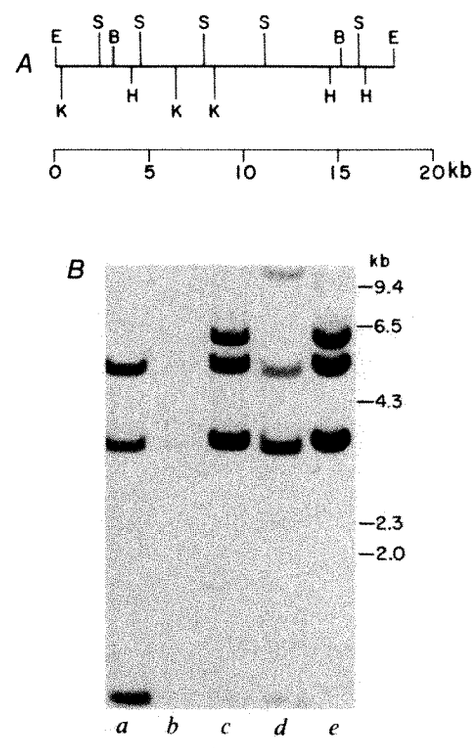


Fig. 5 Sequence linked to *abl* RNA in K562 and EM2 lines is transcribed from the *bcr*. *A*, Physical map of a genomic *Eco*RI fragment cloned from normal (SV80) DNA using the *Eco*RI/*Pst*I probe described in the text. Sites shown are of *Eco*RI (E), *Bam*HI (B), *Kpn*I (K), *Hin*dIII (H), *Sst*I (S). *B*, Hybridization of the same probe to *Sst*I digests of genomic DNAs (30 μg) from normal (*a*) and CMLs (*b*–*e*). Blots were electrophoresed and blotted by standard procedures. An 0.9-kb fragment detected at the bottom of the blot cannot be accounted for in the physical map of SV80 *bcr*, but it could result from the absence of an *Sst*I polymorphic site in SV80 *bcr*.

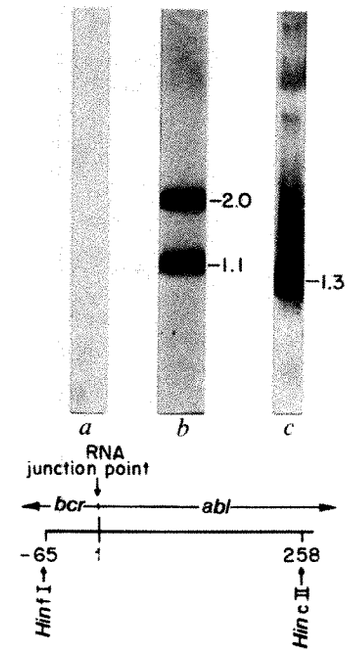


Fig. 6 Localization of sequences at the 8-kb RNA junction point on *abl* and *bcr* loci. The 0.32-kb *Hin*fI/*Hin*cII fragment from the 5′ region of clone K28 (shown at the bottom) was used as a probe in hybridization with a *Bgl*II/*Hin*dIII digest of the λ15 *abl* (lane *a*) and λ2 *abl* clones (lane *b*) shown in Fig. 1. The same probe was hybridized to a *Hin*dIII + *Bam*HI digest (lane *c*) of the genomic *bcr* clone shown in Fig. 5.

in Fig. 3, or a larger polypeptide if initiation is 5′ to this position in as yet uncloned sequence) is substituted with a *bcr* encoded polypeptide. Presumably, this protein corresponds to the M_r 210,000 *abl* protein identified in K562 cells[18,19]. The protein, unlike its normal homologue, has a tyrosine kinase activity. The substitution of the amino terminus could change the conformation of the enzyme to trigger (perhaps constitutively) the phosphorylating activity. The new amino terminus could also change the localization of the enzyme in the cell.

CML provides the first example for a human cancer where a chromosomal translocation results in the production of a modified oncogene-encoded protein which is probably directly involved with the malignant process.

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Note added in proof: Sequence at the RNA junction point is derived from the 3′ end of a 78-bp *bcr* exon and the 5′ end of the 174-bp *abl* exon II. Therefore, the normal splice signals of the two genes are used to form the hybrid transcript.

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LETTERS TO NATURE

EXOSAT observations of a 2,000-s intensity dip in Seyfert galaxy NGC4151

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Variations in the flux from active galactic nuclei (AGN) allow the testing of models of their emission mechanisms. Various suggestions for the energy generation in AGNs require the existence of a compact object, with X rays generated deep in that object's potential well and, therefore, X-ray luminosity related directly to the object responsible for their enormous luminosities. During a 5-h X-ray observation of NGC4151 we observed a significant drop in intensity lasting ~2,000 s. The event is similar to a short flux decline previously observed[1] in NGC6814. Simultaneous intensity monitoring of a quasar with coordinates close to those of NGC4151 revealed no variability in its flux, allowing instrumental causes of the event to be discounted. The short timescale of the intensity dip imposes limits of $2\times10^7\,M_\odot \leqslant M \leqslant 3\times10^8\,M_\odot$ on the mass of the central black hole. We suggest here that the sudden drop in intensity may have been caused by a change in the ionization state of gas clouds surrounding the black hole or, possibly, by the passage of a star across the line-of-sight in the dense nuclear region of the galaxy.

Fourteen extragalactic objects have exhibited variability on timescales $<10^4$ s (ref. 2 and refs therein) comparable with the light travel time across the Schwarzschild radius of a galactic nucleus-sized black hole. But in some of the X-ray observations it has been difficult to discount instrumental causes for the variability. NGC4151, one of the closest Seyfert galaxies, has been intensively studied at X-ray wavelengths[3-5], with a 2-10 keV luminosity of a few $\times10^{42}$ erg s^{-1}, a variable low-energy absorption[6,7] as well as a low-energy excess being reported[8]. Luminosity variations on timescales of days are common[9] and there has been some evidence of more rapid changes but at low statistical significance[10].

The present observations were made on 11 July 1983 with the low-energy imaging telescopes on EXOSAT (ref. 11). The detector used was a channel multiplier array (CMA) with the thin lexan filter giving a sensitivity to X rays between 0.04 and 2.0 keV. No spectral resolution was available. NGC4151 was offset from the centre of the field-of-view by ~10 arc min. The BL Lac object 1E1207.9+3945 (ref. 12) ($z=0.59$) was also observed in the field of view ~4.7 arc min from the Seyfert galaxy. Spatial filters were used to isolate the signals from NGC4151 and 1E1207.9+3945. As the filters were fixed in spacecraft coordinates throughout the observation and defined precisely the relevant areas of sky, they exclude the possibility of all but the most contrived instrumental causes of variability. The average count rate from NGC4151 was 0.022 ± 0.005 c.p.s. and that from 1E1207.9+3945 was 0.010 ± 0.005 c.p.s. The background estimates for both the Seyfert and the BL Lac object were selected from annuli of equal area and concentric to the circular area of 1 arc min radius centred on the objects chosen (the CMA FWHM response is 18 arc). Typical background count rates were a few $\times0.001$ c.p.s. Data obtained with the other low-energy imaging telescope were contaminated with solar-flare emission and cannot be satisfactorily analysed (the reason for the difference in the response of the two telescopes to solar flares is well understood). At the time of the observation reported, the medium-energy detectors were not operating in a mode which allows the comparison of their data with that from the CMAs. NGC4151 was detected in the higher energy gas scintillation proportional counter (GSPC) detector though at such a weak level as to preclude a search for variability in its count rate.

A search was conducted for variability in the flux from NGC4151 by binning the data with bin widths from 1,000 to 9,500 s in steps of 100 s and testing the value of χ^2 against the hypothesis of a uniform counting rate. Such a series of timelines do not represent completely independent trials and the interpretation of the χ^2 results must allow for this. The timelines may be considered independent if the phase difference between the last bins in each data set is greater than one bin width[13]. There were six independent timelines and the probability of obtaining the six values of χ^2 by chance in non-ordered samples was

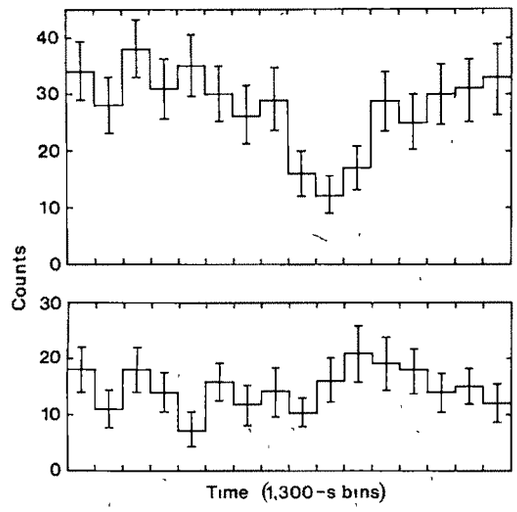


Fig. 1 The background subtracted light curves for NGC4151 (top) and IE1207.9+3945 (bottom) Origin in each case is 7 h 3 min 6 s. Times are in UT.

$<10^{-4}$. The light curves for the observation (Fig. 1) show that the cause of the large χ^2 is a decline in the flux from NGC4151 over a ~2,000-s period.

There are two plausible explanations for this intensity change. Either the opacity of the line-of-sight material within NGC4151 varied, perhaps because of changes in its ionization state, or an essentially opaque body passed between the X-ray source and the telescope. In either case, it is difficult to avoid the conclusion that both the X-ray source and the variable part of the intervening matter have dimensions of the order of, or less than, 2,000 $c = 6 \times 10^{13}$ cm.

Halpern[14] has suggested that variable absorption in QSO MR2251−178 results from changes in the ionization state of the gas surrounding the nucleus. If this model is applied to the current observations, the radial extent of the gas must be $<6 \times 10^{13}$ cm and the ionization parameter[15] ξ must lie between 1 and 100 in order for substantial changes in X-ray transmission to occur. The timescales quoted by Halpern for opacity changes are similar to the timescale of the variability reported here. The low-energy count rate requires a column density $<10^{23}$ atoms cm^{-2}. Using the extreme values allowed by the data, the electron number density in the ionized region must be $\sim 10^9$ cm^{-3}. This places the absorption cloud at 10^{15}–10^{16} cm from the nucleus to achieve the required range of ξ.

The bolometric luminosity of NGC4151, 2.7×10^{45} erg s^{-1} (ref. 2) and the observed variability enable limits to be placed on the mass of the central black hole of $2 \times 10^7 M_\odot \leq M \leq 3 \times 10^8 M_\odot$, model-dependent arguments reduce the range further. The Elliot–Shapiro relationship[16] is satisfied in this case indicating that beaming of the radiation is not required, but the Cavallo–Rees accretion scattering limit[17] is not valid for an occulting object external to the accreting material.

Stars have photospheric radii in the range 5×10^{10}–7×10^{13} cm (ref. 18) and are large enough to occult all, or a large fraction of, the X-ray emitting part of an accretion disk surrounding a black hole with mass in the range 3×10^4–2×10^8 $M_\odot$. The spatial density of stars close to the central objects may be of the order of 10^6 $M_\odot$ pc^{-3} (ref. 19) and therefore such occultations can occur on timescales of months or years. If this is so then the velocity of the occulting object must lie between 4×10^7 and 3×10^{10} cm s^{-1} to perform the occultation in the observed time. This range is appropriate for keplerian velocities within a parsec of the nucleus. We suggest that luminosity dips caused by 'star shadows' will occur, and should be searched for, regardless of whether they caused this event.

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A model of object Geminga as a degenerate white dwarf orbiting around a black hole

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Geminga (2CG195+04) is a very bright γ-ray source with the flux $F_\gamma = 2.4 \times 10^{-9}$ erg s^{-1} cm^{-2} for $E \geq 50$ MeV (refs 1–3). It has been recently identified with X-ray[4] and optical[5] sources. The optical object is the bluest for its magnitude[5], indicating compactness of the emitting region. The high ratios $L/L_X \approx 10^3$, $L_X/L_{opt} \approx 200$ and the absence of radio emission are very unusual. The observed periodicity is $P \approx 1$ min and it changes at a rate corresponding to a doubling time of about several hundred years. I propose here a model which quantitatively explains the values of P and $\dot{P}$. It consists of a degenerate dwarf ($M_d = 0.6\ M_\odot$) filling its Roche lobe and orbiting a black hole ($M_{bh} = 4.4\ M_\odot$) with a period $P = 1$ min. Gravitational radiation dissipates the angular momentum, which leads to mass transfer with a current rate $\sim 10^{-3}\ M_\odot\ yr^{-1}$ and a period change $\dot{P}$ which agrees with the observed value. At a distance of 100 pc the flux of this radiation would be $\sim 5 \times 10^{-3}$ erg s^{-1} cm^{-2} (at $\nu = 1/30$ Hz). This is about seven orders of magnitude greater than from any other known stationary source.

Let R_{12} be the distance between the dwarf and the black hole. Because the dwarf fills its Roche lobe, its radius equals[6]:

$$R_{Roche} = R_d = R_{12} \frac{2}{3^{4/3}} \left(\frac{M_d}{M}\right)^{1/3}, \qquad M = M_d + M_{bh} \tag{1}$$

We can use this and Kepler's laws to describe the orbital period of the system

$$P = \frac{2\pi R_{12}^{3/2}}{(GM)^{1/2}} = \frac{9\pi R_d^{3/2}}{(2GM_d)^{1/2}} \tag{2}$$

On the other hand, for a dwarf made of helium or heavy elements ($\mu_e \cong 2$) the radius R_d depends on the mass M_d through the relation[7]:

$$R_d \approx 7.6 \times 10^8 \left(\frac{M_\odot}{M_d}\right)^{1/3} \text{cm} \qquad \text{for } 0.2 \leq \frac{M_d}{M_\odot} \leq 0.75 \tag{3}$$

Therefore, we can eliminate R_d from equation (2) and write the orbital period P as a function of the mass of the dwarf only

$$P = 36 \left(\frac{M_\odot}{M_d}\right) \text{s} \tag{4}$$

For $P = 60$ s the corresponding value of M_d is 0.6 $M_\odot$ and we can see that the observed period can easily be interpreted as the orbital one. To explain $\dot{P}$ let us note that the gravitational radiation leads to a loss of orbital angular momentum which in turn causes an increase of the mass transfer and an increase of the orbital period. This effect was first applied to explain some

of the dwarf novae properties[6,8]. The orbital angular momentum J and its change $\dot{J}$ due to the gravitational radiation are given by[9]

$$J = M_d M_{bh}\left(\frac{GR_{12}}{M}\right)^{1/2}, \qquad \frac{\dot{J}}{J} = -\frac{32G^3}{5c^5R_{12}^4} MM_dM_{bh} \tag{5}$$

Combining equations (1)-(5) we obtain the equation for M_d and its derivative $\dot{M}_d$, expressed in solar units ($m = M/M_\odot$):

$$\frac{\dot{m}_d}{m_d} = -1.3\times10^{-10}\, m_d^{11/3} m^{2/3} \frac{\left(1-\frac{m_d}{m}\right)^2}{1-\frac{5}{3}\frac{m_d}{m}} \tag{6}$$

The solution of equation (6) when $m_d \ll m$, together with equations (4) and (1), lead to the relations:

$$m_d = 0.6(1+6.3\times10^{-6}\, m^{2/3} t_d)^{-3/11} \tag{7}$$

$$\dot{M}_d = -4\times10^{-4}\, m^{2/3}(1+6.3\times10^{-6}\, m^{2/3} t_d)^{-14/11} M_\odot\ \mathrm{yr}^{-1} \tag{8}$$

$$P = 60(1+6.3\times10^{-6}\, m^{2/3} t_d)^{3/11}\ \mathrm{s} \tag{9}$$

$$R_{12} = 4\times10^9(1+6.3\times10^{-6}\, m^{2/3} t_d)^{2/11}\ \mathrm{cm} \tag{10}$$

where t_d is the time in days with $t_d = 0$ at the moment when $P = 1$ min. The curve $P(t_d)$ given by equation (9) approximately fits the observational points for $m = 5$ (see Fig. 1). The deviations of these points from a straight line may be due to the observational errors or to some phenomena, connected, for example, with the angular momentum exchange between the orbital and intrinsic rotation of the companions. The mass $M_{bh} = 4.4\ M_\odot$ is greater than the usually assumed value for the maximum mass of a neutron star of $2M_\odot$. The absence of radio emission and the very faint X-ray source also implies the absence of a neutron star in this system.

It has been suggested[10] that the evolutionary path leading to the formation of a binary system consisting of a degenerate dwarf and a relativistic star (a black hole or a neutron star) may start with a close pair of main-sequence stars of mass $\sim 11\ M_\odot$.

If the initial mass of the white dwarf in Geminga was $1\ M_\odot$, then the filling of the Roche lobe and the onset of the mass transfer and appearance of the γ-ray source happened ~ 130 yr ago, when the parameters of the system were: $P_{min} \approx 36$ s, $R_{12,min} \approx 3.6\times10^9$ cm. So, Geminga may be a very young γ-ray source. Before the Roche lobe filling phase (for $t \leq 0$) the system had been in a very quiet state for a long time. Gravitational radiation was decreasing the orbital period and the separation, according to equation (5) but with constants M_d and M_{bh}:

$$R_{12} = R_{12,min}\left(1-\frac{256G^3}{5c^5R_{12,min}^4} MM_dM_{bh}t\right)^{1/4}$$
$$= 3.6\times10^9(1-3\times10^{-11}\, mm_dm_{bh}t)^{1/4}\ \mathrm{cm} \tag{11}$$
$$P = P_{min}\left(\frac{R_{12}}{R_{12,min}}\right)^{3/2}$$
$$= 36(1-3\times10^{-11}\, mm_dm_{bh}t)^{3/8}\ \mathrm{s},\ t \leq 0$$

If the formation of the binary system had happened $<10^{17}$ s ago, initially it had parameters:

$$R_{12,0} \leq 1.5\times10^{11}\,(mm_dm_{bh})^{1/4}\ \mathrm{cm}$$
$$P_0 \leq 2.7\,(mm_dm_{bh})^{3/8}\ \mathrm{h} \tag{12}$$

In the future, mass transfer in Geminga will proceed up to the moment when a planet of non-ideal matter is formed and the mass transfer will stop. It follows from equation (7) that the transfer stage will proceed for at least a Hubble time $\sim 3\times10^{17}$ s, after which the period will be $P \approx 2.2$ h, $R_{12} \approx 10^{11}$ cm and $M_d \approx 4.5\times10^{-3}\ M_\odot$.

The present mass transfer rate, $\dot{M} \approx 10^{-3}\ M_\odot\ \mathrm{yr}^{-1}$, is very high. However, if Geminga is at a distance of about 100 pc (ref. 5) then its luminosity $L_\gamma \approx L_\odot$ can be explained by a much smaller accretion rate, $\sim 10^{-9}$-$10^{-11}\ M_\odot\ \mathrm{yr}^{-1}$, suggesting either a low efficiency of accretion or a non-stationary accumulation of matter in the accretion disk. Both these cases have been recently treated by authors (see refs 11, 12 and those cited in ref. 13) who considered geometrically thick, low-viscosity, laminar accretion flows onto black holes ('thick accretion disks').

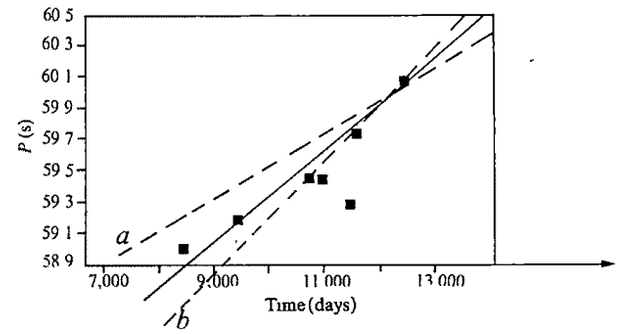


Fig. 1 The dependence of the period (in s) on time (in days). ■, Observational data[19]. The lines are calculated from equation (9). Solid line corresponds to $m = 5$, the dashed line a to $m = 3$, and the dashed line b to $m = 7$. All three lines meet at the point $P = 60$ s, $t = 12{,}120$ days.

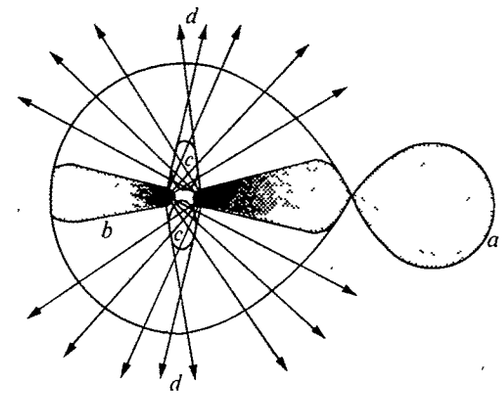


Fig. 2 The model of Geminga *a*, White dwarf filling its Roche lobe; *b*, accretion disk around black hole; *c*, regions of formation of the γ-ray emission due to collisions of relativistic particles; *d*, the beam of relativistic particles.

If the magnetic field is frozen into the matter of the disk, then the induced electric field accelerates the charged particles up to relativistic energies in the funnel of the thick disk which forms along the rotation axis[13]. The interaction of the relativistic particles may lead to the formation of π^0 mesons with the subsequent decay to γ-quanta. The qualitative picture of the model of Geminga is presented in Fig. 2. As in the Crab and Vela[14] the energy losses in the form of relativistic particles may be 100–1,000 times greater than L_γ. The rotation of the thick disk must be nearly uniform so that the magnetic field will not be strongly amplified and the quasi-stationary state of the disk may be preserved for a long time.

If the X-ray flux is formed by the interaction of the relativistic flow with the surface of the dwarf and the disk, then only a small part of the energy flux reaches these surfaces. Due to the reflection effect, we may expect optical variations with the same period $P \approx 1$ min and periodical Doppler shifts of the spectral lines from the dwarf with the amplitude $\approx 4{,}200 \sin i$ km s^{-1}, where i is the unknown angle between the normal to the orbital plane and the line-of-sight. The optical observations may serve as a decisive test for the validity of this model.

The development of turbulence in the accretion disk may lead to a strong X-ray emission $L_X \approx L_{edd} \approx 5\times10^{38}$ erg s^{-1}, but the duration and the frequency of such bursts are impossible to predict within the present model.

If the other unidentified point-like γ-ray sources[15] are of the same origin as Geminga, then we may expect similar L_γ/L_X and L_X/L_{opt} ratios and a periodic modulation of the γ-ray flux with $1\ \mathrm{min} \leq P \leq 3$ h.

The main energy losses by Geminga in this model are due to gravitational radiation. Using the standard quadrupole formula[9] and equation (10) we may write for the gravitational wave luminosity L_G:

$$L_G = \frac{32}{5}\frac{G^4}{c^5}\frac{M_d^2 M_{bh}^2 M}{R_{12}^5} \approx 6\times10^{39}(1+1.8\times10^{-5}\,t_d)^{-10/11}\,\mathrm{erg\,s^{-1}} \quad (13)$$

At a distance of 100 pc, this corresponds to a value of the flux on the Earth of

$$F_G = 5\times10^{-3}\,\mathrm{erg\,cm^{-2}\,s^{-1}} \quad (14)$$

This flux is about seven orders of magnitude greater than the flux from any other known binary system[16].

In the case of supernovae explosions, a high deviation from spherical symmetry and high infall velocities must be assumed to allow a detectable amount of gravitational radiation. There are doubts[17,18] over whether such high values are consistent with real supernovae events. On the other hand, our model of Geminga employs simple arguments and realistic parameters for the binary system. Thus the search for gravitational radiation (at $\nu \sim 1/30$ Hz) from Geminga would probably not be less reasonable than the search for the pulses of gravitational radiation from supernova explosions.

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General relativistic effects on the pulse profile of fast pulsars

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The recent discovery of millisecond pulsars[1,2] has led to several theories about the structure[3–8] and radiation characteristics[9–11] of rapidly rotating neutron stars. The rapid rotation rate implies that such pulsars will be compact[5] (implying large space-time curvature) and that surface emission characteristics will differ significantly from the non-rotational (Schwarzchild) case[11]. We report here the implications of large space-time curvature and large rotation (specifically, the dragging of inertial frames introduced by rotation) on the trajectories of photons, and hence, on the pulse profile of fast pulsars. Space-time curvature leads to substantial amounts of divergence in the pulse width and reduction in the pulse intensity whereas rotation produces a tilt of the pulse cone from its original direction of emission, and deforms the cone, introducing an asymmetry in the (flattened) pulse profile leading to a time delay in the arrival of photons emitted within the pulse cone. We find that the effect of curvature dominates over that of rotation, suggesting a larger brightness temperature than inferred observationally for all pulsars whatever the rotation rate (unless the pulsar is of very low mass).

The full rotational analogue to the Schwarzchild space-time, namely the Kerr metric, does not have a corresponding appropriate interior solution amenable to a straightforward theoretical treatment. However, a rotationally-perturbed interior spherical metric of the following form[12,13] is adequate (signature +−−−)

$$ds^2 = g_{\alpha\beta}\,dx^\alpha\,dx^\beta = e^{2\nu}\,dt^2 - e^{2\psi}(d\phi - \omega\,dt)^2 - e^{2\mu}\,d\theta^2 - e^{2\lambda}\,dr^2 \quad (1)$$

where ω is the angular velocity of the cumulative dragging of inertial frames and ν, ψ, μ, λ depend on the structure of the rotating neutron star. This metric is valid for strong gravitational fields and for a uniform rotation rate that has as its upper bound the critical speed for centrifugal breakup. Neutron star models which rotate at the secular instability limit (assuming the star to be homogeneous) and which are relevant to fast pulsars, are within this bound[6].

Rotating neutron-star structure parameters using the metric (1) (see refs 5, 6) can be used in an external metric (which match at the surface) for calculating the photon trajectories. Space-time curvature will bend a photon trajectory, and rotation will drag it away from its original direction towards the direction of rotation. The net angle of deflection in the trajectory will be given by

$$-\phi_0(\delta) = \int_{r_e}^{D} \frac{d\phi/d\Gamma}{dr/d\Gamma}\,dr \quad (2)$$

where Γ is an affine parameter and r_e and D are respectively the radial locations of the emission and reception of the photon. The deflection angle is a function of δ, the azimuthal angle at which the photon is emitted with respect to the radius vector of the source through the origin of coordinates as seen in the local rest frame of the star ($\delta = 0$ corresponds to a radially outgoing photon and $\delta = 3\pi/2$ to a tangentially-forward photon). We have neglected the polar bending ($d\theta/d\Gamma = 0$) which will be small because we use a perturbed spherical geometry. Therefore, the polar angle of emission, θ_s, made by the line-of-sight through the centre of the star with respect to the rotation axis can be taken to be identical to the angle of inclination seen by a distant observer.

The lagrangian for the metric (1) is

$$L = \tfrac{1}{2}\{e^{2\nu}\dot{t}^2 - e^{2\lambda}\dot{r}^2 - e^{2\mu}\dot{\theta}^2 - e^{2\psi}(\dot{\phi} - \omega\dot{t})^2\} \quad (3)$$

where the overdot indicates derivative with respect to Γ. The equations of motion corresponding to equation (3) provide $\dot{\phi}$ and $\dot{t}$ whereas $\dot{r}$ is obtained from the null condition. Evaluated thus, equation (2) becomes

$$-\phi_0(\delta) = \int_{r_e}^{D} f(r, q_e)\,dr \quad (4)$$

$$f(r, q_e) = \frac{\omega(1+\omega q_e) - q_e\,e^{2\nu-2\psi}}{e^{\nu-\lambda}\{(1+\omega q_e)^2 - q_e^2\,e^{2\nu-2\psi}\}^{1/2}} \quad (5)$$

Here q_e is the impact parameter of the photon at the emission location, and has the form[11]

$$q_e = \left.\frac{e^{\psi-\nu}(v_s + \sin\delta)}{1 + e^{\psi-\nu}(\omega v_s + \Omega\sin\delta)}\right|_{r=r_e} \quad (6)$$

where Ω is the angular velocity of the star as seen by a distant observer and

$$v_s = e^{\psi-\nu}(\Omega - \omega) \quad (7)$$

Because of bending (see Fig. 1), the space-time curvature will cause a divergence in the pulse width. As a measure of this

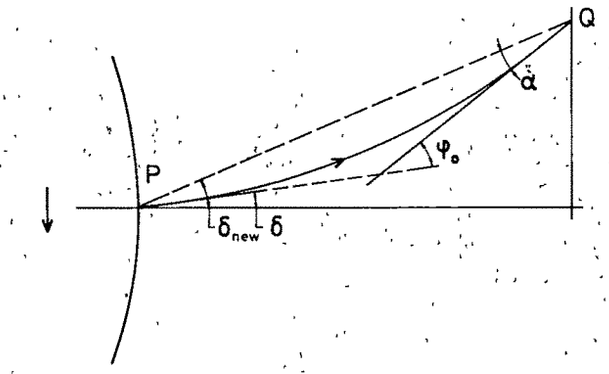


Fig. 1 Schematic illustration of the trajectory of photon starting from P at (or near) the pulsar surface in terms of the azimuthal angle of emission (δ) and bending ($\phi_0 \equiv \delta_{new} - \delta$). For an observer at infinity (denoted by Q), the angle $\alpha \to 0$. The arrow implies the direction of rotation of the neutron star.

divergence effect, we introduce a parameter Δ defined as

$$\Delta = (\mathrm{d}\delta_{new}/\mathrm{d}\delta)_{r=r_e} = (1 + \mathrm{d}\phi_0/\mathrm{d}\delta)_{r=r_e} \quad (8)$$

where

$$\delta_{new} = \delta + \phi_0(\delta) \quad (9)$$

As a consequence of rotation, the impact parameter is asymmetric in $\pm\delta$ and

$$\Delta(\delta, r) \neq \Delta(-\delta, r)$$

implying an asymmetry in the pulse profile (assuming the initial profile to be symmetric). This is unlike the Schwarzchild case, where although the space-time curvature will produce a pulse divergence effect of similar extent, there will be symmetry in $\pm\delta$.

The pulse divergence described above will imply a reduction in the pulse intensity, which can be understood in terms of conservation of energy flux. We denote the intensity deamplification factor by ε:

$$\varepsilon = \frac{I(\delta_{new})}{I(\delta)} = \frac{1}{\Delta}\frac{\sin\delta}{\sin\delta_{new}} \quad (10)$$

The factor ε must be corrected for redshift effects as follows, using Liouville's theorem[14]. If I_ν is the observed specific intensity and I_{ν_0} the emitted (monochromatic) specific intensity (at a given δ), then

$$I_\nu = I_{\nu_0}(\nu/\nu_0)^3 = I_{\nu_0}(1+z)^{-3} \quad (11)$$

where $(1+z)$ is the redshift factor[11]:

$$1 + z = \frac{1 + \Omega q_e}{e^\nu (1 - v_s^2)^{1/2}} \quad (12)$$

The redshift correction to the intensity through a factor $\{1 + z(\delta = 0)\}^{-3}$ is inherent to the pulse. Apart from this, the intensity will become enhanced for forward emitted photons ($\delta < 0$) and reduced for backward emitted photons ($\delta > 0$). We define a net intensity deamplification factor ε' that corrects ε for Doppler boosting (for $\delta < 0$) and dimunition (for $\delta > 0$) as

$$\varepsilon' = \varepsilon\left\{\frac{1 + z(0)}{1 + z(\delta)}\right\}^3 \quad (13)$$

in accordance with Liouville's theorem.

To illustrate the effects of space-time curvature and rotation on the pulse profile, we consider PSR1937+214, assuming its mass to be $1.4\,M_\odot$ ($M_\odot$ = solar mass). For this pulsar, $\Omega = 4.0334 \times 10^3$ rad s^{-1}; the relevant structure parameters for the

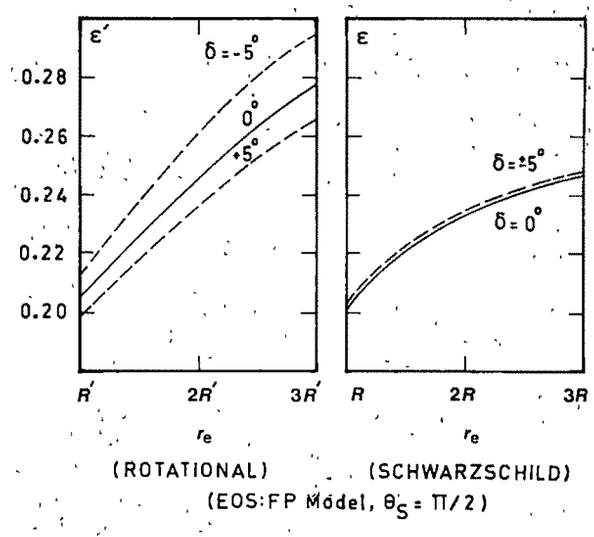


Fig. 2 Net deamplification factor (ε') versus φ radial emission location (r_e). EOS: FP model, $\theta_s = \pi/2$.

calculation have been taken from our earlier work[5,6] (corresponding to the Friedman–Pandharipande equation of state of neutron-star matter). As this pulsar is close to the point of secular rotational instability[5,6] the results will refer to maximal effects of rotation.

There is no general consensus on the mechanism of pulse emission. Here, we have selected one of the more popular models[15], namely, that in which the emission (at a particular frequency) emanates from a point near the surface of the pulsar in the form of a narrow cone (of angle $\leq 10°$) which rotates with the pulsar, sweeping the pattern past the observer. For the purposes of calculation, we take the initial pulse to be a gaussian curve with $-5° \leq \delta \leq +5°$.

The results of our calculations for the rotational (for $\theta_s = 0.3\pi$, 0.5π) and Schwarzchild ($\theta_s = 0.5\pi$ here) cases are summarized in Tables 1 and 2. The initial pulse becomes wider by a factor ~ 2. There is an asymmetry in the pulse profile brought about by rotation which manifests itself in the pulse cone getting compressed for backward emission and stretched for forward emission. This asymmetry, characterized by $|\Delta(+\delta) - \Delta(-\delta)|$, is

Table 1 Azimuthal bending, divergence index (Δ) and deamplification factor (ε') for the rotational and Schwarzchild cases with emission taken at the pulsar surface considered for PSR1937+214

	δ	δ_{new} (deg)		Δ		ε'	
θ_s	(deg)	+	−	+	−	+	−
Rotation case							
0.3π	0	0	0	2.512	2.512	0.158	0.158
	1	2.509	−2 514	2.507	2.517	0.158	0.159
	2	5.014	−5.034	2.503	2.522	0 157	0 160
	3	7.515	−7.558	2 498	2 527	0.157	0.161
	4	10.010	−10.087	2.494	2.532	0.156	0.162
	5	12.502	−12.621	2.489	2.537	0.156	0.164
0.5π	0	0	0	2.213	2.213	0 204	0.204
	1	2.210	−2.215	2.208	2.217	0.203	0.206
	2	4.416	−4 435	2.203	2.222	0.202	0.207
	3	6.617	−6.659	2.199	2.227	0.201	0.209
	4	8.813	−8.889	2.194	2.232	0.200	0.210
	5	11.005	−11.124	2.190	2.237	0.199	0.212
Schwarzchild case							
0.5π	0	0	0	2.226	2.226	0.202	0.202
	1	2.226	−2 226	2.226	2.226	0.202	0.202
	2	4.453	−4.453	2.226	2.226	0.202	0.202
	3	6.679	−6.679	2 227	2.227	0.202	0.202
	4	8.906	−8.906	2.227	2 227	0.202	0 202
	5	11.133	−11.133	2.227	2.227	0.203	0.203

Table 2 Rotation-induced pulse tilt angle and time delay for two values of θ_s and $r_e = R', 2R', 3R'$ considered for PSR1937+214

r_e	θ_s (deg)	Tilt angle (deg)	Time delay for δ (μs) ±1°	±2°	±3°	±4°	±5°
R'	54	10.262	0.2	0 5	0.7	1.0	1.2
	90	10.253	0.3	0.6	1 0	1.3	1.5
$2R'$	54	20.199	0 8	1.6	2.4	3 1	3.9
	90	20.322	1 0	1.9	2 8	3 9	4.7
$3R'$	54	28.823	1.5	3.1	4.6	6.1	7.7
	90	29.290	1.8	3 6	5.4	7.2	9.1

proportional to δ. This is also evident in ε'. Figure 2 illustrates (for $\delta = 0°, \pm 5°$) the variation $\varepsilon'(r_e)$, r_e being taken in units of $R'(R)$ which is the radius of the rotating (non-rotating) neutron star.

One interesting result of our calculations is the 'sweeping' of the entire pulse cone in the direction of rotation of the pulsar, thus imparting a tilt to the cone. The tilt can be understood in terms of the net bending of the radially outward $\delta = 0$ photon, whose values for various θ_s and r_e are given in Table 2.

In Schwarzchild space-time, photons emitted at $\pm\delta$ would have the same time of flight to the distant observer. But with the neutron star rotating rapidly, this symmetry will not be maintained. The backward emitted photons would have larger time of flight than the forward emitted photons. The difference in the arrival times of $\pm\delta$ photons, which we call time delay, can be evaluated from the equations of motion of photons in the external space-time and their redshifts. These values are listed in Table 2. If the time delay between photons with maximum δ and minimum δ equals the pulse duration, the complete pulse will be detected by the observer. If, however, this time delay exceeds the pulse duration, then, photons with δ larger than a critical δ will miss the detector. As a result, the pulse will have a gradual buildup but a steeper falloff. This will be a contributory factor to the asymmetry in the final pulse profile (apart from the effects resulting from inertial frame drag and interstellar/planetary scintillation, if any), and, in principle, will constrain the duty cycle of the pulsar.

The effects of curvature and rotation on the pulse profile presented here are purely general relativistic effects, and will be additional to any (frequency dependent) effect on the propagation of electromagnetic waves through anisotropic/inhomogeneous magnetized plasma. Observationally, most pulsars show narrow profiles. Our calculations, therefore, can be taken to imply that at the emission location, the pulse starts out in the shape of a narrow spike. As the brightness temperature of the source is directly proportional to the intensity of radiation, the important conclusion that follows is that the brightness temperatures of pulsars in general (since the curvature effects are dominant over the rotational effects) are larger by an order of magnitude (in the emitter's frame of reference) than are presently presumed.

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Possible precipitation of ice at low latitudes of Mars during periods of high obliquity

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Most of the old cratered highlands of Mars are dissected by branching river valleys that appear to have been cut by running water[1,2] yet liquid water is unstable everywhere on the martian surface. In the equatorial region, where most of the valleys are observed, even ice is unstable[3,4]. It has been suggested, therefore, that Mars had an early denser atmosphere with sufficient greenhouse warming to allow the existence of liquid water[5]. Here, we suggest instead that during periods of very high obliquities, ice could accumulate at low latitudes as a result of sustained sublimation of ice from the poles and transport of the water vapour equatorwards. At low latitudes, the water vapour would saturate the atmosphere and condense onto the surface where it would accumulate until lower obliquities prevailed. The mechanism is efficient only at the very high obliquities that occurred before formation of Tharsis very early in the planet's history, but limited equatorial ice accumulation could also have occurred at the highest obliquities during the rest of the planet's history. Partial melting of the ice could have provided runoff to form the channels or replenish the groundwater system.

That some mechanism must exist for replenishment of the equatorial inventory of water has been suspected on several grounds: (1) if the valleys formed in a manner analogous to terrestrial river valleys, by slow erosion of running water, then the volumes of water that would have flowed through them would have been so large that some recycling of water is implied; (2) although groundwater seepage has probably had a major role in formation of the valleys[6], several morphological characteristics suggest that precipitation was also involved, so that transfer of water from the atmosphere to the surface occurred[2]; (3) the unique characteristics of several martian landforms, particularly those formed by impact, have been attributed (although not uniquely) to the presence of ground ice in regions where it is unstable.

We have calculated the potential loss of water from the poles on the assumption that sublimation of the seasonal CO_2 cap during summer exposes a residual water-ice cap, as is currently the case for the North Pole[7,8] and may occasionally be the case for the South Pole[9]. The procedure followed here is similar to that used by Toon *et al.*[10]. Temperatures at the surface and down to depths of 7 m are calculated for ±85° lat. taking into account incident solar radiation, long-wave emission from the surface and atmosphere, conduction into the ground, sublimation and condensation of carbon dioxide and evaporation of water. Atmospheric pressures greater than the present value may occur at high obliquities as a result of desorption of CO_2 from the high-latitude regolith[11]. We assumed that the pressure was 40 mbar at 45° obliquity, 25 mbar at 35° obliquity and 10 mbar at the current 25° obliquity[12]. The main difference between our model and that of Toon *et al.* is that we included growth and decay of the CO_2 cap as an integral part of our model rather than assuming that the CO_2 cap disappeared at a specific time. Our results are conservative in that they give estimates of the

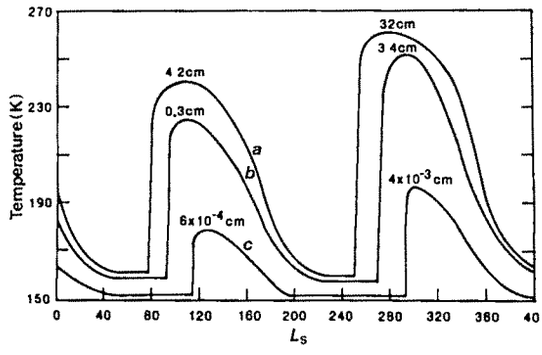


Fig. 1 Seasonal polar cap temperatures calculated for obliquities of 45° (*a*), 35° (*b*) and 25° (*c*). The adjacent numbers refer to the thickness of ice sublimed from the cap (in cm) during the entire summer. For clarity, only temperatures for the warmer pole are shown at each season. Calculations assume the maximum obliquity (0.14) and have periapsis at southern solstice, and thus yield the maximum differences between the two poles; other choices would yield temperatures intermediate between these extremes.

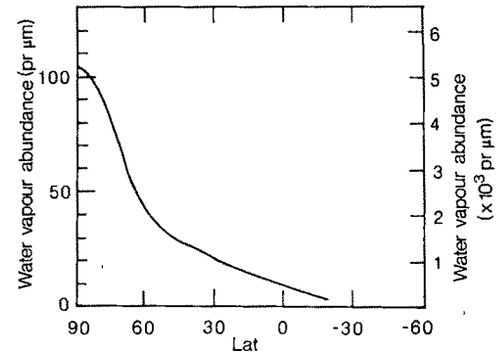


Fig. 2 Latitudinal distribution of water vapour at mid-summer in the north as observed from the Viking space craft (left-hand ordinate) The right-hand ordinate is appropriate for high values for the obliquity, when $\geq 5 \times 10^3$ pr μm water may be present over the cap

amount of water sublimed from the summer cap that are up to a factor of 10 lower than the estimates of Toon *et al.*[10].

Typical results are shown in Fig. 1 for the three values of the obliquity. Although the results are sensitive to factors such as the albedo of CO_2 and water frost, the wind speed and the assumed thermal conductivity, the results, in general, demonstrate that at 45° obliquity a few centimetres to a few tens of centimetres will be evaporated from the poles during each summer. At an obliquity of 35° a few tenths of a centimetre to a few centimetres will be lost, while in present conditions only 10^{-2}–10^{-4} cm are lost.

The results shown in Fig. 1 were calculated assuming sublimation of water into a dry atmosphere. The actual amount of water sublimed and its eventual fate will depend on the rate of atmospheric transport away from the pole. Figure 2 shows the mid-northern-summer latitudinal distribution of water vapour during 1976. Polar abundances of about 100 precipitable (pr) μm were observed, consistent with an almost saturated atmosphere[8,13]. Non-polar atomospheric water indicates transport of water away from the pole and/or exchange of water between the regolith and atmosphere[14,15], although transport from the poles ultimately dominates the process[15]. For equatorial and mid-latitude mixing timescales indicated by dynamic models[16,17], an amount of the order of 5×10^{14} g H_2O can be transported southwards each year, equivalent to removing $\sim 4 \times 10^{-2}$ g cm^{-2} from the cap[15,18]. Given the uncertainties in the models, this value is consistent with the estimated amounts of water that can be sublimed.

At high obliquity, a similar atmospheric circulation pattern should exist, with the exceptions of a poleward shift in the Hadley cell to the subsolar point, and more vigorous cross-equatorial transport (ref. 19 and R. M. Haberle, personal communication). Therefore, if we assume a similar mid-summer distribution of water with latitude due to transport from the polar region, we can use Fig. 2 to show the latitudinal variation at midsummer, reading now from the right-hand scale The estimate of 5×10^3 pr μm over the pole is conservative, based on saturation of the atmosphere over the pole at temperatures that are relatively low for 45° obliquity but higher than that of the present summer cap. By scaling the amount transported to the total abundance, the amount of water transported away from the cap is equivalent to removal of ~ 2 g cm^{-2} from the pole, again consistent with the amounts estimated to have sublimed. From the right-hand scale of Fig. 2, there can be >1,000 pr μm of atmospheric water at mid- and low latitudes. For less conservative assumptions, the amount of water ice sublimed can be as great as 20 cm and the water content of the atmosphere at the equator can be as high as 10,000 pr μm.

At the equator a dust-free atmosphere is capable of holding only as much as 100–200 pr μm of water vapour without saturating[20,21] Thus, the excess above this value will condense and form clouds. Persistent dust storms are, however, likely at high obliquities[5]. The presence of dust causes atmospheric temperatures to rise. As a result, the atmosphere could hold 5,000–10,000 pr μm during the day without saturating, and up to 300 pr μm at night[21]. Thus, even during dust storms water would condense out at night if abundances were higher than ~300 pr μm. If the dust acts as condensation nuclei for the water, then the dust and ice will be removed rapidly to the surface, possibly within several days. Two uncertainties are the effect of the ice clouds on the holding capacity of the atmosphere and the effect of surface ice. Temperatures that occur in the presence of clouds are not well determined; low-altitude clouds will cool the surface dramatically[22,23] while high-altitude clouds could increase surface temperatures[10]. The presence of high-albedo ice on the surface would significantly lower surface and atmospheric temperatures, thereby providing a positive feedback and enhancing the tendency for atmospheric water to condense.

If as much as 20 cm of ice are removed in 1 yr from the summer cap and deposited uniformly between ±45° latitude, the resulting ice thickness is 0.4 cm. It is uncertain, however, where the ice is deposited. The atmospheric circulation pattern and the relative timescales for condensation and settling will determine whether the ice is deposited near the edge of the summer or winter cap or more uniformly over the globe. During the following winter, the non-polar water vapour abundance at saturation will not change significantly, while the winter polar cap will have essentially no water vapour above it. The latitudinal gradient of water during winter is thus towards the pole, but is a factor of 5–50 smaller in magnitude than during summer, so that the rate of transport of water from low latitudes to the winter pole is probably low. Also, at the time when water is seeking to move back to the winter pole, additional water is being supplied from the other summer pole, further inhibiting evaporation. Ice will thus tend to accumulate in non-polar regions

The thickness of ice that could accumulate at low latitudes and the fraction of the obliquity cycle during which it would be stable depends on the amount of ice available at the poles and the range of the obliquity variations. Before the formation of Tharsis the mean obliquity was as high as 35° and the peak value as high as 45° (ref. 24). During the highest obliquities of this epoch, 2 km of ice could be removed from the poles in $<10^4$ yr to form ice deposits tens of metres thick in the non-polar latitudes, if that much polar ice is available. Ice may have returned to the poles only at minimum obliquities. During most of Mars' history, however, obliquities ranged from 15 to 35° (refs 24, 25). Net accumulation of ice in non-polar areas is then likely only at peaks in the obliquity cycle, and removal of as much as 2 km of ice from the poles is unlikely. The polar layered deposits could, however, be extensively reworked, as is suggested by the scarcity of superimposed impact craters[5,10].

Meltwater from the ice deposits may have contributed to the erosion of the branched valley networks. For melting temperatures to be reached at the surface by greenhouse warming, atmospheric pressures near 1 bar are required[26]. However, melting of snow or ice does not necessarily require surface temperatures to be above melting. The low thermal conductivity of snow and its partial transparency to sunlight can result in subsurface melting when surface temperatures are well below freezing[27]. Thus, melting can occur in substantially thinner atmospheres than are required by the atmospheric greenhouse effect alone. Another appealing reason to invoke a mechanism that requires high obliquities to explain the valley networks is that most of the networks formed very early in Mars' history[2,28]. At that time, the Tharsis bulge may not have reached its present size. As Tharsis grew, the maximum obliquities reached decreased, and as a result so did the efficacy of the equatorward transfer of water. Young valley networks are, therefore, rare.

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Onset of the south-west monsoon and sea-surface temperature anomalies in the Arabian Sea

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In 1979, the year of the Global Weather Experiment, the atmosphere and ocean of the monsoon region were particularly well observed. For this reason, the onset period of the monsoon, 11–19 June 1979, was selected for international comparison of numerical prediction models[1]. Previous studies[2–4] have demonstrated that successful predictions for this period are difficult to achieve. I report here two examples of numerical predictions that highlight the role of sea-surface temperatures, namely a control forecast using climatological sea-surface temperatures and an anomaly forecast using more realistic (and warmer) surface temperatures specified for the eastern Arabian Sea. It is shown that the use of the more accurate sea-surface temperatures enables a better prediction of the development of a monsoon depression to be obtained.

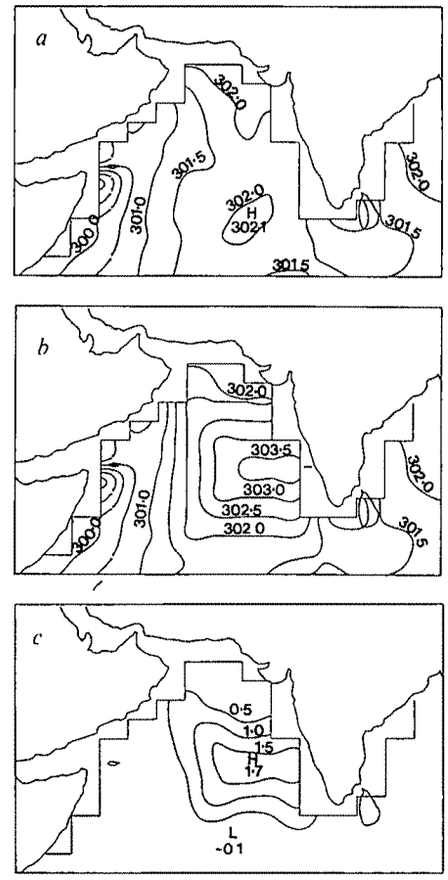


Fig. 1 *a*, Sea-surface temperature for control forecast; *b*, sea-surface temperature for anomaly forecast; *c*, sea-surface temperature anomaly (*b* − *a*). Contour interval, 0·5 K.

For these experiments, the 11-layer global atmospheric general circulation model[5,6] of the UK Meteorological Office was used with a horizontal resolution of 2° lat. and 3° long. The initial conditions for both forecasts were taken from the (level 3B) analysis for 12.00 GMT on 11 June 1979 made by the European Centre for Medium Range Weather Forecasts (ECMWF) using data which had been collected for the Global Weather Experiment. The sea-surface temperatures were fixed throughout the 8 days of each forecast. The values in Fig. 1*a* were derived from a climatological average for the decade 1951–60; those for the eastern Arabian Sea in Fig. 1*b* were based on an analysis by Seetaramayya and Master[7] for 8–15 June 1979. The difference chart (Fig. 1*c*) shows a maximum warm anomaly of 1.7 K in this region.

The onset of the monsoon is characterized by an increase in wind speeds at low levels over the Arabian Sea and the commencement of the rains along the west coast of India. Figure 2*a*, *b* illustrates the changes in the wind field at the 850-mbar pressure level during the onset period. On 11 June the south-westerly jet had a maximum speed of 14 m s^{-1} a few degrees to the east of the Somali peninsula, and the main branch of the monsoon flow was still to the south of India (Fig. 2*a*). During the next 8 days the jet strengthened and extended eastwards to the southern tip of India. A depression formed on the northern flank of the jet and moved first north then west, allowing the monsoon westerlies to advance northwards to cover much of the Indian peninsula and bring the rains to the west coast. By 19 June the maximum speed of the jet was 29 m s^{-1} and the cyclonic vortex of the depression was near the Arabian coast (Fig. 2*b*).

In the control forecast, the jet strengthened and extended eastwards more slowly than actually occurred, to give a maximum speed of only 24 m s^{-1} by 19 June (Fig. 2*c*). A weak vortex developed in the correct location, but the model failed to intensify it and kept it stationary. The anomaly run produced

a much better prediction of the intensification of the vortex and moved it northwestwards. By day 8, 19 June, the centre was at approximately the right latitude but ~5° too far east (Fig. 2*d*). The jet strengthened more than in the control integration, giving a maximum speed of 29 m s^{-1}.

The direct effect of increasing the sea temperatures is to increase the transfer of heat and moisture from the ocean into the atmosphere. Initially the increases are quite small, but after 8 days the differences between the anomaly and control runs (Fig. 3*a*, *b*) show maxima of 54 W m^{-2} for the sensible heat flux and 16 mm per day (460 W m^{-2}) for the rate of evaporation. The higher values in the anomaly forecast are consistent with

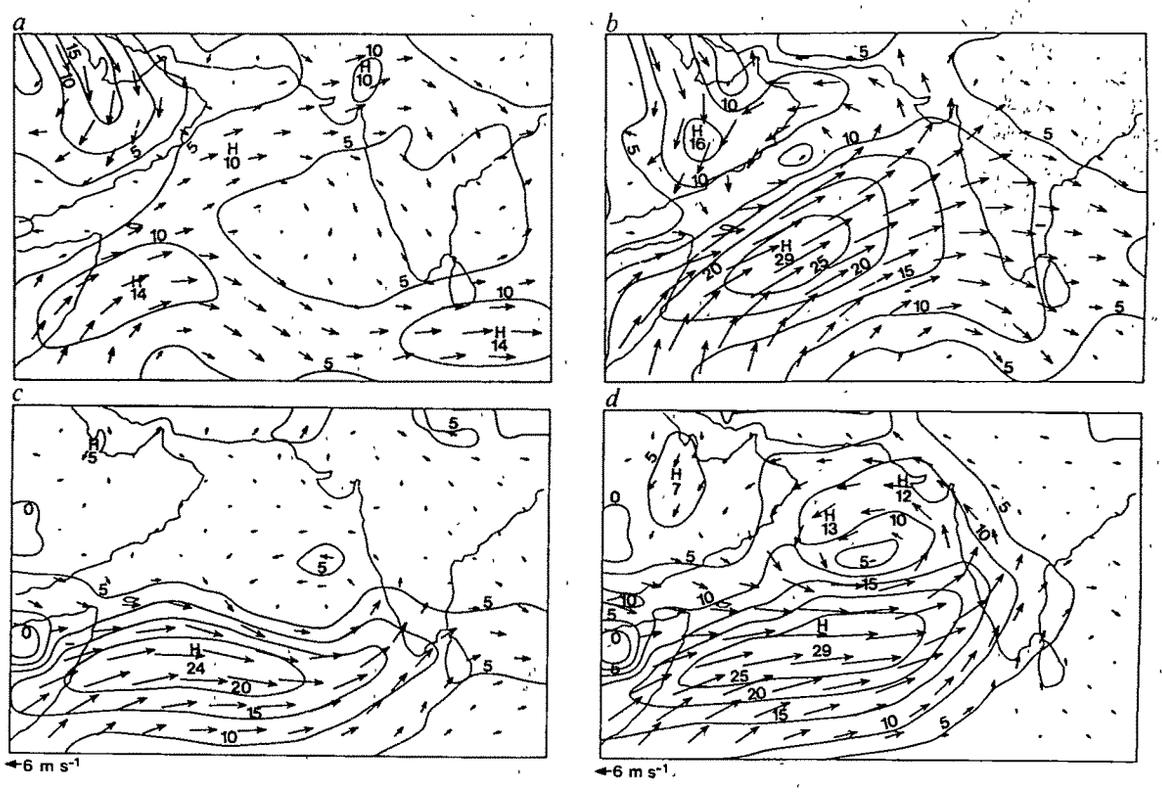


Fig. 2 Wind vectors and isotachs at 850-mbar pressure level (m s^{-1}). *a*, ECMWF analysis for 12.00 GMT 11 June 1979; *b*, ECMWF analysis for 12.00 GMT 19 June 1979; *c*, control forecast for 12.00 GMT 19 June 1979; *d*, anomaly forecast for 12.00 GMT 19 June 1979.

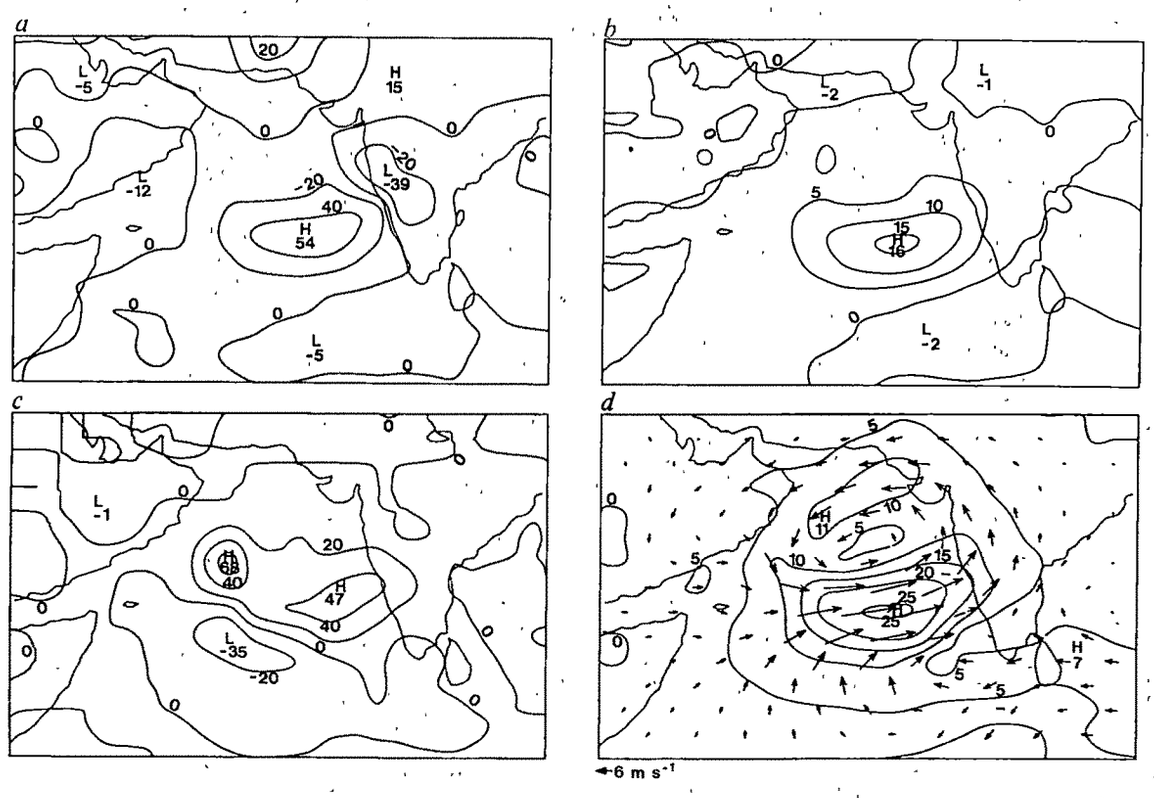


Fig. 3 Anomaly forecast minus control forecast for 12.00 GMT 19 June 1979. *a*, Upward sensible heat flux from surface (W m^{-2}); *b*, rate of evaporation from surface (mm per day); *c*, rate of precipitation (mm per day); *d*, vector wind at 850-mbar pressure level (m s^{-1}).

estimates of the fluxes for the onset period made from ship observations[8]. The changes in surface fluxes induce a substantial increase in precipitation. Figure 3*c* shows a maximum local increase of >65 mm per day after 8 days. This increase in precipitation is sustained partly by the increase in evaporation but mainly by increased moisture convergence. The additional latent heat released into the atmosphere is equivalent to a vertically averaged heating rate of around 16 K per day. This heating is compensated by adiabatic cooling associated with increased ascent. The response of the low-level wind field to these changes is shown in Fig. 3*d*. The main effect is confined to the Arabian Sea, with an asymmetrical cyclone centred to the north of the heating zone; the strongest flow is the westerly directly over this zone, and weak easterlies are induced to the south-east.

Figure 3*d* is qualitatively similar to Gill's[9,10] solutions for the response of a simple linear model to prescribed heating north of the Equator. The vertical structure of the response to the increased sea temperatures is also similar to that imposed in Gill's model. The vertical velocity shows a single peak in mid-troposphere and the flow in the upper troposphere is anticyclonic above the low-level cyclone This correspondence suggests that, in the general circulation model, the latent heat release is the critical factor in promoting the changes in the flow, although the mechanism is not simple. A positive feedback cycle is operating, whereby the increase in latent heat release leads to increased ascent and more moisture convergence at low levels. The ascent causes increased cyclonic flow at low levels, as in the simple model, and the stronger wind increase the surface fluxes of heat and moisture. These effects collectively lead to more precipitation, completing the cycle.

A much better prediction of the development of a monsoon depression over the Arabian Sea was obtained by specifying more accurate sea-surface temperatures in the region where the depression was formed. The strengthening of the low-level jet and the northward movement of the monsoon rains along the west coast of India were also greatly improved in the forecast with the warm anomaly. These features make this prediction the best yet achieved for the onset of the monsoon in 1979. The results have clearly indicated that correct sea-surface temperatures can be essential for accurate numerical weather prediction for the tropics.

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1,200-Myr impact-melting age and trace-element chemical features of the Yamato-790964 chondrite

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Age determinations of impact-produced materials in brecciated meteorites[1] are particulary important for understanding the evolutionary history of meteorites. The age characteristics of these meteorites have been examined mostly by the K–Ar method or, in a few cases[2], by the Rb–Sr method. However, age data are still rare and, except for a few cases[3], the large-ion lithophile (LIL) trace-element chemical features have not been studied in detail. In this respect, the Yamato-79 shocked meteorites are the best specimens for investigation. We report here results of Rb–Sr systematics and trace-element analyses for an almost wholly impact-melted LL-chondrite, Y-790964, one of the Yamato-79 unusual chondrites. The analyses yield an internal isochron age of 1,200±50 (2σ) Myr. The isotopic and trace-element data obtained represent the first clear evidence of the youngest impact-melting and LIL-element fractionations for a complete LL-chondrite, and suggest that the 1,200-Myr-age is a non-trivial marker in the evolution of meteorites.

The meteorite, originally weighing 3.3 kg, has a porous and microcrystalline texture with euhedral, chemically zoned pyroxene and olivine and minor relic chondrules[4,5], and has a similar major-element composition to that of normal LL-chondrites[6]. Two whole rocks from the different portions (A and B) and eight mineral separates from portion A of the meteorite were examined for Rb-Sr systematics, rare-earth elements (REE), Ba, Sr, Rb, K, Mg, Fe and Ca abundances (Tables 1-3). The trace and major elements were determined using the mass spectrometric isotope dilution method similar to that of Nakamura[7] or using an electron probe microanalyser. In additon, major-element compositions in the constituent minerals were analysed for a thin section of the bulk meteorite by an electron probe. Total blanks for all chemical processes were 0.02 ng for Rb and 0.08 ng for Sr, corresponding to <0.2% of the respective elements in most samples except for analyses of the 1 M HCl leached fraction F2-MS. The Rb-Sr data (Table 3), after corrections of the blank, are, in general, quite accurate. Details of our experimental procedures will be published elsewhere[8].

The results for the bulk samples show that abundances of major, minor and trace elements are almost within the range of those in normal LL-chondrites. However, note that two REE patterns, normalized to the average of six normal LL-chondrites, are similar, high and slightly upwardly convex with small negative Eu anomaly, and that abundances of Ba, Rb and K are

Table 1 Elemental abundances in the Yamato-790964 LL-chondrite and the average of LL-chondrites

Element	Yamato-790964, 74 Whole rock A	Yamato-790964, 74 Whole rock B	Average of LL-chondrites
Mg (%)	14.7±0.1	16.1±0.05	15.29*
Ca (%)	1.53±0.01	1.42±0.01	1.25*
K	1220±3	890±5	900†
Rb	3.67±0.01	2.63±0.05	2.5†
Sr	12.09±0.02	11.8±0.1	11.2†
Ba	3.35±0.01	5.45±0.05	3.32
La	0.348±0.003	0.378±0.02	0.331
Ce	0.923±0.008	1.02±0.02	0.865
Nd	0.695±0.005	0.753±0.003	0.623
Sm	0.223±0.002	0.243±0.003	0.200
Eu	0.0794±0.0010	0.0883±0.005	0.080
Gd	0.306±0.005	0.326±0.003	0.274
Dy	0.346±0.006	0.396±0.003	0.338
Er	0.236±0.006	0.257±0.002	0.226
Yb	0.228±0.004	0.256±0.003	0.232
Lu	0.0359±0.007	0.0390±0.006	0.0358

Abundances are expressed in p.p.m. unless otherwise stated.
* Refs 22, 23
† Estimated from frequency distributions using data obtained in this work and from refs 9, 24. Ba and REE abundances are from refs 8, 25

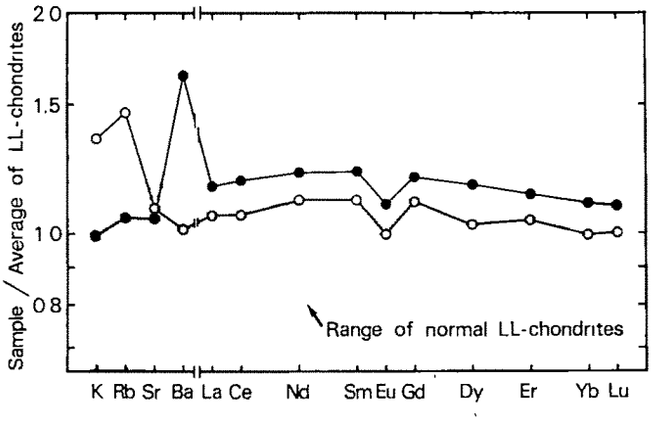


Fig. 1 Trace-element abundance pattern of the Yamato-790964 LL-chondrite. Abundances are normalized to the average of normal LL-chondrites Note that most of the elemental abundances in the meteorite are within the range of normal LL-chondrites but show systematic fractionations in REE, Rb and K from those of normal chondrites WR-A (○) and WR-B (●) are whole-rock samples from portions A and B, respectively.

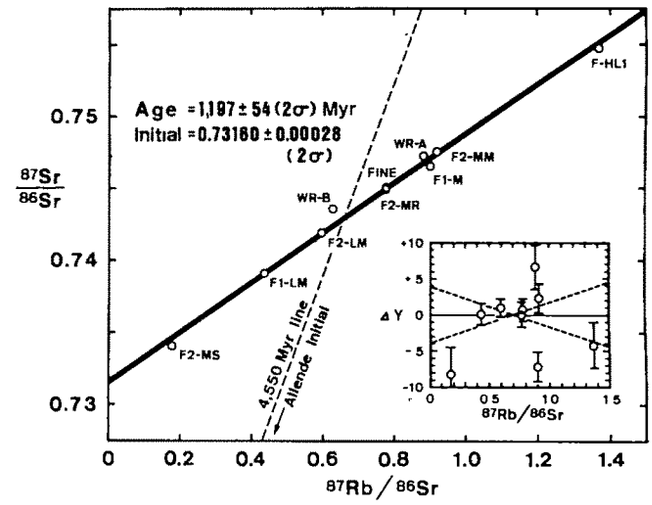


Fig. 2 ^{87}Rb–^{87}Sr evolution diagram of the Yamato-790964 LL-chondrite. Note that data points form roughly an array but show some deviations from the regression line, suggesting the Rb–Sr isotopic system of the meteorite was almost but not completely reset by the 1,200-Myr-melting event.

rather variable. A similar trace-element feature has been found for other Yamato-79 unusual meteorites[8] The variable distributions of alkali metals in LL-chondrites have also been reported for other non-Antarctic LL-chondrites[3,9,24].

A thin section of the meteorite reveals a heterogeneous distribution of numerous vesicles (covering ~20% in the total area) and brown glass or microcrystalline materials (~5% in the area) associated with vesicles. Euhedral, chemically zoned pyroxene ($En_{69}Fs_{24}Wo_7$ at the core, $En_{47}Fs_{15}Wo_{38}$ at the rim) and homogeneous olivine ($Fa_{29}Wo_{71}$) exist within the glass and/or microcrystalline matrix. The glasses show variable chemical compositions, ranging from 50 to 85% of normative Na-rich plagioclase (An30–An10); SiO_2 = 45–61, Al_2O_3 = 23–30, TiO_2 = 0.2–5, FeO = 0.6–6.5, MgO = 0.2–1.6, CaO = 2–7.6, Na_2O = 4–6.5, K_2O = 0.1–0.2%. We could not recognize any particular clast but noted minor relic (radial pyroxene or barred olivine) chondrules and rounded troilite-like opaque minerals in metal grains in some areas. The general petrological features suggest that the meteorite was once heated beyond the metling point of metal/troilite, reaching the melting points of silicate minerals (1,200–1,400 °C)[10] within a short time, and that melting and/or metamorphic recrystallization ensued for the whole stone, followed by rapid solidification.

These chemical and petrological features suggest that the observed variations in absolute abundances of REE, Ba, Rb and K in the bulk samples are due to heterogeneous distributions of glasses which may carry high and variable LIL trace elements. The value of ~10% for the negative Eu anomaly in both bulk samples suggests that the meterorite was related to plagioclase-controlled partial melting. For example, assuming 10–20% equilibrium partial melting of plagioclase+pyroxene (+olivine) in an LL-chondritic source, segregation of the melt from the source and addition (~10%) to a typical LL-chondrite, we would obtain 1.2–1.3 times LL-chondritic REE abundances with 10–15% negative Eu anomaly, which is similar to those of the bulk samples. Hence, the meteorite could represent an impact-produced partial melt that was removed from its point of origin and which assimilated unfractionated material before recrystallization.

In Table 3, except for the leached fraction, the olivine-rich fractions generally have larger $^{87}Rb/^{86}Sr$ ratios while the less olivine-rich and more pyroxene-rich fractions have the smaller $^{87}Rb/^{86}Sr$ ratios. However, the 1 M HCl-leached fraction (F2-MS) with highest normative olivine composition unexpectedly shows the lowest $^{87}Rb/^{86}Sr$ ratio, although the Rb and Sr concentrations are reasonable. There is no obvious explanation for this, but it is possible that low Rb/Sr component(s) were added to the fraction by dissolution (~18%) of the microcrystalline materials. Some minor irregularities of the Rb/Sr system are also noted among other mineral separates, indicating complex effects of Rb–Sr-rich components such as glass on the Rb–Sr systematics of the meteorite. Nevertheless, the relatively large spread of $^{87}Rb/^{86}Sr$ ratios obtained here, ranging from 0.18 to 1.4, is interesting.

In an ^{87}Rb–^{87}Sr evolution diagram (Fig. 2), all the data points obtained roughly show an array. However, the data point of whole rock B deviates markedly from the general trend. Excluding whole rock B, nine data points define a best-fit linear regression line, which yields an age of 1,197 ± 54 (2σ) Myr and an initial $^{87}Sr/^{86}Sr$ ratio of 0.73160 ± 0.00028 (2σ). Despite a large range of values of $^{87}Sr/^{86}Sr$ among the mineral separates, the errors in age and initial ratio obtained here are large. This is apparently caused by dispersion of some data points beyond analytical uncertainties, as shown in the inset to Fig. 2. Note that three samples (F2-MS, F2-MR and F-HL1) were treated with ≤1 M HCl and Clerici's solutions for mineral separation and washing. Although there are minor analytical problems with these samples a close examination of the analytical data suggests that a possible selective leaching of Rb to Sr is not too serious. Also we must consider the potential importance of a weathering effect on the trace-element behaviour in Antarctic samples[11] when evaluating such problems. However, since the specimens analysed in this work were sampled from the inner part of the meteorite and appeared quite fresh under the binocular microscope, the observed isotopic disturbances are considered to be real Specifically, deviations of data points of whole rock (WR) A, B and F1-M are the most significant.

Because the weighted mean ratios of $^{87}Rb/^{86}Sr$ and $^{87}Sr/^{86}Sr$ of mineral separates are similar to those of whole rock A, this rock may roughly represent portion A of the meterotie in terms of Rb–Sr systematics, but not necessarily the total meteorite. Note that the Rb/Sr data of whole rock A ($^{87}Rb/^{86}Sr$ = 0.881), as well as those of whole rock B ($^{87}Rb/^{86}Sr$ = 0.629), fall off significantly from the 4,500-Myr evolution line, suggesting that the meteorite was subjected to severe Rb/Sr fractionation at the time of the 1,200-Myr thermal event. Assuming a two-stage evolution, formation of the meteorite parent body with the same initial $^{87}Sr/^{86}Sr$ ratio as the Allende meteorite (bias-adjusted value = 0.69884)[12] at 4,500 Myr ago, and Rb/Sr fractionation at 1,200 Myr ago, the $^{87}Rb/^{86}Sr$ ratio of the meteroite at the time of formation is calculated to be 0.67. This value is 24% smaller than that of whole rock A but is similar to the $^{87}Rb/^{86}Sr$ ratio

Table 2 Major element and estimated mineral composition for specimens from the Yamato-790964 LL-chondrite

Sample		MgO (%)	FeO (%)	CaO (%)	Ol (%)	Opx (%)	Cpx (%)	Gls (%)	Other (%)
Whole rock									
WR-A	Chip from portion A	24.4	—	2.14					
WR-B	Chip from portion B	27.7	—	2.00					
Mineral separates from portion A									
<300 mesh									
Fine	Suspended in acetone	—	—	—					
F1-LM	Less magnetic	21.8	18.1	3.23	25	18	35	5	17
F1-M	Magnetic	25.1	22.4	1.57	54	11	11	7	17
300< <200 mesh									
F2-LM	Less magnetic	25.2	18.1	3.52	28	25	37	5	5
F2-MB	Magnetic (bulk)	27.5	22.4	1.51	63	8	11	3	15
F2-MR	Magnetic (residue)*	21.8	14.5	3.37	11	37	35	11	6
F2-MS	Magnetic (leached)*	31.4	27.8	0.34	(83	=0	=0	=0	18)
F2-MM	More magnetic	26.3	22.8	1.63	56	12	10	5	17
F-HL1	Density-separate†	31.3	26.4	0.93					

Major elements were determined by an electron probe microanalyser for 100–300 grains from the mineral separates except for samples of WR-A, WR-L, F2-MS and F-HL1, which were analysed by isotope dilution. Mineral compositions were estimated from the distribution of the major-element composition for each grain. Ol, Olivine; Opx, orthopyroxene; Cpx, clinopyroxene; Gls, glass; other, microcrystalline materials + metal + troilite.

* Soluble (F2-MS) and insoluble (F2-MR) fractions obtained by leaching F2-MB with 1M HCl for 80 h at room temperature.

† Separated by Clerici's solution ($3.37 < \text{density} < 3.45\ \text{g cm}^{-3}$) and washed with dilute HCl before analysis.

of most LL-chondrites[9,24]. Therefore, we suggest that the meteorite had a typical LL-chondritic Rb/Sr ratio between 4,500 and 1,200 Myr and that an enrichment of Rb relative to Sr occurred 1,200 Myr ago. Such a Rb/Sr fractionation is in accordance with the melting-assimilation model deduced from the REE data mentioned earlier, and is also consistent with the trace-element data for the associated meteorites[8]. However, the inconsistent behaviour of Rb/Sr with the REE which is observed for whole rock B, and the lack of complete isotopic equilibration of both whole rocks (A and B shift to the left of 1,200-Myr isochron) indicate that the effects of the 1,200-Myr thermal event on the Rb/Sr systematics of the meteorite were very complex. Considering such isotopic evidence, together with the REE, Ba and K data, we suggest that the chemical and petrological characteristics of the meteorite are best explained as the result of intense impact(s) which yielded partial melting and assimilation of the regolith on the LL-chondrite parent body ≤1,200 Myr ago.

It is interesting that the 1,200-Myr age obtained for the Yamato-790964 meteorite is the youngest so far reported for impact-produced lithic materials in brecciated meteorites. Most such meteorites show significantly older ages (>3,400 Myr)[12–14] with the exception of the H-clast in the St Mesmin LL-chondrite which shows a 1,400-Myr K–Ar age[15]. Moreover, except for SNC meteorites, the Yamato-790964 LL-chondrite would be the first complete stone to show the latest crystallization age.

Because no chemical and petrological similarities seem to exist between the LL-related chondritic material mentioned above and SNC meteorites, a possible genetic relation between the two may be ruled out. The 1,300-Myr age obtained for SNC meteorites[16–21] has been explained as a crystallization age which marks extensive volcanic activity on a well-differentiated planet such as Mars[20,21]. On the other hand, the 1,200–1,400 Myr age obtained for the Y-790964 and St Mesmin meteorites is interpreted as signifying a time of strong impact activity on the LL-chondrite parent body (asteroidal planet). How are these facts related? We are increasingly puzzled that all the major young perturbing events observed for meteorites have an age close to 1,200– ~ 1,400 Myr. As the number of results increases, one should examine whether this is a real effect of a major impact-related event on meteorite parent planets or a sampling bias. In the former case, like the 4,000-Myr lunar cataclysm, one should look for collision and storage processes lasting for billions of years.

In any case, in view of the large amount of the similar types of meteorite found in the Yamato Mountain area, Antarctica, we suggest that the 1,200-Myr age obtained here is a non-trivial marker in the evolution of meteorites.

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Table 3 Results of Rb–Sr analyses for the whole rocks (WR) and mineral separates from the Yamato-790964 LL-chondrite

Samples	Weight (mg)	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{86}Sr$
WR-A	52	3.669	12.09	0.881	0.747205 ± 128
WR-B	50	2.559	11.82	0.6285	0.743553 ± 53
Fine	48	3.229	12.02	0.7802	0.745024 ± 32
F1-LM	12	1.517	10.10	0.436	0.739087 ± 59
F1-M	76	3.475	11.19	0.902	0.746516 ± 44
F2-LM	21	2.117	10.28	0.598	0.741913 ± 30
F2-MR	29	7.046	26.33	0.777	0.744918 ± 32
F2-MS	75*	0.0433	0.705	0.178	0.734080 ± 250
F2-MM	54	4.171	13.20	0.9178	0.747503 ± 50
F-HL1	51	2.297	4.90	1.363	0.754679 ± 68
NBS 987 standard (mean of 10 runs)					0.710212 ± 21†

Isotopic analyses were carried out at Okayama University using a mass spectrometer model MAT-261. Precisions for $^{87}Rb/^{86}Sr$ are estimated to be better than 0.8% except for the F2-MS. $^{87}Sr/^{86}Sr$ ratios are normalized to $^{86}Sr/^{88}Sr = 0.1194$. Errors correspond to last digits and are at the 95% confidence limit.

* Calculated weight which contains 10% uncertainty.

† Ratio is in agreement with our previous analysis[19].

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Melting of peridotite to 14 GPa and the genesis of komatiite

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It is generally accepted that the Earth's upper mantle is made of peridotite[1,2]. In volatile-absent conditions, partial melting of mantle peridotite close to the solidus yields a variety of basaltic melts that are progressively more olivine-normative with increasing depth[3,4]. At 3–4 GPa (30–40 kbar), the highest pressures so far investigated, the melts are picritic with up to 20 wt% MgO (ref. 5). Basaltic magmas that are dominant on the Earth's surface today, therefore, are considered to have been generated at relatively shallow depths (< 100 km)[1–6]. Here we extend the pressure range of the melting experiments to 14 GPa and show that melts close to the peridotite solidus at 5–7 GPa are no longer basaltic but are komatiitic with >30 wt% MgO. Based on these results, we describe a model for the genesis of komatiite magmas by partial melting at depths of 150–200 km in the Archaean upper mantle.

The starting materials for the experiments were two lherzolites (Table 1). KLB-1 is a protogranular spinel lherzolite from Kilbourne Hole[7], New Mexico, and PHN1611 is a sheared garnet lherzolite from Thaba Putsoa[8], Lesotho. Both may represent undepleted mantle material and are close to pyrolite-III[9] in composition. Garnet lherzolite PHN1611 has been previously investigated at pressures ≤3.5 GPa (refs 10–13).

Crushed powders (<5 μm) were dried at 1,000 °C at the NNO (Ni–NiO) buffer. Wire loop experiments[14] were performed at 1 atm in a controlled CO_2–H_2 atmosphere close to the NNO buffer. Experiments at 1.5 and 3 GPa were carried out with a piston-cylinder apparatus[15] using a 0.5-inch talc–Pyrex assembly. Run conditions, methods and procedures are similar to those described in ref. 5.

At 5 GPa and above, a split-sphere apparatus[16] at Okayama University was used. Up to 7.5 GPa, a graphite furnace assembly was inserted into a pressure medium of semi-sintered MgO in the shape of an octahedron with an 18-mm edge length. Above 7.5 GPa, $LaCrO_3$ was used as the furnace element in a 14-mm-edge octahedron of MgO (Fig. 1*a*). Temperatures were monitored with W–Re thermocouples and have uncertainties of

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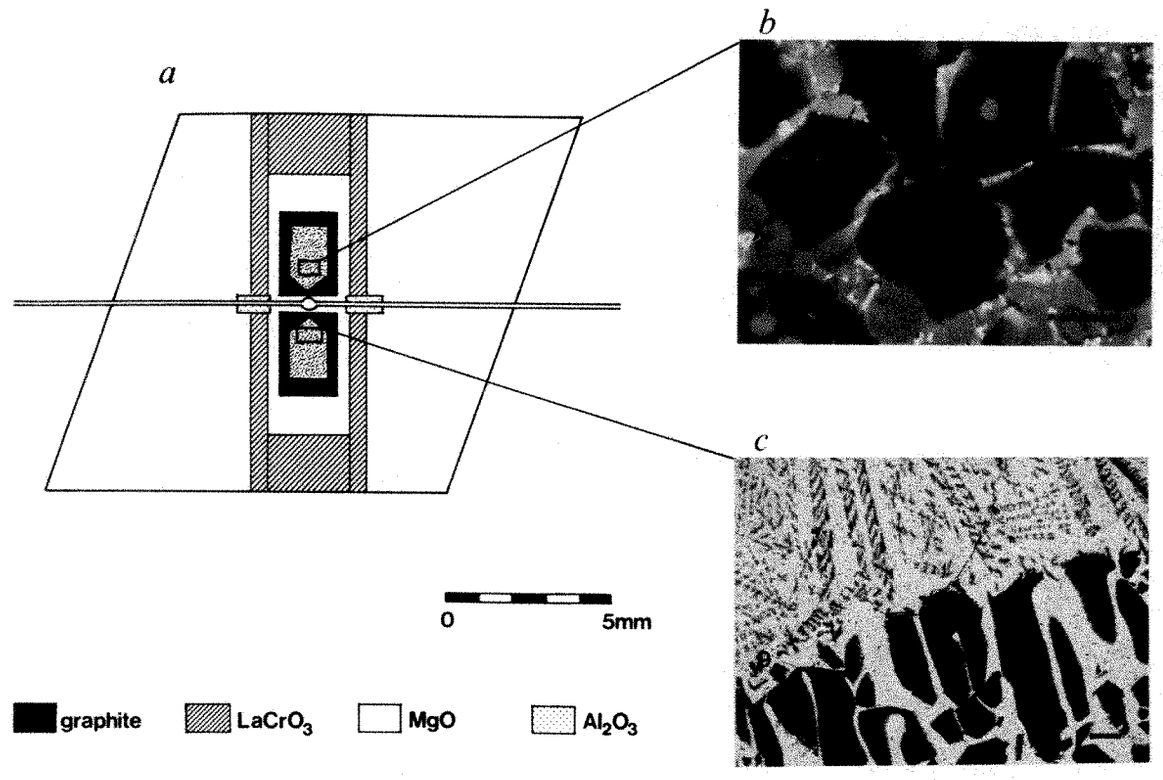


Fig. 1 *a*, Cross-section of the MgO pressure medium with the furnace assembly, thermocouple and sample in place. This design was used at pressures ≥7.5 GPa; below 7.5 GPa a graphite furnace with a fired pyrophyllite sleeve was used. *b*, Back-scattered electron image of the charge from an experiment at 7.5 GPa and 1,900 °C. The melt (now consisting of white areas of iron-rich quench pyroxenes) occurs in pockets between olivine (large dark grains) and garnet (small grey grains). *c*, Back-scattered electron image of the charge from an experiment at 5 GPa and 1,800 °C. The segregated melt is seen at the top with coexisting olivine crystals below the interface. The melt region shows abundant well-developed quench olivine and pyroxene. Scale bars in *b* and *c*, 10 μm.

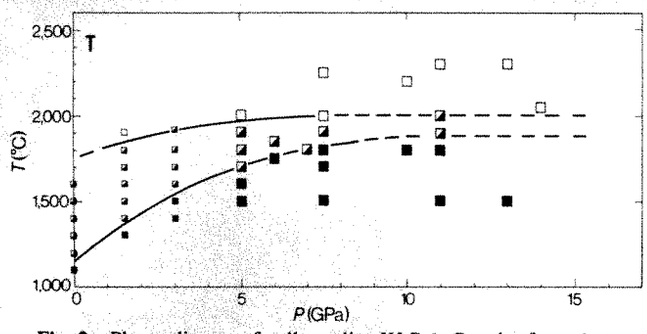


Fig. 2 Phase diagram for lherzolite KLB-1. Results from 1 atm and piston-cylinder experiments indicated by small squares; results from split-sphere experiments indicated by larger squares. ■, Subsolidus; ◪, crystals + liquid; □, superliquidus.

±50 °C due to the temperature gradient within the furnaces[17]. Pressures were calibrated at several fixed points at room temperature [Bi(I–II), Bi(III–V) and Pb(I–II)] and at high temperatures (quartz–coesite, fayalite–γ spinel, coesite–stishovite and forsterite–β spinel). Uncertainties in pressures are estimated to be <0.05 GPa up to 3 GPa, and 0.5 GPa above 3 GPa. Experiments above 5 GPa were typically 10 min in duration.

Figure 2 shows the solidus and liquidus curves for KLB-1. At 1 atm the partial melt region is ~600 °C, whereas it contracts to ~100 °C at 10 GPa as predicted by Herzberg[18]. Olivine is in the liquidus phase at all pressures. The second liquidus phase, however, changes with increasing pressure: chromian spinel (1 atm), Ca-poor orthopyroxene (0.5–3 GPa), pigeonitic clinopyroxene (3–5 GPa) and pyrope-rich garnet (5–11 GPa). Figure 1*b* shows the distribution of melt coexisting with olivine and garnet in an experiment at 7.5 GPa and 1,900 °C. The melt is confined to small isolated pockets in triple junctions between the crystals. Figure 1*c* shows melt, which has segregated from the matrix, coexisting with olivine only at 5 GPa and 1,800 °C. In experiments using the split-sphere type apparatus, segregation of the partial melts during short runs was probably due to the thermal gradient and/or stress gradient in the furnace assemblies.

The chemical compositions of partial melts formed within 50 °C of the dry solidus are shown in Table 1. To avoid the modification of melt compositions, during quenching[5,19], only segregated partial melts were chemically analysed. Because high-MgO melts do not quench to a glass, but crystallize with well-developed spinifex textures (Fig. 1*c*), a defocused electron beam was used for their analysis and the compositions of quench crystals in an area >100 μm were averaged. The melts become increasingly MgO-rich or olivine-normative as pressure increases, and all melts formed above 5 GPa may be classified as peridotitic komatiite in terms of their high MgO content and the chondrite-like abundance of CaO and Al_2O_3 (refs 20, 21). Melts near the solidus at ~5 GPa contain ~30 wt% MgO and are very close to the primary komatiite magma composition estimated by Arndt *et al.*[22] (Table 1).

Preliminary results for PHN1611 are similar, although because of lower MgO/FeO and higher $Na_2O + K_2O$ content, the solidus is displaced to slightly lower temperatures. When compared at the same pressure (P) and temperature (T), partial melts from PHN1611 are enriched in FeO and higher in CaO/Al_2O_3 than those from KLB-1 (Table 1).

The origin of komatiite magmas has been discussed by several authors[6,20–28]. Komatiite has an unusually high liquidus temperature (>1,650 °C)[23] at 1 atm and at low pressures. A very high degree of partial melting (50–70%) of peridotite is required to form a komatiitic liquid[6,21,23]. Both theoretical[29,30] and experimental observation[25] indicate, however, that partial melt will segregate from the peridotite matrix instantly when the degree of partial melting exceeds a critical value which lies between 10 and 30 vol.%. Thus, it is unlikely in static conditions that a very high degree of partial melting can be achieved within the Earth.

A multistage melting model has been proposed by Arndt[25] in which komatiitic magma is formed in a rising diapir after several cycles of partial melting and melt extraction. The possibility of catastrophic magma genesis induced by impacts of large meteorite bodies has also been proposed[24]. Furthermore, a deep-seated magma ocean in the Archaean mantle has been invoked[26] to explain komatiite petrogenesis.

On the basis of the present experimental results, we propose a simple model (Fig. 3) for the derivation of komatiite magma by partial melting of a rising peridotite diapir as it intersects the mantle solidus at a depth of 150–200 km (path A–B). Because initial melts at these depths have komatiitic compositions, it is possible that komatiitic melts segregate and ascend to the surface (path B–C) before the degree of partial melting of the source region reaches a high percentage. Similar models have been proposed for the genesis of picritic (path D–E–F) and basaltic (path G–H–I) magmas at shallower depths[3] and the possibility of komatiite magma generation by partial melting of garnet peridotite at very high pressures (>5 GPa) has already been proposed[27,28].

Because melts of komatiite composition are among the highest temperature magmas known, they may be derived from the deepest levels of magma generation. These depths may be limited

Table 1 Chemical compositions of the starting materials and partial melts

	Starting materials			Peridotite partial melts						Average peridotitic komatiites	
	KLB-1	PHN1611	Pyrolite	P (GPa) ($\times 10^{-4}$)	3.0	5.0	6.0	6.0*	7.0	MgO > 30 wt%	<30 wt%
				T (°C) 1,200	1,550	1,700	1,850	1,850	1,800		
SiO_2	44.48	44.54	45.20	54.2	46.9	47.2	46.3	45.8	48.0	44.9	46.0
TiO_2	0.16	0.25	0.71	0.65	0.92	0.15	0.18	0.38	0.11	0.19	0.32
Al_2O_3	3.59	2.80	3.54	15.1	11.0	5.1	4.8	4.5	3.7	5.3	7.4
FeO(t)	8.10	10.24	8.47	5.6	7.8	9.3	8.9	11.4	7.8	10.4	11.5
MnO	0.12	0.13	0.14	0.09	0.20	0.12	0.17	0.20	0.13	0.18	0.22
MgO	39.22	37.94	37.48	8.4	19.2	31.3	32.9	31.3	35.3	33.6	26.5
CaO	3.44	3.32	3.08	12.3	12.2	4.8	4.4	4.6	3.1	5.0	7.4
Na_2O	0.30	0.34	0.57	2.05	1.20	0.49	0.46	0.52	0.26	0.35	0.45
K_2O	0.02	0.14	0.13	ND	ND	ND	ND	ND	ND	0.08	0.10
P_2O_5	0.03	ND	0.06	ND	ND	ND	ND	ND	ND	ND	ND
Cr_2O_3	0.31	0.29	0.43	0.14	0.43	0.46	0.41	0.37	0.41	0.26	0.39
NiO	0.25	ND	0.20	ND	ND	ND	ND	ND	ND	0.02	0.01
Total	100.02	99.99	100.01	98.5	99.9	98.9	98.5	99.1	98.8	100.28	100.29
				Fo^{oliv}	89.9	92.6	94.1	94.4	92.8	—	
				$K_D(Fe/Mg)^{oliv}_{liq}$	0.30	0.35	0.38	0.39	0.38	—	

Analysis for KLB-1 by H. Haramura. Compositions of PHN1611, pyrolite-III and periodotitic komatiite are from refs 8, 9, 22. Partial melts were analysed by EPMA (electron probe microanalyser) using a defocused 50-μm beam diameter; the coexisting olivines were analysed using a focused-beam, 15-kV accelerating voltage, beam current of 2.0×10^{-8} A and counting times of 40–70 s.

* Peridotite partial melt formed from PHN1611.

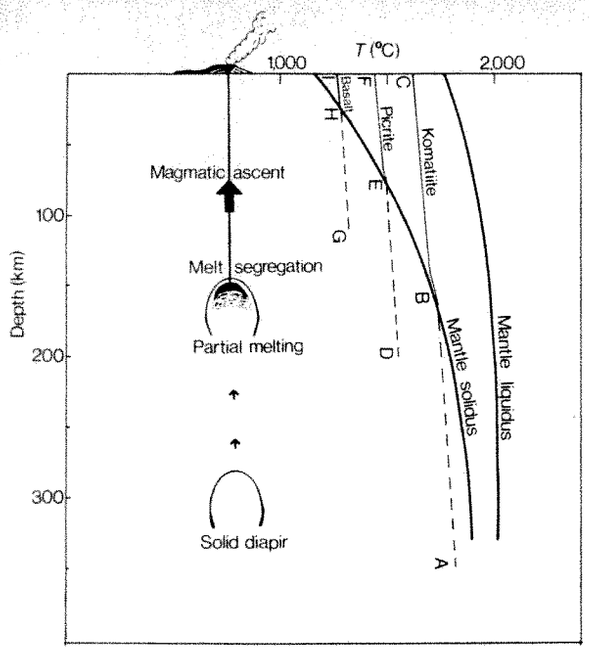


Fig. 3 Diapiric model for the generation of komatiite magma at depths of 150–200 km.

by the density crossover between komatiite melts and coexisitng residual mantle solids[28,29]. Komatiite magmas are not common in the Phanerozoic[21], presumably either because the temperature in the Archaean upper mantle was higher than in more recent geological time, or because diapirs originated from greater depths in the Archaean.

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Preparation and catalytic properties of ionic sodium clusters in zeolites

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Exposure of high-alumina zeolites to sodium metal vapour produces coloured centres[1–5] which have been associated with the formation of ionic sodium clusters, located in the zeolite cages. To form these centres, excess alkali metal is required, which contaminates the reaction vessel and makes the system unsuitable as a heterogeneous catalyst. The pioneering work of Pines[6] in the area of basic catalysis on sodium metal deposited on alumina implies that ionic clusters in zeolites should exhibit interesting catalytic properties, but there have been no reports of such activity because the procedure yields a material which is difficult to reproduce. Here we present a new method for the preparation of such clusters in zeolites, consisting of the impregnation of a dehydrated zeolite with alcoholic solutions of sodium azide, followed by the controlled decomposition of the azide. As demonstrated by electron-spin resonance (ESR) spectroscopy, ionic sodium clusters are formed in the zeolite pores. These clusters exhibit catalytic properties in both isomerization and hydrogenation reactions of alkenes and alkynes. We believe that this represents the first realistic method for the preparation of alkali metal clusters in zeolites as well as the first catalytic characterization of such systems.

In the present experiments we set out to develop a reliable method for the preparation of ionic sodium clusters in zeolites and to determine the catalytic properties of such material. The preparation method followed Fejes *et al.*[7–9] who reported that the residual acidity of Y zeolites, existing after cation exchange with Na^+ ions, could be neutralized on heating a physical mixture of the zeolite with small amounts of sodium azide (amounts up to 1.12 mmol of NaN_3 per g of zeolite Y were used). We found, however, that when increasing quantities of this product are impregnated on the zeolites A, X and Y using the specific procedure outlined below, this zeolite is turned gradually into a very efficient basic heterogeneous catalyst.

Typically, to 1 g of NaY zeolite present in the form of powder or as 0.8–1-mm agglomerates, and dried at 723 K under a flow of inert gas, was added under inert atmosphere a slurry containing 3.85 mmol of sodium azide and 9.12 mmol of methanol. The volume of this slurry is just sufficient to fill the zeolite pore volume. This wet cake was transferred to an ESR cell or a tubular flow reactor. The water-free cake was first activated *in situ* at a temperature of 523 K and then at 673 K, while the system was continuously purged with an inert gas. In the tubular reactor, the following catalytic conversions were followed: geometrical and double-bond isomerization of *cis*-2-butene and hydrogenation of acetylene, benzene and *cis*-2-butene. The ratio of the product isomers in the isomerization of *cis*-2-butene can be used as a criterion to determine whether the reaction is acid or base-catalysed[6,10–14].

Figure 1*A* illustrates how the isomerization activity of NaY first declines on addition and decomposition of increasing amounts of NaN_3, and then increases. When an average of eight molecules of NaN_3 are impregnated per supercage of zeolite (3.85 mmol per g of zeolite Y), an activity pattern with two maxima is obtained (Fig. 1*B*). The catalysts with an activity maximum at low temperature have a bright-red colour and exhibit an ESR spectrum having 13 lines with $g = 2.003$ and $a = 3.35 \pm 0.10$ mT (Fig. 2*A*). This spectrum has been ascribed to an ionic Na_4^{3+} cluster[2]. The cluster signal is accompanied by a single broad line at $g = 2.078$. This signal has not been reported in previous work and is, for the moment, assigned to large alkali metal particles located in the zeolite supercages or at the outer surfaces of the zeolite crystals. On thermal sintering, a single line at $g = 2.003$ appears (Fig. 2*B*); this signal is close to that

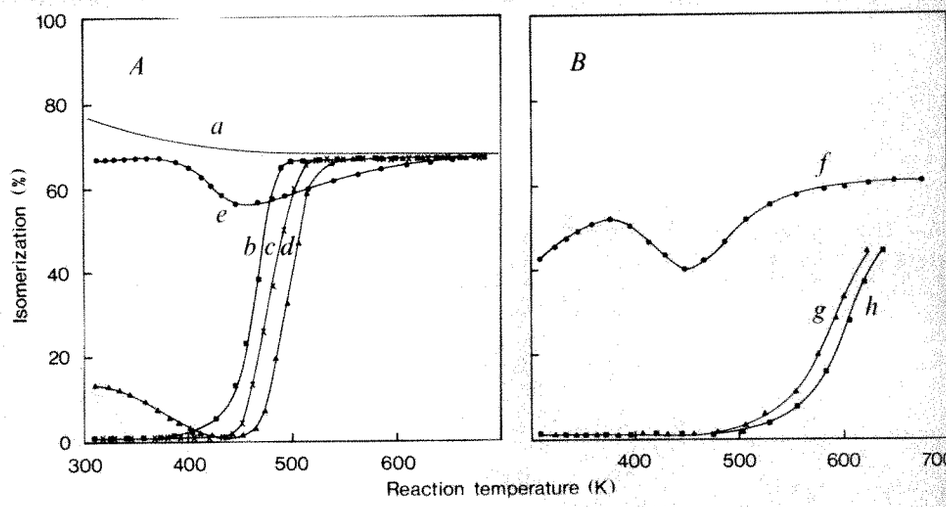


Fig. 1 Isomerization of *cis*-2-butene over dehydrated NaY zeolites: *A*, impregnated with increasing amounts of NaN_3, using a volume hourly space velocity (VHSV) of 36, and *B*, impregnated with 3.85 mmol of azide per g of zeolite at a VHSV of 100. *a*, The thermodynamically expected equilibrium conversion; *b*, the isomerization activity of the parent NaY and its activity after addition of *c*, 1.54; *d*, 2.88; and *e* or *f*, 3.85 mmol of azide per g of zeolite; *g*, after thermal treatment of *f* at 700 K; *h*, after treatment of *f* with oxygen at room temperature and heating in an inert atmosphere at 673 K.

reported for bulk metal[5]. Extended sintering ultimately causes the disappearance of the signal ascribed to the ionic cluster. During this treatment, the broad line at $g = 2.078$ is unaffected while the isomerization activity varies in parallel with the intensity of the signal, ascribed to the existence of the ionic cluster. On exposure to oxygen of the red catalyst (at room temperature) its colour, low-temperature catalytic activity and the 13-line ESR spectrum vanish instantly. The disappearance of the other ESR signals requires longer exposure times.

The initial 1- to *trans*-2-butene product ratios from *cis*-2-butene at low conversions are given in Table 1. The values of these ratios increase in parallel with the amount of NaN_3 added to zeolite Y. If the initial 1-butene to *trans*-2-butene ratios are >2, the *cis*-2-butene isomerization is catalysed by basic sites[6,10–14]. This behaviour confirms that on addition of increasing amounts of NaN_3 to NaY zeolite, residual acidity first has to be neutralized and eventually basic catalytic centres form. We associate this high base-catalysed activity with the presence of the ionic sodium clusters. The residual activity observed after exposure of the catalyst to oxygen is associated with the sodium metal particles located either in the supercages of the Y zeolite or at the external surface of the zeolite crystals. The presence of ionic clusters, as evidenced by the 13-line ESR spectrum and the high catalytic activity, is extremely sensitive to the presence of residual oxygen and water molecules. Therefore, the catalytic sites are definitely sodium clusters and not sodium oxide or hydroxide species which are dispersed in the zeolite cages.

The 13-line ESR spectrum was observed by Harrison *et al.*[5] only for zeolites containing sodalite units on exposure to sodium

Table 1 Initial ratios (R) of 1-butene to *trans*-2-butene from *cis*-2-butene at low conversions (~1%) and low reaction temperature (325 K)

Catalyst*	*R*
a	0.05
b	0.98
c	0.83†
d	2.63†
e	8.65‡

* Catalysts of Fig. 1. † VHSV = 36. ‡ VHSV = 250.

Table 2 Hydrogenation activity of NaY zeolites containing initially 3.85 mmol NaN_3 per g of zeolite

Reaction	VHSV	Reaction temperature (K)	% Conversion
Hydrogenation butenes	125	348	36
Hydrogenation acetylene	80	343	10
Hydrogenation benzene	76	345	0

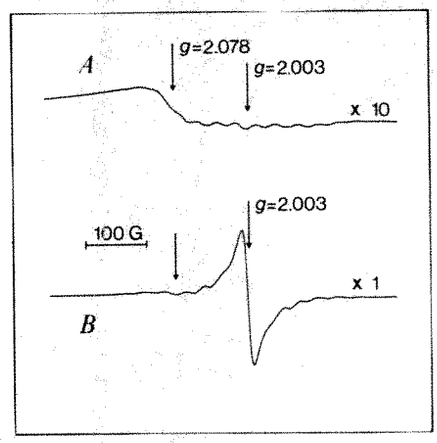


Fig. 2 ESR signals from dehydrated NaY after impregnation with 3.85 mmol NaN_3 per g of zeolite and after subsequent thermal activation (*A*) or after sintering of *A* in an inert atmosphere at 673 K (*B*).

metal. They concluded, therefore, that the sodium ionic clusters are located in the hidden cages of these zeolites. At this stage, it is not clear how electron transfer from the hidden ionic clusters to the butene molecules occurs, or how this view can be reconciled with the high catalytic activity, but we have ample evidence that the high-base catalysed activity in the butene isomerization appears in parallel with these ionic clusters.

Table 2 shows that the catalysts containing ionic clusters are also able to hydrogenate butene and acetylene at non-negligible rates, while benzene in the same conditions cannot be hydrogenated. Peculiar properties of these catalysts are: (1) 50% of the products obtained from *cis*-2-butene, are saturated (butane); the other 50% are feed isomers. This indicates that hydrogenation as well as isomerization occurs at comparable rates. (2) Hydrogenation of acetylene on these basic centres is highly selective as the ethylene/ethylene + ethane ratio is close to 85%.

When the NaN_3 method is used with zeolite X, an intense blue colour, a 19-line ESR spectrum associated with a Na_6^{5+} cluster[1] and a pronounced low-temperature catalytic activity is found. To a minor extent, this is also true for zeolite A, while with other zeolite supports such as Na-ZSM-5 and mordenite, no colour is observed, nor is an ESR signal with $g = 2.003$ detected. Such samples also show no low-temperature basic activity.

In this respect, we have been unable to reproduce the catalytic activity of mordenite containing only Na^+ ions in hydrogenation reactions, as reported by Minachev[15]. These results confirm that either the ionic clusters are stabilized in the sodalite cages as suggested by Harrison *et al.*[5], or they are not formed in high-silica zeolites, so the virtue of A, X and Y zeolites may be the close

presence of several acid sites, due to the low Si/Al ratio, which can form a suitable counterion to the ionic cluster Na_4^{3+}. In view of the latter possibility, it is no longer necessary to assume the presence of such clusters in hidden locations and direct contact of the reacting hydrocarbons is possible with these clusters.

We have shown that on impregnation of appropriate quantities of NaN_3 into the high-alumina zeolites A, X and Y, and on its subsequent thermal decomposition in the specific conditions described, ionic sodium clusters are formed which exhibit typical basic catalytic properties at low reaction temperatures.

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Novel pathway connecting the outer and inner vertebrate retina

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In many fish retinas, thin axons from the external horizontal cells extend through the inner nuclear layer and expand into large terminal processes that lie along the border of the inner nuclear and inner plexiform layers[1]. Although the horizontal-cell axon terminals are structurally very prominent, their function is unknown[2]. Here we report morphological and functional evidence that signals from catfish (*Ictalurus punctatus*) horizontal-cell axon terminals can be transmitted directly to amacrine cells. Current injected into horizontal-cell axon terminals produces responses from both transient and sustained amacrine cells very similar to those elicited by light stimuli. Electron microscope observations show chemical synapses from the axon terminals onto amacrine cell perikarya and processes. These data suggest that amacrine cells in the catfish retina receive two inputs, one from bipolar cells and the other from horizontal-cell axon terminals.

For electron microscopy, standard fixation and staining procedures were used[3]. Fourteen sets of serial sections (200-400 sections per set) from five retinas were examined. The horizontal-cell axon terminals are easily recognized in the electron microscope because of their size and the abundance of microtubules[4]. We observed typical chemical synapses made by the terminals onto postsynaptic elements in the inner nuclear layer and along the border of the inner plexiform layer. Approximately two-thirds of the postsynaptic elements were identified as amacrine cell processes or perikarya because they contained pale cytoplasm and scattered synaptic vesicles and, occasionally, made conventional synapses. Furthermore, serial section tracing did not reveal any synaptic ribbons in the postsynaptic elements, or processes associated with these elements which extended towards the outer plexiform layer. Figure 1 shows an example of a horizontal-cell axon terminal synapsing onto the cell body of a presumed amacrine cell in the inner nuclear layer. The synapse is characterized by an aggregation of round synaptic vesicles associated with increased density on both the pre- and postsynaptic membranes. A subsurface cistern frequently occurred along the postsynaptic site. One such synapse was found, on average, in each 60-μm^2 area of contact made by the horizontal-cell axon membrane with the amacrine cell perikarya and elements in the inner plexiform layer. We have so far observed a total of 18 such contacts, 2 on cell perikarya and 16 on processes.

For physiological experiments, extrinsic current was injected into horizontal-cell axon terminals in the eyecup preparation while recordings were made from amacrine cells. Two intracellular electrodes with tips separated by ~0.4 mm were used; one electrode injected current into the horizontal cells while the other recorded responses from amacrine cells. In the catfish retina only one type of horizontal cell receiving inputs from cones has been reported; its perikaryal response shows a monophasic receptive field of ~0.5 mm in halfwidth and its axon-terminal response shows a receptive field with a halfwidth of ~1 mm (refs 5, 6). This difference in receptive-field size, which may be demonstrated by either a travelling or random bar of light[6], enabled us to identify unambiguously the site of recording. Two types of amacrine cell response can be identified in the catfish retina; one type produces a sustained response and is referred to as sustained or type-N amacrine cell and the other produces a transient response and is termed transient or type-C amacrine cell[7-9].

We performed current injection experiments in six retinas and observed that current injected into the horizontal-cell axon terminals always produced responses in both the transient (15 pairs) and sustained (15 pairs) types of amacrine cells[8-10]. Examples are shown in Fig. 2 in which responses from both types of cells were produced by both light and current injected into the horizontal-cell axon terminal. Records *a* and *b* were from a depolarizing sustained (type-NA) amacrine cell

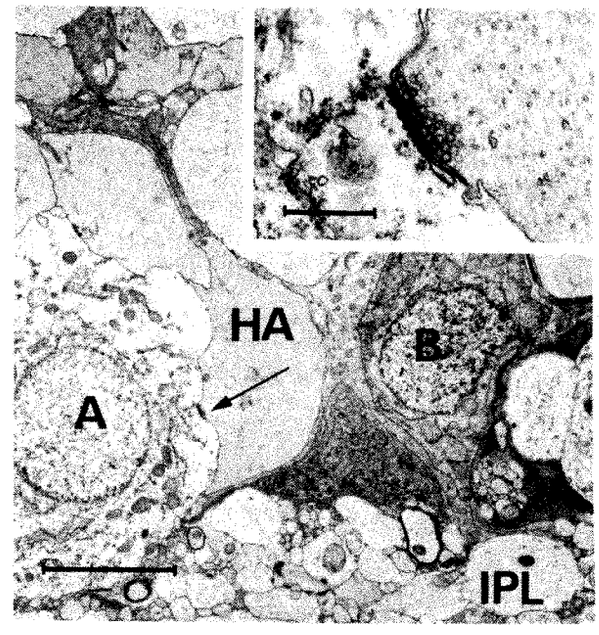


Fig. 1 Electron micrograph of cross-section of a catfish retina showing a synapse from a horizontal-cell axon onto the amacrine-cell perikaryon. Arrow, synapse; A, amacrine cell; HA, horizontal-cell axon; B, bipolar cell; IPL, inner plexiform layer. Scale bar, 5 μm. Inset, high magnification of the synapse indicated by the arrow. Scale bar, 0.5 μm.

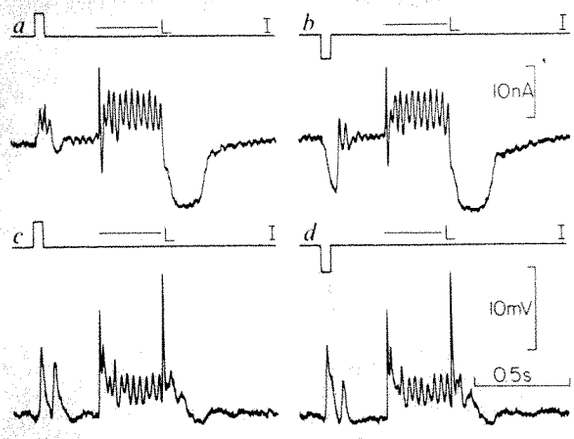


Fig. 2 Responses recorded from sustained or type-N (*a*,*b*) and transient or type-C (*c*,*d*) amacrine cells. The responses are typical of catfish amacrine cells as reported previously[7,8]. Responses were evoked by a current (I) pulse followed by a light flash, the approximate timing of which is shown by the bar marked L. Quantitative analyses of latency of current or light responses have been described elsewhere[6]. The type-N cell shown is the depolarizing variant (type NA) but current injected into the horizontal-cell axon produced similar responses (with the polarity of slow potential reversed) from the hyperpolarizing variants (type NB). Note that, in the type-N cell, the polarity of the current-induced slow potentials reversed when the polarity of current was reversed. Oscillations were superposed on the depolarizing phase of the response. Some type-C cells produced oscillations as seen in *c*, *d*, but some did not. Calibrations apply to all records.

whose light-evoked responses were characterized by an initial depolarizing transient followed by oscillatory wavelets. There was also a very large afterhyperpolarization at the cessation of illumination. A depolarizing current injected into the axon terminal (Fig. 2*a*) also produced a depolarizing response on which a few oscillatory wavelets were seen, whereas a hyperpolarizing current (Fig. 2*b*) produced a hyperpolarizing response with wavelets seen on the off-depolarization. Light-evoked responses from a transient (type-C) cell are shown in Fig. 2*c*, *d*. Illumination elicited large on- and off-transient depolarizations with oscillations between, whereas depolarizing (Fig. 2*c*) and hyperpolarizing currents (Fig. 2*d*) both produced on- and off-transient depolarizations.

There are three possible pathways by which the horizontal-cell axon terminals can communicate with amacrine cells: (1) via horizontal-cell perikarya which synapse onto the bipolar cells and then contact amacrine cells; (2) via horizontal-cell axon terminals synapsing onto the bipolar cells which contact amacrine cells; or (3) via synapses directly onto the amacrine cells. The first and second possibilities seem unlikely because: (1) current ($\leq$40 nA) injected into axon terminals failed to evoke a response in bipolar cells (10 pairs), whereas current ($<$10 nA) injected into horizontal-cell perikarya always evoked responses from bipolar cells (32 pairs); (2) current ($\leq$40 nA) injected into the axon terminals produced only very small responses (0.7 mV) in the horizontal-cell perikarya (12 pairs)[6]; and (3) current of $<$10 nA injected into the axon terminals produced responses from the amacrine cells (Fig. 2). Furthermore, axon terminals have not been observed to synapse onto the bipolar cells in catfish, whereas synapses made by the axon terminals onto the amacrine cells have been found.

We conclude that there are two independent pathways for signal transmission from the outer to inner plexiform layers in the catfish retina. One is the established pathway through bipolar cells and the other is a new pathway through the horizontal-cell axon terminals. Two recent anatomical studies have also identified synapses made by horizontal-cell axonal processes in goldfish (ref. 11 and D. W. Marshak and J. E. Dowling, personal communication); hence such a synaptic pathway may not be unique to the catfish.

Note that the current-evoked responses elicited in the amacrine cells have similar characteristics to those of the light-evoked responses, showing that complex responses such as oscillations in the type-N cell and transient depolarizations in the type-C cell are produced when signals are transmitted directly from the axon terminals to the amacrine cells. This suggests that the complex responses of amacrine cells reflect intrinsic properties of the cell and not the properties of the specialized bipolar–amacrine cell synapses.

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Acetylcholine receptor-aggregating factor is similar to molecules concentrated at neuromuscular junctions

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The basal lamina in the synaptic cleft of the vertebrate skeletal neuromuscular junction contains molecules that direct the formation of synaptic specializations in regenerating axons and muscle fibres[1–4]. We have undertaken a series of experiments aimed at identifying and characterizing the molecules responsible for the formation of one of these specializations, the aggregates of acetylcholine receptors (AChRs) in the muscle fibre plasma membrane. We began by preparing an insoluble, basal lamina-containing fraction from *Torpedo californica* electric organ, a tissue which has a far higher concentration of cholinergic synapses than muscle, and showing that this fraction caused AChRs on cultured chick myotubes to aggregate[5–7]. A critical step is learning whether or not the electric organ factor is similar to the receptor-aggregating molecule in the basal lamina at the neuromuscular junction. The importance of this problem is emphasized by reports that clearly non-physiological agents, such as positively charged latex beads[8], can cause AChR aggregation on cultured muscle cells. We have already shown that *Torpedo* muscle contains an AChR-aggregating factor similar to that of electric organ, although in much lower amounts[6]. Here we demonstrate, using monoclonal antibodies, that the AChR-aggregating factor in our extracts of electric organ is, in fact, antigenically related to molecules concentrated in the synaptic cleft at the neuromuscular junction.

A mouse was injected with 300 μg of an electric organ extract enriched >1,000-fold in AChR-aggregating activity[7] and hybridomas were generated by standard procedures (see Table 1 legend). To identify hybridomas that produced antibodies against electric organ material, we used indirect immunofluorescence histochemistry and screened for antibodies that bound to formaldehyde-fixed frozen sections of electric organ. We then tested the antibodies that stained electric organ for those that could immunoprecipitate the AChR-aggregating activity from our electric organ extract. Thus far, we have cloned two

hybridomas producing such antibodies. Both antibodies, 6D4 and 2F6, also immunoprecipitated the activity in muscle extracts and blocked AChR aggregation when added to myotube cultures together with either muscle or electric organ extract (Table 1).

To determine the distribution of muscle antigens recognized by 6D4 and 2F6, formaldehyde-fixed frozen sections of *Torpedo* skeletal muscle were examined by immunofluorescence histochemistry. Figure 1 shows that 6D4 intensely stained the neuromuscular junctions. Staining was also detected in the walls of arteries and around individual pre-terminal axons, and there were occasional lightly stained patches at the extrajunctional surface of some muscle fibres (not shown). No other structures in muscle were labelled. Thus, the AChR-aggregating factors in our extracts of electric organ and muscle are antigenically related to molecules associated with more than one tissue component of muscle, but on myofibres, such molecules are concentrated at neuromuscular junctions.

Sections of muscle labelled with 2F6 had a staining pattern similar to that of 6D4, suggesting that both monoclonal antibodies bind to the same antigens. However, 2F6 did not stain sections exposed to fixative for longer than 1 min, even at a 100-fold greater concentration than that sufficient to stain ≥ nfixed tissue. Binding of 6D4, on the other hand, was not affected by our routine fixation; the titre required for staining was the same for unfixed sections and sections fixed for 5 min. Thus, 6D4 and 2F6 recognize epitopes which can be distinguished from one another by their sensitivity to fixation.

We are now performing experiments to determine whether antigens recognized by 6D4 and 2F6 at the neuromuscular junction are components of the synaptic basal lamina. As a first step, we checked the distribution of basal lamina in *Torpedo* muscle by staining cross-sections with an antiserum[9] against laminin, a common constituent of basal lamina sheaths. As expected, basal lamina envelops the same structures in *Torpedo* muscle as in mammalian muscle[10], including muscle fibres, arteries, capillaries, veins, perineurium and Schwann cells (which surround axons). Thus, 6D4 and 2F6 do not stain all structures that have basal lamina, but basal lamina is associated with all structures stained by these antibodies. To determine whether or not epitopes recognized by 6D4 are extracellular, muscles were fixed, incubated with the antibody and examined in whole mounts by immunohistochemistry. The plasma membranes remained intact during these procedures; thus, the antibodies could interact only with the extracellular matrix and the external surface of plasma membranes. Fluorescence microscopy revealed that all the structures labelled in cross-sections were also stained in the whole mounts, including neuromuscular junctions (Fig. 1*c*). Some whole-muscle preparations incubated with 6D4 and a horseradish peroxidase (HRP)-conjugated second antibody were examined by electron microscopy to determine the distribution of 6D4 epitopes at neuromuscular junctions. Figure 1*d* demonstrates that HRP reaction product occupied much of the synaptic cleft, including the synaptic portion of the myofibre basal lamina; no labelling was detected beyond the synaptic cleft. Thus, epitopes recognized by 6D4 at neuromuscular junctions are concentrated in the synaptic cleft and are a part of or adjacent to the synaptic basal lamina.

Extracts that cause AChR aggregation on cultured myotubes have been obtained by others from neurone cultures[11] and neurone-rich tissues including brain, spinal cord and peripheral nerves[12–17]. None of the active factors in these extracts has been shown to be related to molecules concentrated at the neuromuscular junction or any other synapse, as we demonstrate here for the AChR-aggregating factor from *Torpedo* electric organ.

It is possible that the electric organ AChR-aggregating factor is related to a known component of the basal lamina at the neuromuscular junction. Such components include acetylcholinesterase[18], a heparan sulphate proteoglycan[19], and one or more uncharacterized antigens[20], all of which are either absent from, or in much lower concentrations in, extrajunctional regions of the myofibre basal lamina sheaths. The ability of these molecules to cause receptor aggregation has not been tested. Other components of synaptic basal lamina, including type IV collagen, fibronectin and laminin, are common to synaptic and extrasynaptic basal lamina[10]. Of these shared components, only laminin causes AChR aggregation when applied to cultured myotubes[21]. As reported previously, gel filtration of our solubilized electric organ material did not reveal any AChR-aggregating activity in the size range of laminin (relative molecular mass 880,000)[9]; most of the activity eluted at a position characteristic of proteins of lower relative molecular mass (50,000–100,000)[7]. Moreover, as illustrated in Table 2, antiserum against laminin, which stained basal lamina in our sections of *Torpedo* muscle, did not immunoprecipitate AChR-aggregating activity from electric organ extracts. These observations, coupled

Table 1 Monoclonal antibodies block and immunoprecipitate AChR-aggregating activity in extracts of electric organ and muscle

Additions	Activity (%) Immunoprecipitation	Blocking
Electric organ extract		
+control	100 ± 12	100 ± 13
+6D4	−5 ± 2	−2 ± 2
+2F6	23 ± 5	9 ± 0
Muscle extract		
+control	100 ± 7	100 ± 29
+6D4	3 ± 4	31 ± 12
+2F6	3 ± 0	18 ± 8

A *p*H 5.5 citrate extract[6] containing 1–1.5 units of AChR-aggregating activity prepared from *Torpedo* electric organ or tail muscle was mixed with 120–200 μl of hybridoma supernatant for 2 h at 37 °C in a volume of 200–465 μl. To determine blocking, aliquots were applied directly to cultured chick myotubes. To determine immunoprecipitation, the mixture was incubated for an additional 0.5–2 h with goat anti-mouse IgG-conjugated Sepharose beads (see ref. 6), centrifuged to remove the bound immune complexes, and aliquots of the supernatant were assayed on cultured myotubes. AChR-aggregating activity was determined by counting the number of patches of AChRs on the myotubes as described previously[6]. Control supernatants were taken from either the parent myeloma or a hybridoma producing antibodies that did not stain electric organ. Each value represents the mean ±s.e.m. of triplicate determinations. Generation of monoclonal antibodies: A female BALB/c mouse was given an intraperitoneal (i.p.) injection of 20 μg of purified electric organ extract[7] emulsified with an equal volume of Freund's complete adjuvant; after 4 weeks, the mouse was injected i.p. with an additional 16 μg of this extract emulsified with Freund's incomplete adjuvant. After 4 more weeks, 260 μg of extract without adjuvant was injected beneath the capsule of the spleen. The spleen was removed 3 days later and the cells were fused with a mouse myeloma cell line, P3X63-Ag8.653, by the procedure of Oi and Herzenberg[23]. Viable hybridomas were found in 500 of the original 572 wells plated. Of these, the supernatant from 105 wells stained sections of electric organ.

Table 2 Antiserum against laminin does not immunoprecipitate AChR-aggregating activity

Additions	Activity (%)
None	100 ± 16
Normal serum (20 μl)	122 ± 13
Antiserum against *p*H 5.5 citrate extract (2 μl)	−5 ± 12
Antiserum against laminin (20 μl)	104 ± 13

An aliquot of a *p*H 5.5 citrate extract[6] containing 1.5 units of AChR-aggregating activity prepared from *Torpedo* electric organ was mixed with the indicated amount of heat-inactivated rabbit serum in a total volume of 70 μl for 2 h at 37 °C. The mixture was incubated for an additional 0.5 h with 10 μg of fixed *Staphylococcus aureus* (Pansorbin [Calbiochem], washed and resuspended in 50 μl of 1 M Tris buffer, *p*H 8.5), then centrifuged to remove bound immune complexes and assayed as described in Table 1 legend. Antiserum against *p*H 5.5 citrate extract[6] was used to confirm the effectiveness of *S. aureus* immunoprecipitation.

with the finding that 6D4 and 2F6 staining co-localized with only a fraction of anti-laminin staining in muscle, indicate that the electric organ AChR-aggregating factor is not laminin. However, the fact that two different monoclonal antibodies against the electric organ AChR-aggregating factor recognize cell-surface antigens in the walls of arteries and in nerves as well as at neuromuscular junctions may mean that the factor is structurally related to one or a family of basal lamina molecules that have a broad distribution in muscle.

Taken together, our studies provide several lines of evidence that the electric organ AChR-aggregating factor is similar to the component of the synaptic basal lamina that directs the accumulation of AChRs on regenerating muscle fibres: the factor is found in an extracellular matrix-rich fraction of the electric

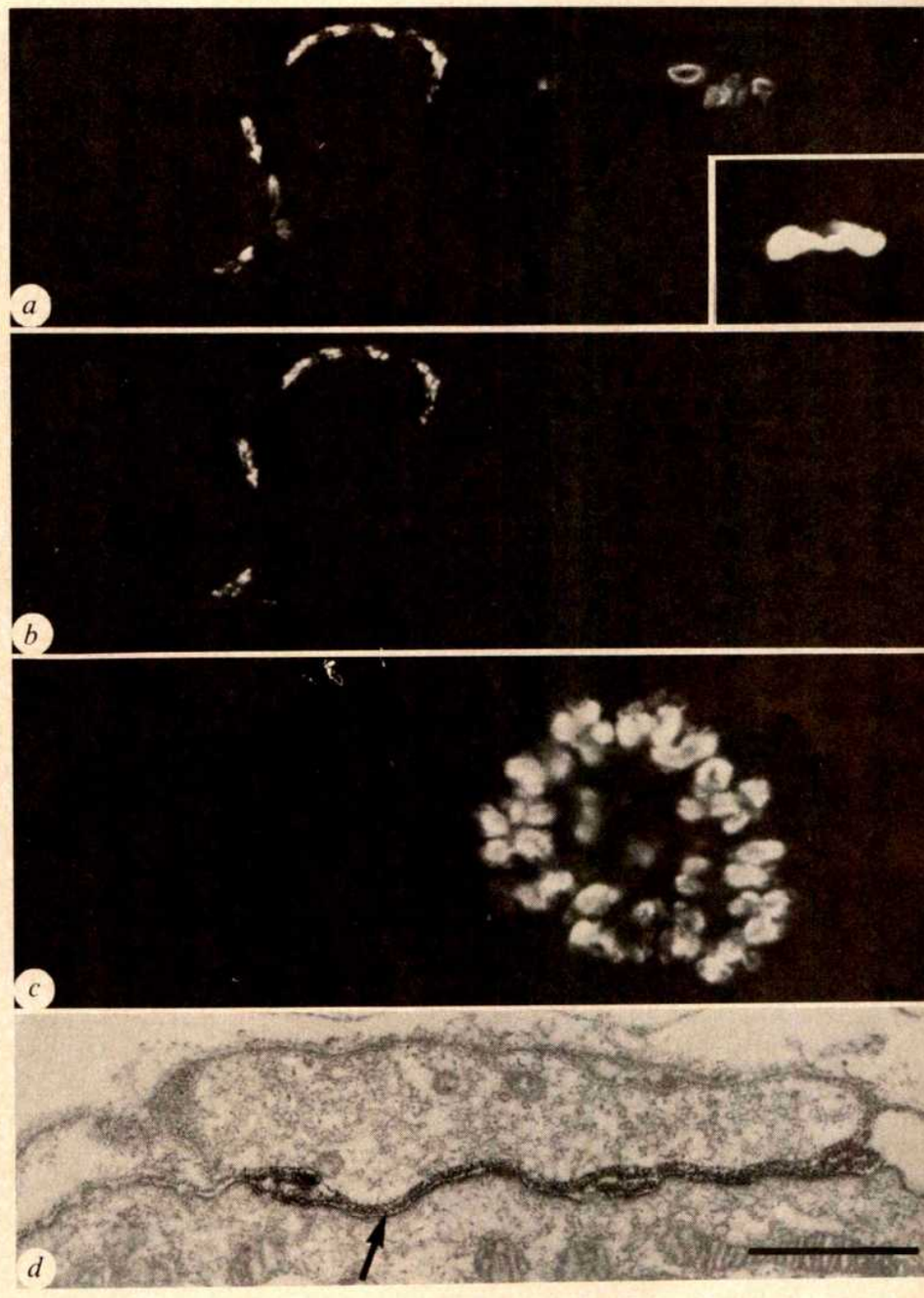


Fig. 1 6D4 binds to antigens concentrated at neuromuscular junctions in skeletal muscle. ***a***, Frozen section of *Torpedo californica* muscle viewed with fluorescein optics to show the position of 6D4-binding sites. ***b***, Same field as in *a* but viewed with rhodamine optics to reveal the location of neuromuscular junctions. 6D4 binding is confined to neuromuscular junctions, the wall of a collapsed artery (inset), and the perimeter of each of several axons. ***c***, A whole-mount of a fascicle of *Torpedo* muscle fibres viewed with fluorescein optics to reveal bound 6D4. A neuromuscular junction, seen from above and similar in layout to that of other vertebrates[24], is intensely stained; preterminal axons are faintly stained. ***d***, Electron micrograph of a neuromuscular junction from a *Discopyge ommata* fin muscle incubated with 6D4 and then with an HRP-conjugated second antibody. Electron-dense HRP reaction product occupies much of the synaptic cleft, including the synaptic portion of the muscle fibre basal lamina (arrow). We used the fin muscles from *D. ommata* (an electric ray like the *Torpedo* but less than one-quarter the size) because they were smaller than any muscles we encountered in *Torpedo* and, thus, were easier to process for electron microscopy. Fluorescence immunohistochemistry on frozen sections and on whole mounts of these muscles revealed the same 6D4 staining pattern as in muscles from *Torpedo*; no 6D4 staining was observed at neuromuscular junctions in frog, salamander and chick muscles. Scale bar: *a*, *b*, 75 μm; *c*, 33 μm; *d*, 1 μm.

Methods. Frozen sections *a*, *b*: sections (8–10 μm thick) of back muscles of *Torpedo* were incubated for 10 min in 10% normal goat serum in phosphate-buffered saline (PBS), washed briefly in PBS, fixed for 5 min in 1% formaldehyde in PBS, washed for 5 min in 0.1% Triton X-100 in PBS (PBS-T), and incubated overnight at 4 °C in 6D4 supernatant diluted 1:200 in PBS containing 20% normal goat serum and 0.5% Triton X-100 (PBS-ST). (The same staining pattern was observed at dilutions of 6D4 supernatant ranging over 1–20,000-fold.) The sections were then washed for 3 h in PBS-T and incubated for 1 h in PBS-ST containing fluorescein-conjugated goat anti-mouse IgG (Cappel) to label bound 6D4 and rhodamine-α-bungarotoxin to stain AChR aggregates at neuromuscular junctions. Coverslips were mounted with Citifluor mountant medium AF1 (City University, London). Whole mounts (*c*): Abdominal muscles bathed in *Torpedo* Ringer's[25] were dissected down to single fascicles three to five muscle fibres thick. The muscles were then fixed for 1 h with 1% paraformaldehyde in Ringer's solution, incubated overnight at 4 °C in 6D4 supernatant diluted 1:200 with 0.3 M sodium phosphate buffer *p*H 7.1 (PB), washed for 8 h with several changes of PB, incubated overnight in fluorescein-conjugated goat anti-mouse IgG (Cappel) diluted 1:800 with PB, washed for 3 h in phosphate buffer with 0.1% Triton X-100 (PB-T), refixed for 0.5 h in 1% paraformaldehyde, washed for 2 h with PB-T, and mounted with mountant medium AF1 between glass coverslips. Electron microscopy (*d*): Muscles were pinned out in Ringer's solution, incubated for 3 h in 6D4 diluted 1:50 with Ringer's solution, washed for 45 min with Ringer's, incubated in Ringer's containing HRP-conjugated goat anti-mouse IgG (Cappel), washed for 45 min in PB, fixed for 30 min in 1% glutaraldehyde in PB, washed for 30 min with 0.25 M cacodylate buffer *p*H 7.1, incubated for 30 min in 0.05% 3,3-diaminobenzidine (Sigma) and 0.02% H_2O_2 in cacodylate buffer, washed for 30 min in cacodylate buffer, immersed for 1 h in 1% osmium tetroxide in PB, dehydrated with ethanol and embedded whole in a mixture of Epon and Araldite[3].

organ[6]; the corresponding fraction of *Torpedo* muscle contains a similar activity[6]; antiserum against partially purified electric organ factor blocks and immunoprecipitates AChR-aggregating activity from muscle and stains muscle basal lamina[7]; as demonstrated here, two monoclonal antibodies recognizing different epitopes block and immunoprecipitate the active component in extracts of both electric organ and muscle and bind in high concentration at the neuromuscular junction; and, at least one of the monoclonal antibodies recognizes molecules that are in or adjacent to synaptic basal lamina. Thus, monoclonal antibodies against the electric organ AChR-aggregating factor, such as 6D4 and 2F6, may be useful reagents for identifying and purifying the components of synaptic basal lamina that direct the aggregation of AChRs on regenerating myofibres, for studying their mechanism of action, for learning how they are regulated and for determining whether they also direct the formation of receptor aggregates in developing muscles or the maintenance of such aggregates in normal adult muscles. It is now known that synaptic basal lamina directs the aggregation of acetylcholinesterase as well as that of AChRs in regenerating muscle[4]. As described in the following report[22], 6D4 and 2F6 have been used to provide evidence that the electric organ factor which causes AChR aggregation also causes the accumulation of acetylcholinesterase on the surface of cultured myofibres, raising the possibility that a single molecule in the basal lamina directs the differentiation of both of these postsynaptic specializations in regenerating muscle.

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Aggregates of acetylcholinesterase induced by acetylcholine receptor-aggregating factor

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Basal lamina-rich extracts of *Torpedo californica* electric organ contain a factor that causes acetylcholine receptors (AChRs) on cultured myotubes to aggregate into patches[1-3]. Our previous studies have indicated that the active component of these extracts is similar to the molecules in the basal lamina which direct the aggregation of AChRs in the muscle fibre plasma membrane at regenerating neuromuscular junctions *in vivo*[2,4,5]. Because it can be obtained in large amounts and assayed in controlled conditions in cell culture, the AChR-aggregating factor from electric organ may be especially useful for examining in detail how the postsynaptic apparatus of regenerating muscle is assembled. Here we demonstrate that the electric organ factor causes not only the formation of AChR aggregates on cultured myotubes, but also the formation of patches of acetylcholinesterase (AChE). This finding, together with the observation that basal lamina directs the formation of both AChR and AChE aggregates at regenerating neuromuscular junctions *in vivo*[6], leads us to hypothesize that a single component of the synaptic basal lamina causes the formation of both these synaptic specializations on regenerating myofibres.

First, we investigated whether extracts of *Torpedo* electric organ would affect the distribution of AChE on myotubes. For this, aliquots of a saline- and detergent-insoluble fraction of electric organ[3] were added to 5-day-old chick myotube cultures; this fraction is rich in components of basal lamina and causes AChRs on cultured myotubes to aggregate[3]. After 24 h, treated and control cultures were fixed and stained for cholinesterase (ChE) activity by the histochemical method of Karnovsky[7]. Figure 1*a*, *b* shows that the insoluble fraction caused a marked increase in the number of patches of reaction product on the myotubes. Few patches were seen on myotubes stained in the presence 10^{-5} M eserine (Table 1), indicating that the reaction product was due to cholinesterase activity. Patches were induced when both the culture medium and the insoluble fraction were pretreated with methane sulphonyl fluoride (MSF), an irreversible inhibitor of ChE[8] (Table 1); therefore, the ChE in the patches must have been produced by the myotubes. To determine

Table 1 Histochemically stained patches contain myotube-derived ChE

	Addition	Aggregates per myotube segment
Expt *a*		
Control	None	1.1 ± 0.1
	Eserine	0.5 ± 0.2
Insoluble fraction	None	8.9 ± 0.1
	Eserine	0.9 ± 0.3
Expt *b*		
Control	None	2.1 ± 0.2
	MSF-treated medium	2.1 ± 0.5
Insoluble fraction	None	4.2 ± 0.3
	MSF-treated medium	4.5 ± 1.2

Expt *a*, 40-μl aliquots of a MSF-treated insoluble fraction (6.9 μg) or MSF-treated medium were added to cultures (see Fig. 1 legend for MSF treatment). The cultures were incubated overnight and stained for cholinesterase in the presence or absence of 10^{-5} M eserine. Eserine was added 10 min before the histochemical reaction was begun and was present throughout the 1½-h incubation. Expt *b*, MSF (final concentration, 2 mM) was added to culture medium and the mixture was incubated at room temperature for 1 h. Aliquots of the insoluble fraction, treated with MSF as in *a*, were then added to control cultures and cultures that had been fed with MSF-treated medium. The cultures were incubated overnight and stained for cholinesterase. Data are expressed as mean ±s.e.m. of triplicate determinations.

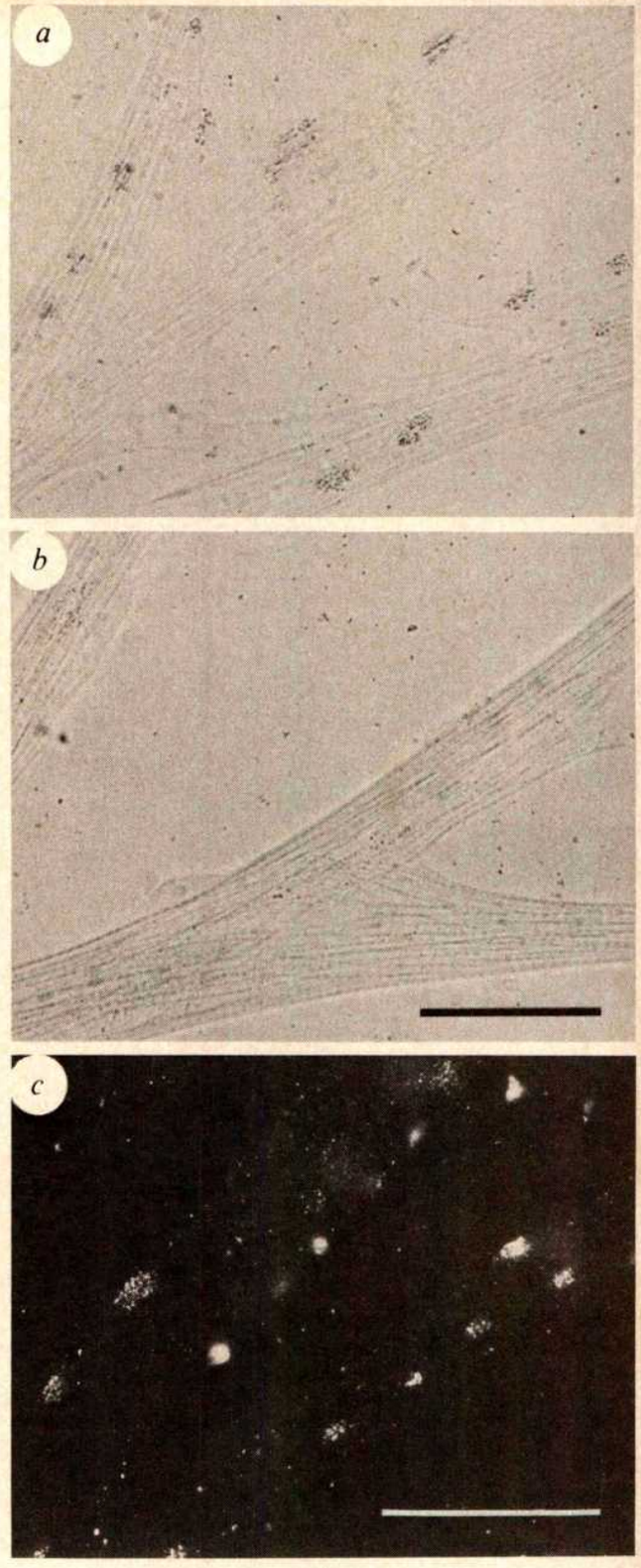


Fig. 1 Formation of AChE aggregates by *Torpedo* electric organ extracts. ***a***, ***b***, Bright-field photographs of 5-day chick myotube cultures[3], incubated for 24 h with medium containing 16 μg of an insoluble fraction of electric organ (*a*) or normal medium (*b*) and stained for ChE activity. ***c***, Fluorescence micrograph of a 5-day culture exposed for 24 h to 45 ng of agarose pool and lablled with anti-AChE and fluorescent second antibody. Scale bars, 50 μm.
Methods. ***a***, ***b***, The cultures were rinsed with buffer containing 150 mM NaCl, 10 mM sodium phosphate, *p*H 7.4 (PBS), fixed for 5 min in 1% paraformaldehyde in 90 mM sodium phosphate, 130 mM sucrose, *p*H 7.4, rinsed with PBS and then reacted for cholinesterase by the method of Karnovsky[7] for 1½ h at room temperature. Cultures were rinsed with 0.1 M sodium maleate, *p*H 6.0, with water, then dehydrated in graded ethanols and mounted in glycerol. Methane sulphonyl fluoride (final concentration 2 mM) was added to the insoluble fraction and the mixture incubated for 1 h at room temperature before adding it to the cultures. The MSF treatment inhibited cholinesterase in the insoluble fraction by >99.8%, as assayed by the method of Ellman *et al.*[26]. The rapid spontaneous hydrolysis of MSF prevented it from inhibiting ChE on the myotubes. ***c***, AChE was labelled with a monoclonal antibody to chick AChE and fluorescent second antibody as follows. Cultures were rinsed with PBS, fixed for 30 min in 1% paraformaldehyde in 90 mM sodium phosphate, 130 mM sucrose, *p*H 7.2, rinsed with PBS and incubated for 1 h at room temperature with a mouse monoclonal antibody specific for chick AChE, 1A2 anti-AChE[9] (6 μg ml^{-1} in PBS supplemented with 10% horse serum). The cultures were then rinsed with PBS, incubated for 1 h with a 1/200 dilution of rhodamine-conjugated goat anti-mouse IgG (Cappel) in PBS + 10% horse serum, rinsed with PBS, dehydrated in graded ethanols and mounted in glycerol. No immunohistochemical staining was seen when cultures were incubated with antibodies secreted by the parent myeloma (not shown).

Table 2 Comparison of ChE histochemistry and AChE immunohistochemistry

	Aggregates per myotube segment	
	ChE histochemistry	Anti-AChE immunohistochemistry
Expt *a*		
Control	3.0 (2.6, 3.4)	3.3 (2.8, 3.8)
Insoluble fraction	6.4 (6.3, 6.6)	5.3 ± 0.6
Expt *b*		
Control	2.0 (1.8, 2.1)	4.8 ± 0.8
*p*H 5.5 extract	6.9 (6.2, 7.6)	10.5 ± 0.8
Expt *c*		
Control	1.6 ± 0.3	1.3 ± 0.2
Agarose pool	7.2 ± 0.7	5.7 ± 0.8

Cultures were treated with the insoluble fraction, *p*H 5.5 extract or agarose pool and either stained for ChE activity or labelled immunohistochemically to reveal AChE (see Fig. 1 legend). Labelled patches on the bottom surface of the myotubes were counted (see Fig. 2 legend). The numbers of patches on 10 myotube segments 400 μm long examined at ×250 magnification were averaged for each culture histochemically. As the patches of rhodamine-labelled anti-AChE were difficult to see at this magnification, antibody-labelled patches were counted at ×400 magnification and the numbers of patches on 20 myotube segments were averaged and multiplied by 1.6. Expts *a*, *b* and *c* were done on different sets of cultures. Data are expressed as the mean of duplicate or triplicate determinations. For experiments done in duplicate, the individual values are given in parentheses; for experiments done in triplicate, the s.e.m. is shown.

whether the patches induced by the electric organ fraction contained true AChE, control cultures and cultures treated with the insoluble fraction were fixed and labelled with a monoclonal antibody specific for chick AChE[9]. The electric organ fraction caused an increase in the number of patches of antibody-labelled AChE on the surface of the myofibres similar to the increase seen with histochemical staining (Fig. 1*c*, Table 2). Because the labelling protocol used for these experiments did not allow antibodies to penetrate plasma membranes, the AChE patches detected must be on the myotube surface. Thus, the insoluble fraction of electric organ causes not only the aggregation of AChRs on cultured myotubes, but also the formation of aggregates of AChE.

We next sought to establish a quantitative assay for AChE-aggregating activity, so that its level could be compared with that of the AChR-aggregating factor. Figure 2 shows that as the amount of the insoluble fraction added to cultures was increased, the number of AChE patches increased and then reached a plateau. Although the magnitude of the response to saturating amounts of the insoluble fraction varied over 2–9-fold from plating to plating, there was little variability in the dose depen-

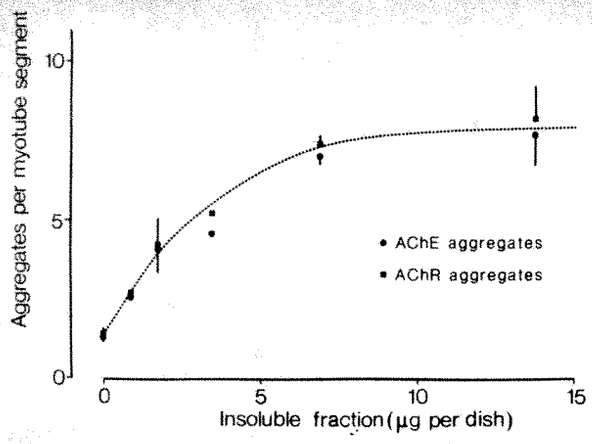


Fig. 2 Dose dependence of the formation of AChR (■) and AChE (●) aggregates on cultured myotubes. Cultures, treated for 24 h with the indicated amount of insoluble fraction, were incubated with 6×10^{-8} M rhodamine-α-bungarotoxin[27] in culture medium for 1 h at 37 °C. The cultures were then rinsed twice with PBS and fixed and reacted for cholinesterase activity as described in Fig. 1 legend. AChR and ChE clusters were counted at ×250 magnification with a Nikon microscope equipped with phase and fluorescence optics. The ChE-stained patches and α-bungarotoxin-labelled AChR clusters were often seen to be partially or totally overlapping; however, there was no indication that the ChE staining ever completely obscured a toxin-labelled AChR aggregate. For each culture, myotubes were chosen at random and the numbers of patches of cholinesterase reaction product along unbranched 400-μm-long segments of the myotubes were counted under phase optics; then the number of AChR clusters was counted under fluorescence optics. Only patches on the bottom of the myotubes were counted because most factor-induced clusters of AChRs and ChE occurred on this surface of the myotube. Counts from 10 myotubes, evenly spaced over the surface of the dish, were averaged. We counted as an aggregate any distinct fluorescent or cholinesterase-stained patch whose longest axis was >2 μm. Data are expressed as the mean ±s.e.m. ($n=3$); error bars are shown where they exceed the size of the data point. To quantitate AChR- and ChE-aggregating activity, curves were drawn through the points by eye (the two curves superimpose in this figure) and one unit of activity was taken as the amount of the fraction required to give a half-maximal response (in this case 2.58 μg per dish).

dence of the response. Thus, by defining one unit of activity as the amount of material required to cause a half-maximal response, AChE-aggregation could be measured reproducibly between experiments.

The dose dependence of the insoluble fraction-induced formation of AChE patches on cultured myofibres was the same as for AChR aggregation (Fig. 2), suggesting that a single factor caused both effects. If this were the case, it should be possible to extract the AChE-aggregating activity from the insoluble fraction and purify it by procedures that solubilize and purify the AChR-aggregating factor. Accordingly, the insoluble fraction was extracted with *p*H 5.5 citrate buffer[3], the solubilized material was fractionated by gel filtration chromatography on agarose[2] and the column fractions causing AChR aggregation were pooled. Both AChR- and AChE-aggregating activities were measured in the insoluble fraction, the *p*H 5.5 extract and the 'agarose pool'. Table 3 shows that the AChR- and AChE-aggregating specific activities were increased to the same extent by extraction and gel filtration chromatography and that the dose dependence for AChR aggregation was the same as that for the formation of AChE aggregates at each step in the purification. (Formation of AChE aggregates was routinely assayed by ChE histochemistry; however, immunohistochemical experiments confirmed that each fraction induced patches that contained true AChE [Table 2]). Thus, AChE- and AChR-aggregating activities co-purify. We also examined whether or not two different monoclonal antibodies, 6D4 and 2F6, directed against the AChR-aggregating factor (see accompanying report[10]), would recognize the AChE-aggregating factor (Table 4). When bound to Sepharose beads, 6D4 and 2F6 each removed AChR- and AChE-aggregating activity from the agarose pool. Moreover, when added directly to cultures together with the agarose pool, 6D4 and 2F6 inhibited the extract-induced formation of both AChR and AChE aggregates. From the observations that (1) the AChR- and AChE-aggregating activities co-purify, (2) the dose dependence for the induction of AChR and AChE aggregates is the same at each stage of the purification and (3) two different monoclonal antibodies immunoprecipitate and inhibit both activities, we conclude that a single factor in our extracts of electric organ causes the formation of AChR and AChE patches.

Table 3 Co-purification of AChE- and AChR-aggregating activities

Fraction	Aggregating activity (units per mg protein) AChR	AChE
Insoluble fraction	374	382
*p*H 5.5 extract	811	952
Agarose pool	49,800	56,200

For each fraction, AChE- and AChR-aggregating activities were measured as described in Fig. 2 legend. The values are the mean of two separate determinations.

Table 4 Immunoprecipitation (*a*) and inhibition (*b*) of AChE- and AChR-aggregating activities

	Addition	Aggregates per myotube segment (% control) AChE	AChR
Expt *a*	Control	100 ± 16	100 ± 15
	6D4	−2 ± 2	−1 ± 4
	2F6	−1 ± 4	7 ± 3
Expt *b*	Control	100 ± 5	100 ± 5
	6D4	2 ± 11	11 ± 4
	2F6	22 ± 4	10 ± 3

Expt *a*: An aliquot of agarose pool (7.2–8.2 units, 134–150 ng) was mixed with 0.3–1.3 ml hybridoma supernatant and incubated for 1½ h at 37 °C. Immunoglobulins and immune complexes were precipitated by adding 30–50 μl of a suspension of goat anti-mouse antibodies covalently coupled to Sepharose beads[3] and 100 μl aliquots of the supernatant were assayed for aggregating activity. Control supernatant was from a hybridoma secreting antibodies of unknown specificity. Expt *b*: An aliquot of agarose pool (6.5–8.2 units, 120–150 ng) was mixed with 0.35–1.3 ml myeloma-conditioned medium or hybridoma supernatant, incubated for 1 h at 37 °C and 0.12–0.4 ml aliquots were assayed for aggregating activity. Data for both experiments, expressed as mean ±s.e.m. ($n=3$), are per cent of maximal response.

AChR and AChE aggregates form part of the postsynaptic apparatus at both normal and regenerated neuromuscular junctions and are the only components of the apparatus known to be directly involved in synaptic transmission. The formation of these cell-surface specializations at developing neuromuscular junctions is induced by the axon terminal[11–13]. On the other hand, in adults, the synaptic portion of the myofibre's sheath of basal lamina can direct the aggregation of AChRs and AChE on regenerating myofibres[4–6]. Thus, the synaptic basal lamina in the adult contains synaptogenic information similar to that provided by the axon in the embryo.

The identity and mechanism of action of the molecules that induce the postsynaptic specializations at developing and regenerating neuromuscular junctions are unknown, although *in vivo* and *in vitro* studies indicate that electromechanical activity of muscle fibres is necessary for the accumulation of high levels of AChE[14–16]. Crude extracts from several neurone-rich tissues such as brain, spinal cord and nerve have been shown to cause AChR aggregation on cultured myotubes, and the active components of some have been partially purified[17–25]. Furthermore, many of the AChR patches formed in response to at least one of the brain extracts are associated with synapse-specific anti-

gens, AChE and invaginations in the myotube plasma membrane which resemble the junctional folds found at normal and regenerating neuromuscular junctions[25]. The results reported here extend the findings of such studies by showing that, in the basal lamina-containing fraction of electric organ, the same factor that causes AChR aggregation also causes the formation of aggregates of AChE. We have also demonstrated (see accompanying report[10]) that two different monoclonal antibodies against this AChR/AChE-aggregating factor bind in high concentration at the neuromuscular junction and that the molecules recognized by at least one of the monoclonal antibodies are in or adjacent to the synaptic basal lamina. Thus, it is reasonable to suggest that the molecules in the synaptic basal lamina which direct the formation of AChR aggregates on regenerating myofibres also cause aggregation of AChE. We are now performing experiments to test this monomolecular induction hypothesis for the formation of AChR and AChE aggregates at both regenerating and developing neuromuscular junctions and to discover whether the electric organ AChR/AChE-aggregating factor causes the formation of other components of the postsynaptic apparatus.

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Inhibition of the Prausnitz–Küstner reaction by an immunoglobulin ε-chain fragment synthesized in *E. coli*

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The Prausnitz–Küstner (P–K) reaction is a sensitive test for the presence and activity in the skin of immunoglobulin E, an important class of immunoglobulin mediating allergic reactions. A fragment of the human myeloma ND ε-chain gene, encoding the second, third and fourth domains of the IgE constant region (Cε2–4) was assessed here for its ability to inhibit the P–K reaction *in vivo*. Injection of the fragment in skin sites of healthy human adults prevented subsequent sensitization with serum containing IgE antibody to ragweed antigen. Inhibition of the P–K reaction required a 200-fold molar excess of the C_ε fragment over the IgE present in the sensitizing serum. The efficacy of the C_ε fragment in inhibiting the P–K reaction compared favourably with that of natural myeloma IgE (PS) in terms of both blocking concentrations and duration of the blocking effect. The inhibition of the P–K reaction by C_ε2–4 fragments was specific and probably caused by the saturation of IgE receptors on mast cells[1,2] by the recombinant gene product.

The C_ε2-4 gene fragment used encodes amino acids 218–547 of the IgE molecule and was subcloned in *Escherichia coli*, producing a polypeptide of relative molecular mass 40,000[3]. Preparations consisting of >95% affinity-purified C_ε2-4 dimeric chain fragments, when injected into skin sites of a human volunteer, completely blocked the passive sensitization of these sites by serum (E.C.) containing IgE antibody to ragweed antigen (Table 1). This blockade was prevented by treating the C_ε2-4 fragments at 56 °C for 1 h or by reduction and alkylation. Skin sites injected with C_ε2-4 fragments remained normally responsive to histamine (data not shown). More importantly, skin sites sensitized with reaginic serum (E.C.) 1 h before C_ε2-4 fragment injection retained their full ability to support a P-K reaction.

The P-K reaction under study was caused by IgE antibody to ragweed antigen in the sensitizing serum. Heat treatment (56 °C, 1 h) of serum E.C. abolished its ability to sensitize normal skin (data not shown), as would be the case for an IgE antibody[4,5]. Table 2 shows that when an immunoglobulin-enriched fraction of serum E.C. was fractionated over an anti-IgE (PS) Sepharose-4B column, only the IgE-containing eluate, but not the IgE-depleted effluent, was able to induce a P-K reaction. This was blocked by pretreatment of the skin sites with C_ε2-4 fragments.

The P-K reaction induced by a 1:100 dilution of serum E.C. (1:100) containing 5×10^{-11} M IgE was abolished completely

Table 1 Inhibition of Prausnitz-Küstner reaction by C_ε2-4 fragments

	Inhibitor	Area (mm²) of wheal and flare at sites sensitized with Serum E.C. diluted 1:10	Serum E.C. diluted 1:100
Expt 1	Diluent	87/480	42/257
	C_ε2-4 (100 μg ml^{-1})	0/0	0/0
	C_ε2-4 56 °C 1 h (100 μg ml^{-1})	76/455	38/271
Expt 2	Diluent	103/516	54/268
	C_ε2-4 (10 μg ml^{-1})	0/0	0/0
	C_ε2-4 (10 μg ml^{-1}) reduced, alkylated	92/472	44/240

Values are the areas of the wheal/the area of the flare. Reduction and alkylation were performed as described in ref. 3. The same subject was used for passive sensitization in both experiments; the serum IgE of this subject was 4 IU ml^{-1} (~10 ng ml^{-1}). The sensitizing serum (E.C.) contained 380 IU ml^{-1} of IgE (~912 ng ml^{-1}), of which 8.7% was directed against ragweed antigen, as determined by the specific drop in serum IgE following absorption of the serum over a Sepharose 4B ragweed antigen column compared with a control Sepharose 4B human serum albumin[7]. Serum E.C. was free of detectable hepatitis B antigen and of antibodies to that antigen. C_ε2-4 fragments were injected into skin sites 1 h before the injection of serum E.C. Skin sites were challenged 48 h later with ragweed antigen (1,000 protein nitrogen units ml^{-1} of a mixture of giant and short ragweed; Hollister-Stier). Twenty minutes later the skin sites were examined for the presence of wheal and erythema. The surface area of the reactions was estimated as follows. Transparent tape was used to transfer the outline of the reactions to paper[8] which was cut and weighed on an analytical balance. The area was read from a standard curve. Diluent consisted of 0.15 M NaCl and 0.03% human serum albumin. All injections were intradermal and 0.02 ml in volume. In each experiment a set of skin sites were also sensitized with diluent. None of these sites showed a wheal or flare reaction when challenged with ragweed antigen.

by concentrations of C_ε2-4 fragments as low as 1×10^{-8} M and concentrations of IgE (P.S.) as low as 1×10^{-9} M. Thus, a 200-fold excess of C_ε2-4 and a 20-fold excess of IgE (PS) over IgE present in serum E.C. was required to block the P-K reaction. The latter result agrees well with the results of Stanworth *et al.*[6], who found that a 10-100-folds excess of myeloma IgE (ND) over reaginic serum IgE was required to block the P-K reaction. When serum E.C. was diluted to 5×10^{-12} M IgE, it lost its ability to produce a P-K reaction. As 1×10^{-8} M C_ε2-4 completely inhibited the ability of 5×10^{-11} M IgE in E.C. serum to cause a P-K reaction, it is likely that the 200-fold excess of C_ε2-4 inhibited serum IgE to skin mast cells *in vivo by* 90%. *In vitro*, a 100-fold molar excess of C_ε2-4 fragments inhibited ^{125}I-IgE PS binding by 80% (ref. 3). Thus, the ability of C_ε2-4 fragments to inhibit IgE binding to mast cells *in vivo* and *in vitro* is comparable.

Comparison of the relative amounts of C_ε2-4 fragments and IgE (PS) needed to produce 50% reduction of the area of skin erythema induced by the P-K reaction shows that the inhibitory activity of the C_ε2-4 fragments was, on a molar basis, 30% that of natural IgE (Fig. 1). This is close to the value of 35% obtained for the relative efficacy of native IgE fragments in inhibiting ^{125}I-IgE binding to basophils *in vitro*[3].

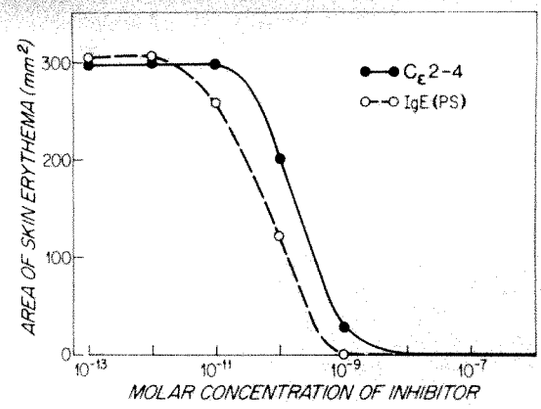


Fig. 1 Dose-response curve of inhibition of the Prausnitz-Küstner reaction. Skin sites in the forearm of a normal donor, whose serum IgE was <4 IU ml^{-1}, were injected 1 h before sensitization with decreasing concentrations of C_ε2-4 (——), IgE (PS) (- - -) or diluent (△). A 1:100 dilution of the reaginic serum E.C. containing 5×10^{-11} M IgE was used to sensitize the sites which were challenged 48 h later with ragweed antigen as described in Table 1 legend. The size of the skin erythema (flare) is shown.

Table 2 Induction of the Prausnitz-Küstner reaction by IgE-enriched fractions of reaginic serum and its inhibition by C_ε2-4 fragments

Serum (E.C.) fraction	IgE content (ng ml^{-1})	Area (mm^2) of wheal/flare at skin sites preinjected with diluent
Serum (1:100)	10	34/312
Ig enriched	7	22/210
IgE depleted	≤0.2	0/0
IgE enriched	7	27/238

An immunoglobulin-enriched fraction of serum E.C. was obtained by precipitation with 50% $(NH_4)_2SO_4$. The precipitate was reconstituted with 0.15 M saline to the initial serum volume, extensively dialysed against 0.15 M saline and diluted 1:100 in standard diluent before testing. An aliquot of the immunoglobulin-enriched fraction was circulated for 18 h at 4 °C over a Sepharose anti-IgE (PS) column[9]. The anti-IgE (PS) was raised in a goat and renderd IgE specific by absorption with human IgM, IgA, IgG myelomas and κ, λ Bence-Jones proteins coupled to Sepharose. This was followed by absorption and elution on an IgE (DAZ) column. The effluent IgE-depleted fraction was absorbed twice more over other columns of anti-IgE (PS) Sepharose. IgE was eluted from the first absorbing column with glycine-HCl buffer, *p*H 3. Eluate and effluent were dialysed extensively against phosphate-buffered saline (PBS), then diluted in standard diluent and sterilized by Millipore filtration before use. The IgE content was assayed by a solid radioimmunoassay using monoclonal mouse anti-human IgE (gift of Dr R. Siraganian) to coat the plastic polyvinyl plates and ^{125}I-anti IgE ND (Pharmacia) as a developing reagent. The assay was carried as described in ref. 9. The sensitivity of the assay was 200 pg ml^{-1}. The recipient of the P-K reaction had a serum IgE of 50 IU ml^{-1} (~120 ng ml^{-1}).

Table 3 Duration of inhibition of the Prausnitz-Küstner reaction

Inhibitor	Inhibitor concentration 10^{-7} M	10^{-6} M
C_ε2-4	12	17
IgE (PS)	14	21

Values given are days elapsed following injection of inhibitor before a successful P-K reaction could be achieved. Multiple skin sites of a normal donor with a serum IgE of 50 IU ml^{-1} were injected at day 0 with C_ε2-4 fragments, IgE (PS) or diluent. At intervals of ≥2 days (days 0, 4, 9, 12, 14, 17, 19, 21), individual skin sites were sensitized with serum E.C. (1:100 dilution), then challenged 48 h later with ragweed antigen. Sites pretreated with diluent always gave a positive wheal and flare reaction with a mean ±s.d. area of the wheal of 56±9 mm^2 and a mean ±s.d. area of the flare of 400± mm^2 for the eight successive determinations. The days shown represent the time of the first appearance of any flare and/or erythema at the challenged skin sites.

Table 3 shows that inhibition of the P-K reaction lasted for 12 days at sites that had received C_ε2-4 fragments, compared with 14 days at sites that had received IgE PS, when each was used in a 2,000-fold molar excess over IgE present in serum E.C. This inhibition lasted 17 and 21 days, respectively, at sites that had received a 20,000-fold molar excess of C_ε2-4 fragments or IgE PS.

The present experiments demonstrate that, in man, the P-K reaction can be blocked by a bacterial expression product of a human C_ε2-4 gene fragment. The inhibitory efficacy of C_ε2-4 fragments compared favourably with that of myeloma IgE (PS) both in terms of concentrations needed to inhibit the P-K reaction and in the duration of their blocking effect. As C_ε2-4 fragments are not glycosylated, glycosylation of the IgE molecule may not be essential for mast cells binding *in vivo*.

The C_ε fragment used in the present study contained the tryptophan control region of the expression plasmid pWT211 (ref. 3). Smaller C_ε fragments lacking plasmid-derived peptides are currently being constructed and tested for inhibition of the P-K reaction. Because there are no known allotypic markers for C_ε, such fragments should not be immunogenic to man. Lack of immunogenicity, potential availability in unlimited quantities and capacity to inhibit the P-K reaction for a relatively long period should make such fragments useful in attempts to prevent allergic reactions.

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Increased MHC *H-2K* gene transcription in cultured mouse embryo cells after adenovirus infection

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The class I major histocompatibility complex (MHC) antigens are highly polymorphic[1] cell-surface proteins[2] whose expression is essential for the cellular immune response against virus-infected, abnormal and foreign cells[3,4]. Transformation of primary rat cell cultures by the oncogenic adenovirus 12 (Ad12) results in suppression of the transplantation antigens[5], thus enabling the transformed cells to escape the immune response and efficiently form tumours *in vivo*[6]. In contrast, transformation of the same cells with the non-oncogenic adenovirus 5 (Ad5) does not suppress the transplantation antigens[5] and, consequently, they elicit an effective (MHC-restricted) immune response[6]. Here, however, we show that infection of mouse embryo cells with both viruses initially increases the level of transcripts from the *H-2K*^{b} transplantation antigen gene. Both the adenovirus E1a (12S RNA) and E1b genes are required for activation of the *H-2K* gene and measurement of the relative rate of transcription indicates that the increase in the level of *H-2K* messenger RNA following infection is at least in part due to a gene-specific transcriptional activation. The newly transcribed *H-2K*^{b} mRNA is then properly transported to the cytoplasm.

Cell cultures from 16-day-old C57BL/10 mouse embryos were infected after second or third passage with wild type or mutants of Ad5. RNA was extracted 50 h after infection and the level of transcripts from the *H-2K*^{b} gene measured by S_1 nuclease protection analysis using a 600-nucleotide *Ava*II probe which spans the exon III-intron III boundary of the *H-2K*^{b} gene[7]. As indicated by the relative intensities of the 230-nucleotide S_1 nuclease-protected fragment (Fig. 1*a*), infection with Ad5 resulted in a 10–15-fold increase in the level of *H-2K*^{b} mRNA (lane 2) compared with mock-infected cells (lane 1). In contrast, infection with either the Ad5 *dl*312 E1a deletion mutant or the Ad5 *dl*313 E1b deletion mutant[8,9] did not effect an increase in the level of *H-2K* mRNA (Fig. 1*a*, lanes 3, 4). To determine whether Ad5 infection caused a nonspecific change in the rate of gene transcription and/or RNA stability, we also measured the level of histone H4 gene transcripts in the above experiments. The level of histone H4 RNA increased only 2–3-fold after Ad5 infection under conditions in which the level of *H-2K*^{b} gene transcripts increased 15-fold (Fig. 1*b*).

Measurement of the relative rate of transcription of *H-2K*^{b} in nuclei isolated from mock-infected and Ad5-infected cells indicated that the increased level of *H-2K* mRNA following Ad5 infection was at least in part due to transcriptional activation. The activation appeared to be specific for *H-2K* as the rate of transcription of the type IV collagen gene did not increase on Ad5 infection (Fig. 1*c*).

Infection of mouse embryo cultures with Ad12 also resulted in a 10–15-fold increase in the level of total cellular *H-2K*^{b} mRNA (Fig. 2*a*, lane 1) compared with mock-infected cells (lane 9). Infection with a non-tumorigenic Ad12 E1a mutant (*in* 751), which lacks functional protein products from both the 12S and 13S mRNAs because of a stop codon in the first exon, did not activate the *H-2K*^{b} gene (Fig. 2*a*, lane 8). Similarly, infection with a non-tumorigenic Ad12 mutant (*hr*1121) which does not produce the 52K (relative molecular mass 52,000) E1b protein[10] did not induce *H-2K* gene expression (Fig. 2*a*, lane 5). Infection with a weakly tumorigenic Ad12 E1a mutant

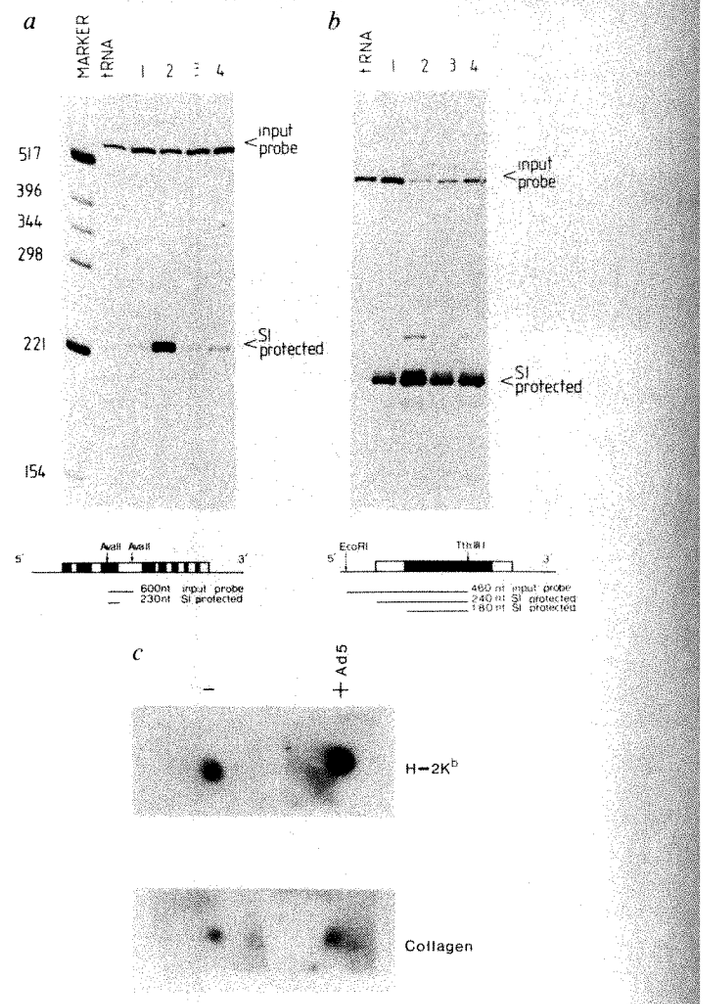


Fig. 1 *a*, *b*, S_1 nuclease analysis of *H-2K*^{b} (*a*) and histone H4 (*b*) mRNA from C57BL/10 mock-infected (lane 1), Ad5-infected (lane 2), *dl*312-infected (lane 3) and *dl*313-infected (lane 4) mouse embryo cells. Markers are ^{32}P-labelled pBr 322X Hinf. *c*, Dot hybridization of labelled nuclear transcripts from mock-infected (–) and Ad5-infected (+) C57BL/10 mouse embryo cells to *H-2K*^{b} and type IV collagen gene fragment.
Methods. *a*, A 600-nucleotide probe spanning the exon III/intron III boundary of the *H-2K*^{b} gene was end-labelled with reverse transcriptase and hybridized with 15 μg total RNA at 60 °C for 12 h in 10 μl of 80% formamide, 40 mM PIPES *p*H 6.4, 1 mM EDTA, 400 mM NaCl. The mixture was digested with 3,000 units of S_1 nuclease (Boehringer) in 300 μl of 300 mM Na-acetate *p*H 4.8, 200 mM NaCl, 2 mM $ZnSO_4$ at 20 °C for 1 h, followed by 40 °C for a further hour. S_1 nuclease-protected DNA was ethanol-precipitated, denatured and electrophoresed on a 7 M urea/7% acrylamide gel[30,31]. *b*, A 460-nucleotide probe spanning the 5′ end of the histone H4 gene was end-labelled with T4 polynucleotide kinase and hybridized with 15 μg total RNA at 52 °C as described for *a*. Samples were digested with S_1 nuclease for 2 h at 20 °C, then treated as for *a*. *c*, Nuclei were isolated from mock-infected and Ad5-infected cells. RNA synthesized in 5×10^6 isolated nuclei was labelled for 20 min with 200 μCi [α-^{32}P]UTP (3,000 Ci mmol^{-1}) in a 300 μl reaction volume as described in ref. 32. Plasmids containing the *H-2K*^{b} gene and the type IV collagen gene were linearized by digestion with *Hin*fI and *Eco*RI respectively. DNA (15 μg) was bound to a 0.45-μm nitrocellulose filter[33] and hybridized to labelled RNA from 2×10^6 isolated nuclei in a 400-μl volume[32]. After 46 h, hybridized filters were washed at 54 °C in 0.1 × S.S.C., 0.1% S.D.S. for 45 min, air-dried and exposed for 2 h.

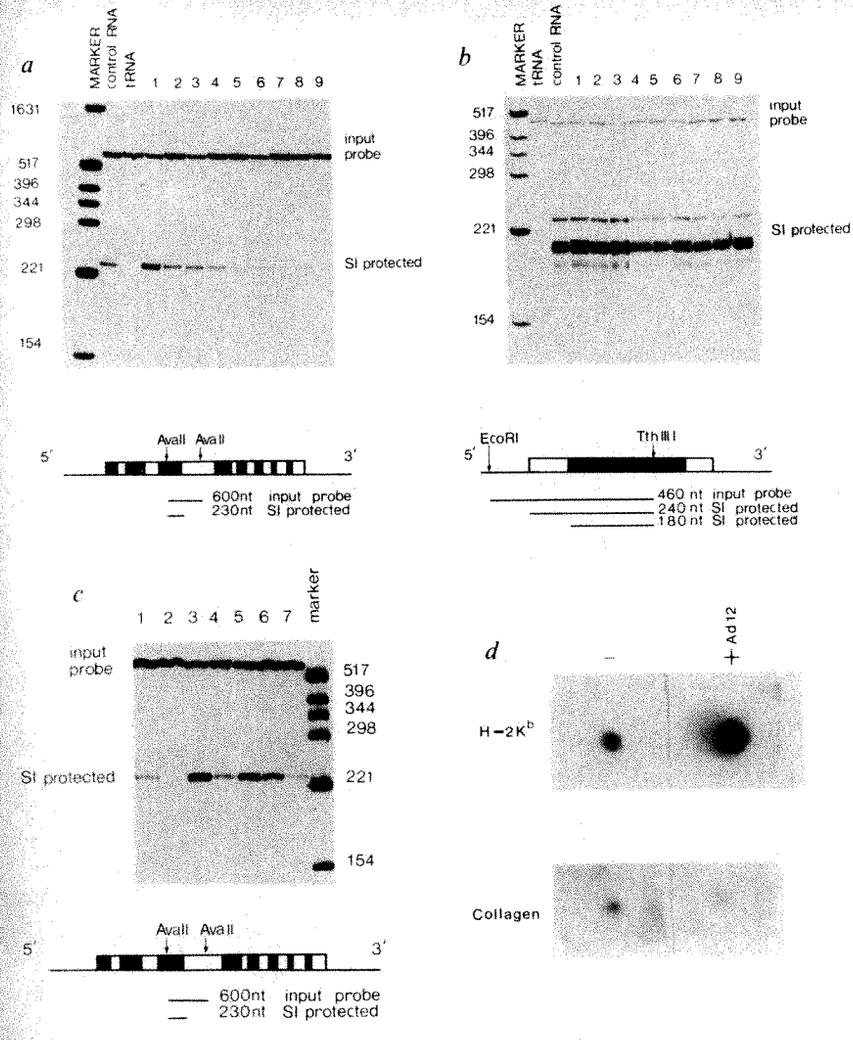


Fig. 2 *a*, *b*, S_1 nuclease analysis of $H\text{-}2K^b$ (*a*) and histone H4 (*b*) mRNA from mock-infected (lane 9) C57BL/10 cells and from cells infected with 10–20 PFU per cell of Ad12 E1a mutant *hr*751 (lane 8), Ad12 E1a mutant *hr*771 (lane 7), Ad12 E1a mutant *in*600 (lane 6), Ad12 E1b mutant *hr*1121 (lane 5), *in*751 plus *hr*1121 (lane 3), *hr*751 plus *in*600 (lane 4), *in*600 plus *hr*1121 (lane 2) and Ad12 wild type (lane 1). S_1 nuclease analysis was done as described for Fig. 1*a*, *b*. *c*, S_1 nuclease analysis of $H\text{-}2K^b$ mRNA from mock-infected C57BL/10 cells (lane 1), control tRNA (lane 2) and from cells infected with Ad12 (lane 3), *in*751 (lane 4), *hr*771 (lane 5), *in*600 (lane 6) and Ad12 *hr*1121 (lane 7) at a titre of 50 PFU per cell. S_1 nuclease analysis was done as described for Fig. 1*a*. *d*, Dot hybridization of labelled nuclear transcripts from mock-infected (–) and Ad12-infected (+) C57BL/10 mouse embryo cells to $H\text{-}2K^b$ and type IV collagen gene fragments. Methods as for Fig. 1*c*.

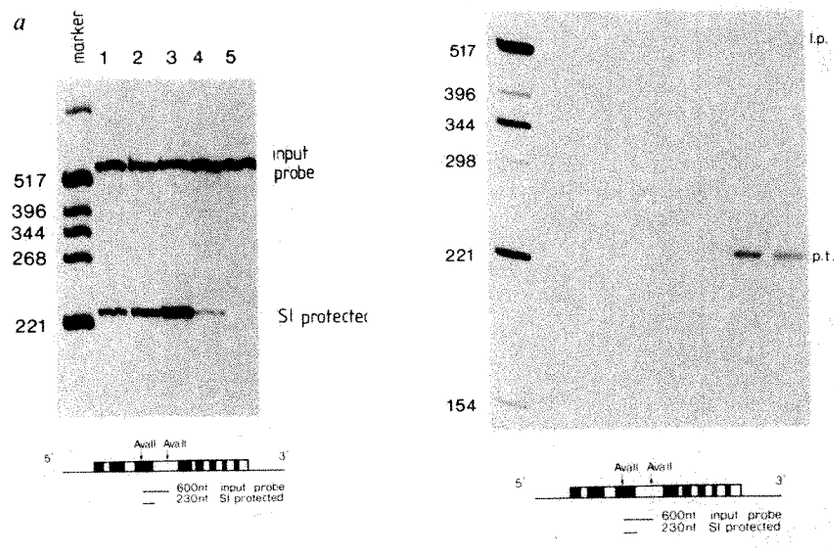


Fig. 3 *a*, S_1 nuclease analysis of $H\text{-}2K^b$ gene transcripts from mock-infected C57BL/10 mouse embryo cells (lane 4), control tRNA (lane 5) and from cells infected with polyoma virus (lane 1) or SV40 (lane 2) at a multiplicity of infection of 10–50 PFU per cell. Lane 3 contains control RNA isolated from C57BL/10 cells treated with 1,000 U ml^{-1} mouse γ-interferon. *b*, S_1 nuclease analysis of cytoplasmic(cyt) and nuclear (nuc) $H\text{-}2K^b$ mRNA from mock-infected (lane 2), Ad5-infected (lane 3, 10 PFU per cell) and Ad12-infected (lane 4, 10 PFU per cell) C57BL/10 mouse embryo cells. Lane 1 contains a tRNA control. S_1 nuclease analysis was carried out as described for Fig. 1. All lanes contain an equal amount of RNA (20 μg).

(*hr*771) which produces some E1a protein products only after a delay in permissive cells[10], or with an Ad12 E1a mutant (*in*600) which contains the normal E1a 12S RNA and a truncated 13S RNA[10] resulted in, respectively, no increase (Fig. 2*a*, lane 7) or only a marginal increase (lane 6) in the level of $H\text{-}2K$ gene transcripts at the multiplicity of infection used (10 plaque-forming units (PFU) per cell). Co-infection of E1a mutants *hr*751 and *in*600 with each other or with the *hr*1121 E1b mutant resulted in an increase in the level of $H\text{-}2K$ mRNA (Fig. 2*a*, lanes 4, 3 and 2 respectively); this effect could be caused by either complementation or an increased number of virus particles (10 PFU per cell of each; see below). The levels of histone H4 transcripts in these infections were only 1.5–2 times higher than in mock-infected cells (Fig. 2*b*).

Failure of adenovirus E1a mutants to activate the $H\text{-}2K^b$ gene may result from the lack of production of other adenovirus early proteins which are normally activated by the E1a gene products[11,12]. We therefore repeated the Ad12 mutant infections at a higher multiplicity of infection (50 PFU per cell) to allow production of other adenovirus early proteins in the absence of

the E1a proteins (ref. 13 and R. Grand and P. Gallimore, personal communication). As shown in Fig. 2*c*, infection with a high titre of E1a mutants (*in*600 and *hr*771) resulted in activation of the *H-2K*^{b} gene equal to that effected by wild-type virus. This result indicates that it is not the protein products of the E1a 13S transcript alone that activate the *H-2K*^{b} gene. The E1b 52K protein and the products of the E1a 12S RNA, or 13S exon 1 RNA, are directly or indirectly necessary for *H-2K*^{b} gene activation since high-titre infection with either the E1b 52K mutant (*hr*1121) or the E1a mutant which lacked protein products of both 12S and 13S RNAs (*hr*751) failed to significantly (less than twofold) increase the level of *H-2K*^{b} gene transcripts (Fig. 2*c*). Measurement of the relative rate of transcription of the *H-2K*^{b} gene in mock-infected and Ad12-infected cells indicated that the increase in the level of *H-2K* mRNA following viral infection was at least in part due to transcriptional activation (Fig. 2*d*).

This result may reflect the *trans*-acting enhancing properties of the E1a protein products, which can activate viral[11,12,14], newly introduced[15–18], integrated[19] and endogenous genes[20] either directly or through inactivation of cellular repressor proteins[13,21]. Alternatively, the gene activation may be mediated by a general anti-viral state induced by cellular proteins in response to either viral replication or viral proteins[21,22]; this seems unlikely, as Ad12 virus infections at 10 PFU per cell did not result in detectable levels of interferon (<4 U ml^{-1}) in the cell culture (data not shown).

The results presented here indicate that infection of mouse embryo cells with either Ad5 or Ad12 results in transcriptional activation of the MHC *H-2K* gene. Viral protein products from the 12S E1a mRNA or the first exon of the E1a gene, which is common to both 13S and 12S mRNAs, and the 52K E1B protein are necessary for this activation (Fig. 2). As yet, we do not know whether viral protein products from both the E1a and E1b regions are mediating the activation directly or via interaction with other (early) viral or cellular proteins. For example, the Ad5 E1b 58K protein binds the same p53 cellular protein as simian virus 40 (SV40) large-T antigen[24]. To determine whether other DNA tumour viruses such as SV40 mediate induction of the class I MHC genes using similar mechanisms[25] and whether induction is related to permissiveness of the virus/cell system, we infected mouse embryo cells with either SV40 (non-permissive, as is adenovirus) or polyoma virus (permissive). Infection with either of these viruses resulted in activation of the *H-2K* gene but at a lower level than that resulting from the adenovirus infections, which itself was lower than that induced by interferon in a control experiment (Fig. 3*a*).

Our results are in contrast to those of Schrier *et al.*[5] who reported a decrease in the level of cytoplasmic class I mRNA and suppression of MHC antigen expression in Ad12-, but not in Ad5-infected cells. As Schrier *et al.*[5] measured cytoplasmic mRNA, the difference could be due to a block in the transport of the newly synthesized mRNA from the nucleus to the cytoplasm. To test this possibility, the cells were infected with Ad5 and Ad12 (10 PFU per cell) and both the nuclear and cytoplasmic mRNA were quantitated. The results (Fig. 3*b*) show that the *H-2K* mRNA in the cytoplasm is increased between 10 and 15-fold in both cases, while the mRNA level in the nucleus is much lower and only visible after a longer exposure, which excludes the possibility of a block in mRNA transport.

However, preliminary experiments using radioimmune labelling of the Ad5- and Ad12-infected cells indicate that the amount of H-2K^b antigen on the cell surface increases only marginally, if at all, depsite the large increase in cytoplasmic *H-2K*^{b} mRNA (data not shown). Thus, the results suggest that infection first induces a stimulation of MHC transcription, which normally would enable a better cellular immune response against infected cells. In the case of viruses, for example Ad5 and Ad12, this may not result in an increased expression of MHC antigens on the cell surface because of other viral factors[23]. In the case of oncogenic viruses such as Ad12, this may be followed by a suppression of MHC class I gene transcripts to allow escape from immune surveillance. Whether this is caused by an immunity from natural killer cells[26–28] alone and/or from cytotoxic T cells[6] as part of an MHC-restricted response[29], is unclear.

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Irreversible swelling of secretory granules during exocytosis caused by calcium

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The fusion of the limiting membrane of a secretory granule with the plasmalemma during exocytosis is equivalent to the fusion and release of contents that occurs when phospholipid vesicles fuse with planar bilayers[1,2]. Experiments with bilayers demonstrate that phospholipid vesicles must swell if they are to fuse[3]. Also, inhibition of exocytosis in solutions of high osmolarity occurs in several types of secretory cell[4–8]. We report here experiments on the cortical granule exocytosis of sea-urchin eggs. Exocytosis is prevented when the osmolality of the medium surrounding the eggs is raised from 1 to 2 osmol kg^{-1}. High osmolality also prevents calcium-dependent exocytosis *in vitro*. Prior treatment with calcium at high osmolality triggers fusion when normal osmolality

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is restored, even if calcium is removed before dilution. Addition of calcium causes the cortical granules to swell. The large increase in membrane capacitance which normally accompanies fusion[9] is absent in eggs activated in solutions of high osmolarity. Our data are consistent with the idea that a secretory granule must swell to fuse with the plasma membrane and support the hypothesis[2,10] of an osmotically driven fusion step during exocytosis.

When a sea-urchin egg is fertilized or activated with the calcium ionophore A23187, there is a rapid and complete exocytosis of the cortical granules that lie immediately beneath the plasmalemma[11,12]. This secretory event is stimulated by an increase in cytoplasmic free calcium[13]. If eggs are treated with calcium ionophore in sea water to which sucrose, sodium sulphate or stachyose is added to produce an osmotic pressure increase corresponding to 1 osmol per kg, they retain their full complement of cortical granules (Fig. 1). The inhibition is reversible in that eggs returned to sea water after treatment with ionophore in the presence of high-osmolarity solution undergo a complete exocytosis. Pretreating the eggs with osmoticant and returning them to sea water before adding calcium ionophore does not prevent exocytosis. The similar effects of 0.7 M stachyose, 0.55 M sodium sulphate and 0.85 M sucrose suggest that it is the osmotic rather than the pharmacological properties of the three substances which are responsible for the inhibition of secretion. In the presence of solutions of high osmotic pressure the granules shrink, suggesting that if swelling of the granule is hindered then exocytosis is inhibited.

We wished to determine whether solutions of high osmolality were indeed inhibiting the fusion of the secretory granule membrane with the plasma membrane or whether fusion had, in fact, taken place. Under normal conditions the granule contents take up water and salts as they disperse[14,15]. It was possible that the granule and plasma membranes had fused with the formation of an exocytotic stoma and that solutions of high osmolality were preventing granule dispersal.

One consequence of exocytosis of the cortical granules of the egg is a two- to threefold increase in the membrane capacitance which is the result of addition of cortical granule membrane to the plasmalemma[9,16]. Figure 2 shows that under conditions in which exocytosis (by which we mean both membrane fusion and the dispersal of the granule contents) is prevented, only a very small increase in membrane capacitance is observed, ~10%

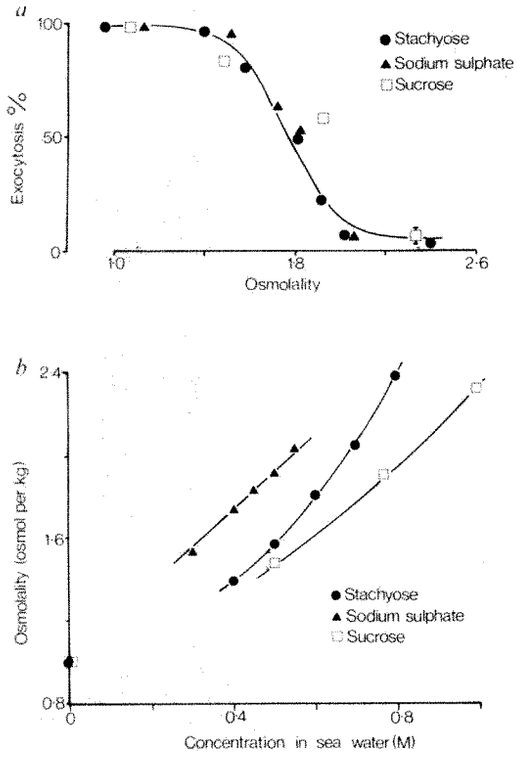


Fig. 1 *a*, Effect of increasing osmolality on cortical granule exocytosis in *Lytechinus pictus* eggs. Jelly-free eggs were resuspended in artificial sea water[19] (1.02 osmol per kg) or filtered sea water (0.90 osmol per kg) containing various concentrations of sucrose, stachyose or sodium sulphate. *b*, Osmolality was measured using a Wescor vapour pressure osmometer. Resuspended eggs were mixed with an equal volume of solution of identical composition containing 20 μM A23187 (Calbiochem), and 5 min after mixing, the extent of exocytosis of the cortical vesicles was assessed in the light microscope by gently flattening the eggs against a coverslip. The cortical granules are identified easily by their size and degree of association with the egg cortex. The granules in a disk of 50 μm radius were counted in 10 eggs selected at random. Each experiment was recorded on photographic film (Zeiss, Nikon or Leitz DIC optics with ×100, 1.25 or 1.4 NA objectives). The osmolarity of the solutions being tested was not known to the observer. Inhibition by stachyose solutions was time-dependent. After resuspension in stachyose the eggs shrank rapidly then swelled slowly towards their original size. Exocytosis eventually occurred at all stachyose concentrations tested. Stachyose did not merely reduce the rate of exocytosis, as in 0.8 M stachyose sea water, for example, no detectable exocytosis occurred for 25 min after addition of the calcium ionophore, although exocytosis was extensive at 30 min after addition. In untreated preparations, exocytosis is complete in 1 min. Temperature, 20 °C.

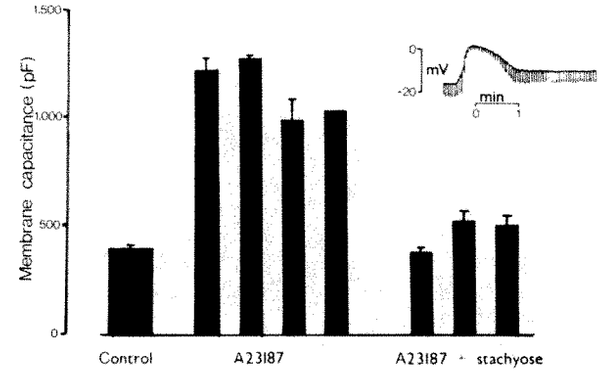
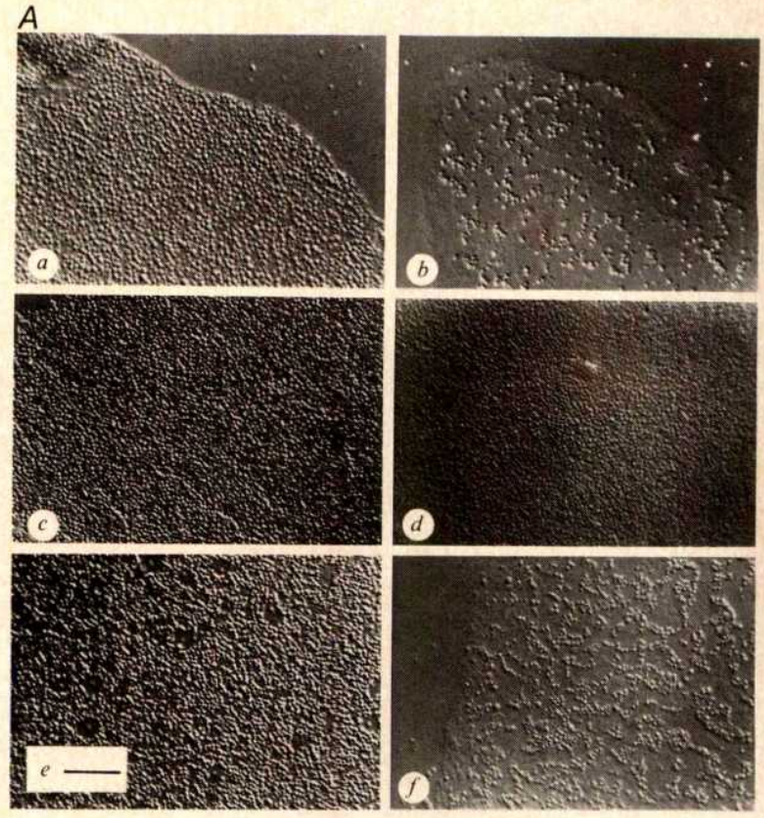


Fig. 2 Effect of stachyose on the increase in egg membrane capacitance resulting from membrane fusion. The inset shows the membrane potential alterations when the calcium ionophore A23187 (50 μM in ethanol) is added in the presence of 0.8 M stachyose in artificial sea water (2.39 osmol per kg). Membrane capacitance was measured by injecting constant current pulses through one microelectrode while recording membrane potential with a second microelectrode. Data were recorded on magnetic tape and the membrane time constant and resistance measured from five consecutive current pulse records made from tape with a chart recorder, shown (A23187 and A23187 + stachyose) as single experiments (mean ± s.e.m.). Controls are shown as mean and s.e.m. of 22 estimates of membrane capacitance in six unfertilized eggs. The rise time of the recording system was 3.5 ms. Egg input resistance before activation, 66–180 MΩ; after activation, 20–60 MΩ; current pulse, 100 or 200 pA; electrode resistance, 50–100 MΩ (KCl-filled). Temperature, 16 °C. The small increase in membrane capacitance observed when eggs are treated with A23187 in the presence of 1 M stachyose (10% of the change seen in control eggs) may result from exocytosis of 10% of the granules. The converse assumption is that it results from a decrease in the membrane resistance (R_s) between the interior of each granule and the external solution. The decrease in R_s could be distributed across the area of contact of granule and plasma membranes or could result from the appearance of a small aqueous pore or channel between the interior of each granule and the external medium. Our data indicate that the capacitance of the granule membrane (C_g) is 800 pF and that of the cell membrane (C_m) 400 pF. We have assumed that the granule membrane resistance (R_g) and granule membrane capacitance are linked by series resistance R_s to the plasma membrane resistance (R_m) and capacitance. Thus, $I = ((R_s + R_g + R_m)/R_m R_g + R_s))\ V + ((C_m R_m[R_g + R_s] + C_g R_g[R_m + R_s])/R_m(R_g + R_s))\ dV/dt + (C_g C_m R_s R_m R_g/R_m(R_g + R_s))\ d^2V/dt^2$. I, membrane current; V, membrane potential; t, time. Solving for V and t with a step change of membrane current we find from the apparent increase in membrane time constant in the presence of 1 M stachyose that if R_m is ≤200 MΩ then R_s cannot be <50 MΩ. This sets an upper limit on the size of the pores. There are ~30,000 cortical granules in a *L. pictus* egg[8], which gives a resistance per pore of 1.5×10^{12} Ω. This resistance is of the order of the resistance of the smallest ion channels so far discovered in biological membranes; we therefore believe that fusion and the formation of an exocytotic stoma have occurred in ≤10% of the cortical granules of the egg under these conditions.



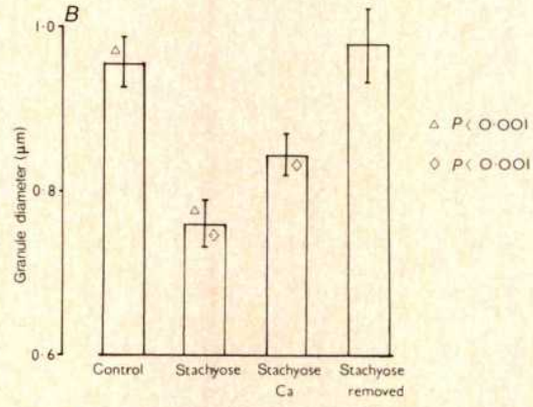


Fig. 3 ***A***, Effects of stachyose on cortical vesicle exocytosis *in vitro*. Cortical fragments consisting of vitelline coat plasma membrane and attached cortical vesicles were prepared by attaching eggs by their vitelline coat to glass coverslips with polylysine and shearing away the bulk of the egg using a jet of isolation medium (220 mM potassium glutamate, 500 mM glycine, 5 mM $MgCl_2$, 10 mM NaCl, 2.5 mM ATP, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulphonyl fluoride, 1 mM EGTA, *p*H 6.7; ref. 19). Cortical fragments on the right (*b*, *d*, *f*) have been treated with calcium (6 μM; Ca/EGTA = 0.838; ref. 19). *a*, Cortical fragment in isolation medium; *b*, fragment in isolation medium 2 min after addition of 6 μM calcium; *c*, fragment in isolation medium containing 1 M stachyose showing granules of decrease size; *d*, fragment in isolation medium containing 1 M stachyose 5 min after treatment with 6 μM calcium showing inhibition of exocytosis of cortical vesicles; *e*, fragment returned to isolation medium after treatment with 1 M stachyose-containing isolation medium (vesicles swell to their original size and will undergo exocytosis if exposed to calcium, not shown); *f*, fragment returned to isolation medium after treatment for 2 min with 1 M stachyose-containing medium containing 6 μM Ca, then for 2 min with 1 M stachyose-containing medium ($<10^{-8}$ M Ca) before returning to isolation medium ($<10^{-8}$ M Ca); exocytosis occurs in the absence of calcium when stachyose is removed. As in whole eggs, the inhibition of exocytosis is time dependent. Exocytosis occurred in the presence of stachyose after 15–20 min. At normal osmolarities, exocytosis occurs completely within 1 min of calcium addition. Temperature, 20 °C; scale bar, 10 μm. ***B***, Effects of stachyose and calcium on the size of cortical vesicles *in vitro*. Control, preparation in isolation medium; Stachyose, in 1 M stachyose-containing medium; Stachyose Ca, in 1 M stachyose-containing isolation medium containing 6 μM calcium; Stachyose removed, preparation treated with 1 M stachyose-containing medium and returned to stachyose-free medium. Photomicrographs similar to those shown in *A* were printed at ×10,000 and the diameter of 100 granules estimated from the measurements of granule perimeter made using a digitizing tablet. Mean diameter ±s.e.m. are shown. The statistical significance of the differences in mean diameter were assessed using a one-tailed Kolmogaroff–Smirnoff test. The dispersion in measured granule diameters seems to result from the dispersion of apparent granule sizes and not from errors in measurement, as the measuring procedure was precise, giving granule diameters of 0.98 ± 0.038 and 0.98 ± 0.041 μm (mean ±s.e.m.) in two separate experiments in which granules were treated with high-osmolarity stachyose and returned to normal osmolarity.

of that seen at normal osmolarity. Under these conditions, it seems that no exocytotic stoma form and that inhibition of granule swelling prevents fusion as well as dispersal. These experiments also indicate that stachyose is not preventing the ionophore-induced internal calcium transient, as we observe the calcium-associated membrane depolarization.

Inhibition of exocytosis by applying solutions of high osmolarity to the outside of the egg may be the consequence of alterations in ionic strength or viscosity in the cytoplasm caused by the shrinkage of the egg. Therefore, we investigated the effects of high osmolality on exocytosis *in vitro*.

A preparation consisting almost entirely of plasma membrane with attached cortical granules can be made by attaching eggs to glass coverslips with polylysine and shearing away the bulk of the egg[17]. This preparation gives access to the cytoplasmic face of the plasma membrane. Granules undergo exocytosis in response to micromolar calcium concentrations[18–20]. It is known that high-osmolarity sucrose solutions reduce the rate of exocytosis *in vitro* and that under these conditions the effect of calcium seems to be irreversible[21]. Inhibition by sucrose is incomplete because the cortical granules have a substantial sucrose permeability, so we used stachyose, a tetrasaccharide to which the granules are less permeable. Figure 3*A* shows the result of an experiment in which calcium was added to the preparation in the presence of stachyose (1 M in isolation buffer; 3 osmol per kg) and in which exocytosis is prevented. If calcium is removed and the preparation is allowed to remain for 5 min in EGTA-containing 1 M stachyose isolation buffer before dilution into isolation buffer containing EGTA, then exocytosis occurs on dilution to normal osmolarity. Exposing a preparation to stachyose in the absence of calcium and returning it to normal isolation medium does not result in a substantial loss of cortical granules; >90% are unaffected by this treatment. These experiments demonstrate that, even in the presence of 1 M stachyose, calcium is exerting an effect whose results persist after its removal. That is, the calcium-dependent step in exocytosis is irreversible in stachyose-treated cortices.

The planar array of cortical granules on the isolated plasma membrane forms an excellent optical image. Figure 3*B* shows that granules shrink considerably when exposed to 1 M

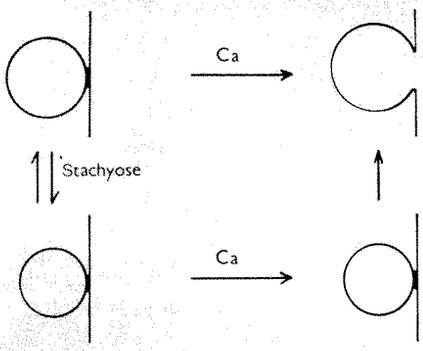


Fig. 4 Diagrammatic representation of the changes thought to be occurring in our experiments. Calcium promotes swelling and exocytosis whereas stachyose and other osmoticants cause reversible shrinkage of the cortical vesicles. If calcium is added in the presence of stachyose, the vesicle swells slightly but the swelling is insufficient to cause fusion. However, the state of the vesicle is altered irreversibly by calcium. Removal of stachyose after treatment with calcium leads to exocytosis.

stachyose in isolation medium and appear to swell slightly when they are also exposed to calcium. We suppose that calcium-induced swelling can only cause exocytosis if granules swell beyond their usual size.

Cortical granule exocytosis *in vitro* is not affected by large increases in ionic strength. We find that it occurs in solutions containing 1 M KCl, which is four times the ionic strength of cytoplasm[22]. Nor is a solution of high viscosity (30% Ficoll, relative molecular mass 400,000, 96 cP) inhibitory *in vitro*[21]. Therefore, it seems likely that high osmolality prevents exocytosis in whole eggs, as it does *in vitro*, by preventing granule swelling[21,23]. We find that higher concentrations of stachyose are needed to inhibit exocytosis *in vitro* (1 M in isolation buffer; 3.0 osmol per kg) than in whole eggs (0.7 M in sea water; 2.2 osmol per kg). We attribute this to a small but significant permeability of the granule membrane to stachyose, because a proportion (~10%) of the granules burst when returned to normal osmolarity after treatment with stachyose in the absence of calcium. This interpretation is supported by the observation that inhibition of exocytosis by stachyose is time-dependent (see Figs 1, 3 legends).

A diagram consistent with our results is shown in Fig. 4, in which calcium induces a swelling of the secretory granule which under normal circumstances leads to fusion. In the presence of solutions of high osmolarity, calcium-induced swelling is insufficient to cause fusion. But calcium has irreversible effects and fusion occurs when the granule swells further on exposure to normal osmotic pressures. We do not know why the cortical granule swells on exposure to calcium, nor why granule swelling leads to fusion. Granule swelling may result from an increased influx of ions and water across the granule membrane as a consequence of calcium-induced permeability changes or as a consequence of calcium-induced alterations of the osmotic properties of the granule contents. Fusion may occur because the granule ruptures preferentially at its point of contact with the plasma membrane, as has been suggested to explain the fusion of phospholipid vesicles with planar bilayers[24]. A mechanism in which calcium stimulates an ion exchange across the apposed granule and plasma membranes[25] seems to us less likely because exocytosis in sea-urchin eggs will occur in the absence of external monovalent ions[26].

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Movement of myosin-coated beads on oriented filaments reconstituted from purified actin

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Although the biochemical properties of the actin/myosin interaction have been studied extensively using actin activation of myosin ATPase as an assay[1,2], until recently no well-defined assay has been available to measure the mechanical properties of ATP-dependent movement of myosin along actin filaments. The first direct measurements of the rate of myosin movement *in vitro*[3,4] used a naturally occurring, biochemically ill-defined array of actin filaments from the alga *Nitella*. We report here the construction of an oriented array of filaments reconstituted from purified muscle actin and the use of this array in a biochemically defined quantitative assay for the directed movement of myosin-coated polystyrene beads. We demonstrate for the first time that actin alone, linked to a substratum by a protein anchor, is sufficient to support movement of myosin at rates consistent with the speeds of muscle contraction and other forms of cell motility.

We have used severin, a protein from the amoeba *Dictyostelium*[5,6] which binds with very high affinity to the barbed end of actin filaments[7] (Fig. 1*a*). Using biotinylated severin, actin filaments can be bound to avidin immobilized on a carbon-coated electron-microscope grid, which serves as an optically clear substratum (Fig. 1*b*). In appropriate flow conditions, the bound filaments should orient with their free pointed ends in the downstream direction (Fig. 1*c*). According to H. E. Huxley's model[8], myosin moves along actin from the pointed end to the barbed end. Therefore, myosin beads placed on the actin/severin/avidin array should move upstream against the flow of buffer. The use of electron-microscope grids allows the actin array to be visualized by transmission electron microscopy after measurement of bead movement.

We followed the active movement of 40 myosin beads over distances of 10–50 μm on actin arrays demonstrated by electron microscopy to be markedly oriented (Fig. 2). The average velocity of skeletal myosin beads was 4 μm s^{-1} (26 beads, range 0.5–6 μm s^{-1}), which is close to the measured maximum rate of contraction of intact muscle under zero load (6 μm s^{-1})[9]. The average velocity of *Dictyostelium* myosin beads was 0.6 μm s^{-1} (14 beads, range 0.3–1.3 μm s^{-1}). These velocities measured with

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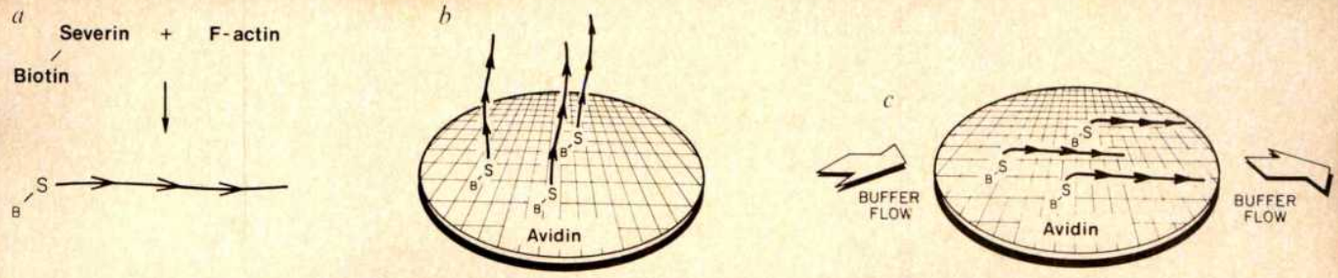


Fig. 1 Schematic representation of the method for preparing the oriented actin substratum. *a*, Severin was biotinylated as follows: 800 μl severin at 0.25 mg ml^{-1} in 50 mM sodium phosphate, *p*H 7.5, was combined with 15 μl 1 mg ml^{-1} *N*-hydroxysuccinimido biotin in dimethyl sulphoxide for 90 min at 22 °C, and the reaction quenched with 5 μl 0.1 M glycyl glycine, *p*H 7.5. The mixture was dialysed against F buffer (10 mM imidazole, *p*H 7.0, 25 mM KCl, 4 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol) containing 4 mM EGTA at 4 °C for 36 h. Severin/biotin was mixed with fresh 0.25 mg ml^{-1} F-actin[13] in F buffer with 1 mM ATP and 0.2 mM $CaCl_2$ to a ratio of severin:actin 1:500 to produce a population of filaments with their barbed ends blocked by severin. *b*, *c*, A carbon-coated electron-microscope grid (200 mesh), coated with 100 μg ml^{-1} avidin in 50 mM $NaHCO_3$, *p*H 9.5, for 10 min at 22 °C, and washed with F-buffer containing 0.2 mM $CaCl_2$, was pinned onto the bottom of a channel (3 mm wide, 1 mm deep) moulded in silicone polymer. Severin/actin filaments were applied to the grid surface with laminar flow in the channel at 10 ml min^{-1} for 10 min. Unattached actin was washed from the grid surface in a flow (5 ml min^{-1}) of F buffer containing 25 μg ml^{-1} actin, 4 mM EGTA and 1 mM ATP, leaving a mat of ordered filaments.

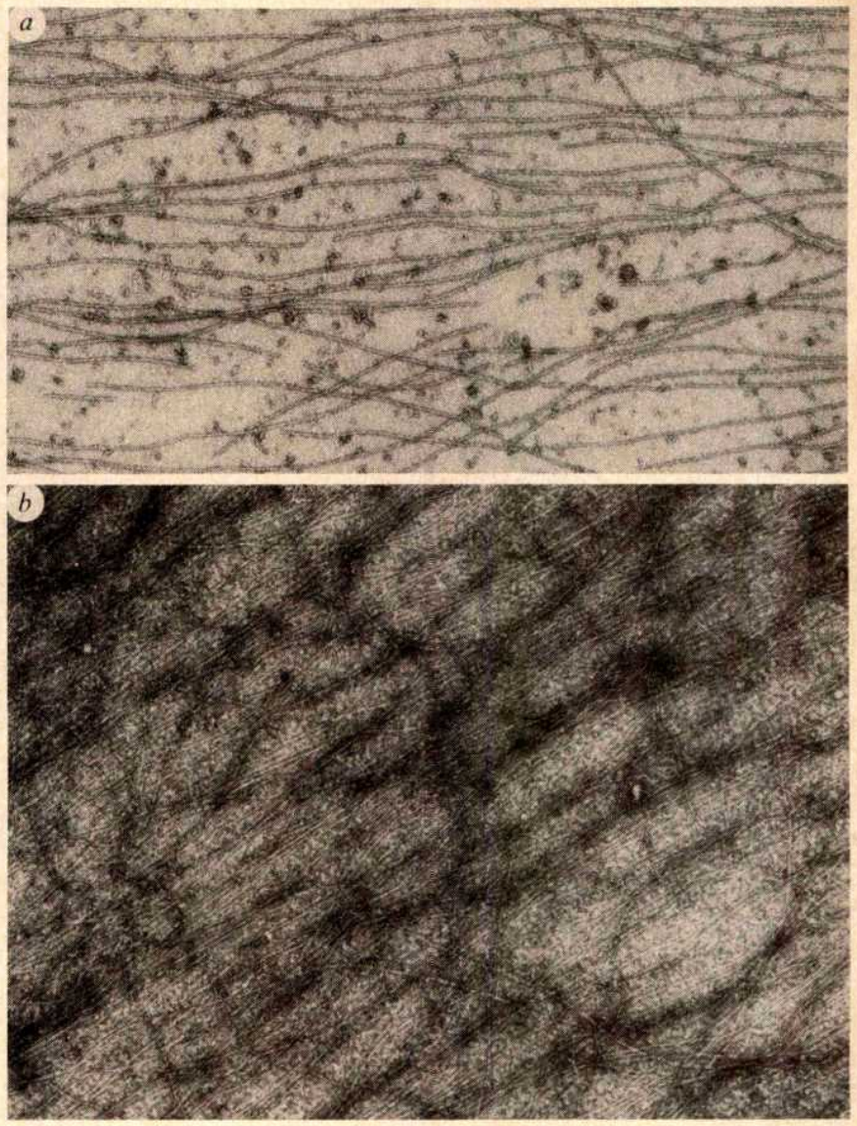


Fig. 2 Electron micrographs of F-actin oriented as described in Fig. 1 legend. *a*, Image less densely populated with actin filaments than *b*, revealing the orientation of the filaments resulting from the flow. ×58,500. *b*, Image of a dense meshwork of oriented filaments taken from a grid that supported movement of myosin beads. ×52,000. To obtain the images shown, a grid was carefully removed from the channel, noting its orientation with regard to the buffer flow. Aqueous uranyl acetate (1%) was flowed in the same direction across the grid, the excess being drawn off with filter paper from the downstream edge.

the synthetic actin array are remarkably similar to those observed using the *Nitella*-based assay[4] (2–6 μm s^{-1} for skeletal muscle myosin and 0.5–1.5 μm s^{-1} for *Dictyostelium* myosin).

These results show that, to support myosin movement, actin filaments need not be in the form of bundles (as they are in *Nitella*), nor are accessory proteins required (other than a filament anchor such as severin). In addition, our results suggest that the specific myosin type (skeletal muscle or *Dictyostelium*) is the principal determinant of bead velocity, rather than the type of actin (muscle or *Nitella*).

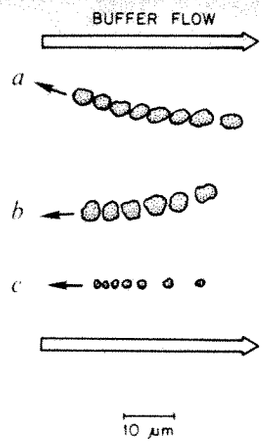


Fig. 3 Movement of myosin beads in the upstream direction with respect to the buffer flow. *a*, *b*, The two larger distinct bead aggregates were coated with 200 μg ml^{-1} *Dictyostelium* myosin and their positions shown every 10 s, corresponding to a rate of movement of ~0.5 μm s^{-1}. *c*, The smaller bead aggregate was coated with 200 μg ml^{-1} skeletal muscle myosin and its position shown every 2 s, corresponding to a rate of movement of ~2 μm s^{-1}. Myosin beads were deposited on the actin array by microinjection, suspended in F buffer containing 1 mM ATP, 0.2 M sucrose, 1 mM dithiothreitol and 4 mM EGTA. With F buffer containing 25 μg ml^{-1} actin, 4 mM EGTA and 1 mM ATP flowing in the channel at 0.04 ml min^{-1}, myosin beads and distinct bead aggregates attached to the grid surface and moved upstream. Myosin bead preparation and analysis of movement were as described previously[4]. The diagram shows typical bead movements. To be scored as an active event, bead movements had to be: (1) upstream to the direction of buffer flow; (2) at the plane of focus of the surface of the grid; (3) associated with a distinct binding event; (4) characterized by sliding rather than tumbling. In several cases, the rate of buffer flow was changed in the course of active bead movement and no effect on the rate of bead movement was observed. In contrast, movements resulting from bulk flow were: (1) downstream; (2) generally not in the plane of focus of the surface of the grid; (3) not associated with binding to the grid surface; (4) characterized by tumbling when the beads were close to the surface of the grid; (5) dependent on the presence and rate of buffer flow.

Drag on a sphere in laminar flow at low Reynolds's number is directly proportional to the bulk fluid velocity. As discussed previously[3], sufficient power is generated by a single myosin molecule to move a 1-μm bead 5 μm s^{-1} upstream against a flow of 5 μm s^{-1}. Because the flow rate near the beads in the present experiments is ≤1 μm s^{-1} (determined by measuring the velocity of unattached beads moving downstream near the substratum), it is probable that the limit on the speed of myosin movement over actin in this assay is determined by the kinetics of the biochemical reaction. The finding that some beads move slower than this maximum may reflect the presence of disorder in the array of actin filaments. Two observations are consistent with the presence of local regions of disorder. Only one-third of the myosin beads that attach to the substratum move, and those move over distances of 10–50 μm and stop, apparently tightly bound to the electron microscope grid.

Several biochemically defined actin/myosin-based *in vitro* models of cell motility have been suggested[10–12], but none of these has offered a quantitative measure of myosin movement. Many types of experiment are now possible with the biochemically defined assay described in the present study. One important application will be in the identification and purification of factors that regulate actin/myosin-mediated cell motility. This assay can also be used to study the effects of genetic manipulation of both actin and myosin structure on myosin movement along actin filaments. It should be possible to locate and define the amino-acid residues critical for the transduction of chemical energy liberated by ATP hydrolysis into the mechanical work of cellular motility.

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Note added in proof: We have also observed movement on actin filaments applied by flow to severin nonspecifically bound to an electron-microscope grid surface.

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Compartmentalization of sickle-cell calcium in endocytic inside-out vesicles

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Much recent interest in the mechanism of dehydration of the dense subpopulation of sickle-cell anaemia (SS) red cells, including the 'irreversibly sickled cells' (ISCs), stems from the view that these relatively rigid cells have a major role in the two main clinical features of the disease, namely haemolytic anaemia and microvascular occlusion[1]. The discovery that SS red cells have an elevated calcium content and accumulate Ca^{2+} during deoxygenation-induced sickling[2–4] suggested a working hypothesis of wide appeal for the mechanism of cell dehydration: retained calcium would activate the red cell Ca^{2+}-sensitive K^+ channels[5], causing progressive net loss of KCl and water[6–8]. However, retained calcium, which seemed as weakly bound to cytoplasmic buffers as in normal red cells[9], failed to show any measurable activation of K^+ channels or Ca^{2+} pumps in metabolically normal SS cells, despite the apparent functional normality or near-normality of these transport systems[9–17]. We now offer a possible explanation for this failure. We show that, contrary to the traditional views, SS cells, and to a lesser extent normal human red cells, possess intracellular vesicles with ATP-dependent Ca^{2+}-accumulating capacity, and that nearly all the measurable calcium of fresh SS cells is contained within such vesicles, probably in the form of precipitates with inorganic or organic phosphates.

Intracellular vesicle-like structures have been described previously in electron micrographs of single sections of fixed normal red cells[18,19]. In such sections, however, true vesicles are indistinguishable from artefacts resulting from occasional membrane infoldings. To demonstrate that the vesicles are fully contained within the cell, it is necessary to examine the three-dimensional structure of a representative number of cells in serial section. Figure 1 shows typical runs of consecutive sections of fresh SS cells and ghosts chosen to illustrate genuine and artefactual vesicular images. Analyses of 75 SS cells, 59 SS ghosts and similar studies (not shown) with 36 normal red cells and 62 normal ghosts, revealed that both SS and normal red cells possess fully enclosed vesicles. In at least 84% of the normal cells, one to four tiny vesicles were found, which were ~0.1 μm in diameter. The vesicles were usually contained within one or two consecutive 0.10–0.15-μm sections, but occasionally extended over up to seven such consecutive sections, with tubular or

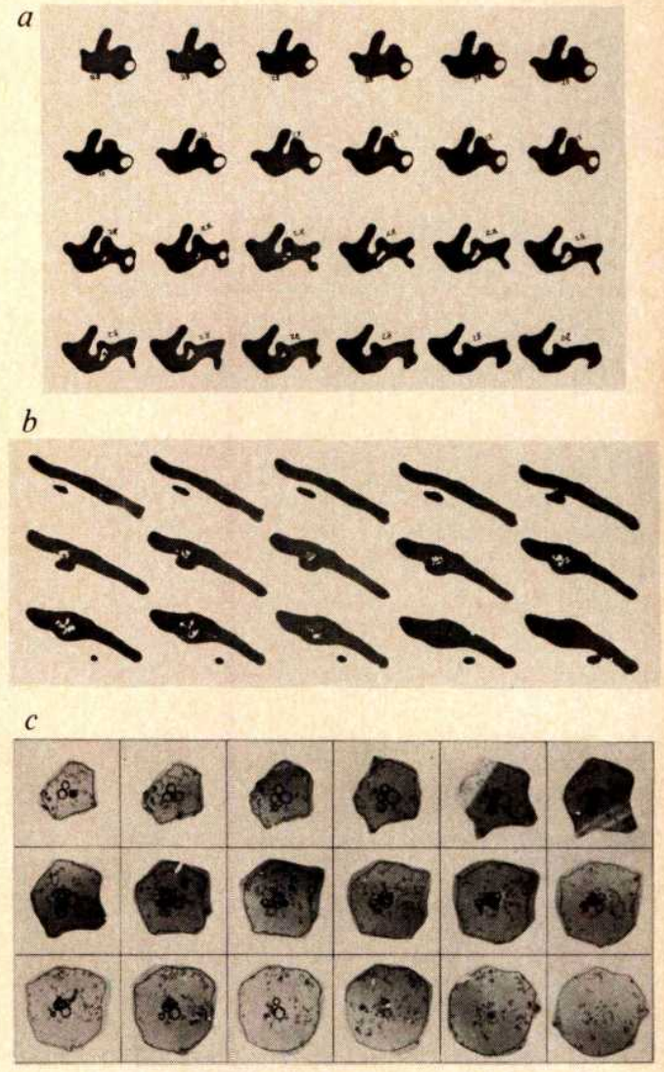


Fig. 1 Genuine and artefactual vesicular images. The cell in *a*, which is not an ISC, illustrates the appearance of a genuine, fully-enclosed vesicle (near the centre) containing some electron-dense material, as well as a long tubular artefact with open communication to the medium (on the right). The cell in *b* is part of an ISC and illustrates the complex structure of a large vesicle also containing electron-dense material. The images in *c* are from an SS ghost. The mottled background is characteristic of SS cells alone and may be caused by haemoglobin (Hb) aggregates. (Following hypotonic lysis, SS ghosts viewed under phase microscopy contained many dense aggregates which moved about within the ghosts; these were rare and much smaller in normal ghosts, and probably represent clumps of denatured Hb.) Many of the vesicles seen in the SS ghosts also contain, or are associated with, electron-dense material. The relative vesicular volume in individual ghosts was estimated by weighing cut-outs of photocopies of the serial sections of SS ghosts and the vesicles contained within them; it varied from <0.02% to 19%, with typical values of ~0.1–0.4%. The high electron density of the cytoplasm and vesicle-associated precipitates, as well as the convoluted shape of most vacuolar spaces, precludes such estimates in the serial sections of intact SS cells.

Methods. Blood was drawn from normal subjects and from sickle-cell anaemia patients, into heparinized containers. Only SS cells are shown in this figure. The red cells were washed four times in saline and packed at 1,200*g* for 1 min in small microfuge tubes. 20 μl of packed cells was fixed by addition to 1 ml of buffer containing 2.5% glutaraldehyde and (in mM): KCl, 75; NaCl, 75; HEPES-K (*p*H 7.2), 10. After 30 min at room temperature, the cells were washed twice with buffer alone. The cell pellets were post-fixed in 1% osmium tetroxide, dehydrated in graded ethanol solutions and embedded in Araldite resin. Serial sections (0.10–0.15 μm) were cut, mounted on Pioloform support films and slotted grids and stained with lead citrate[26]. Similar images were obtained with red cells fixed in 0.15 M cocodylate buffers, or by using 1% osmium tetroxide with or instead of glutaraldehyde. With all solutions, similar low proportions of deformed cells, such as those illustrated in *a*, were seen with preparations of SS cells but not with normal cells. Phase-contrast inspection of specimens before and after fixation did not reveal major differences other than in the overall rigidity of fixed cells. Ghosts were prepared by lysing packed cells in 50 vol. of ice-cold medium as described in Fig. 4 legend. Intact red cells were packed and 20 μl transferred to microfuge tubes containing 1 ml of lysing medium with 1% osmium tetroxide. After 1 h at room temperature, the ghosts were washed once with lysing medium alone, and the pellets processed as for the intact cells.

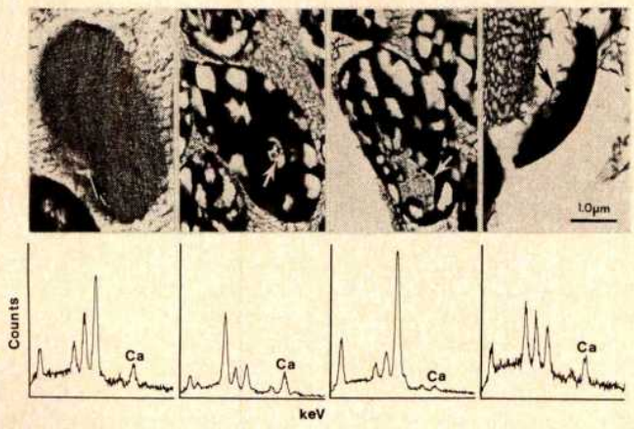


Fig. 2 Top, frozen dried cryosections of the dense ISC-rich fraction of SS cells (see Fig. 4 legend), showing four typical examples of Ca^{2+}-containing vacuoles. The vacuoles are indicated by arrows, and the spectra obtained when the electron beam was focused on the content of the vacuoles are shown beneath each micrograph. The holes in the two red blood cells in the centre are a result of ice crystal damage typically observed in the deeper part of the specimens. Analysis of the walls of the holes gave phosphorus concentrations similar to those of the adjacent cytoplasm, whereas the walls of the calcium-containing vacuoles were high in phosphorus, consistent with their being membrane-enclosed. Occasional cells, such as that illustrated in the extreme left-hand panel, were much less electron-opaque than the majority and, while some also showed vacuoles with high Ca content, the ionic composition of their cytoplasm (not shown) suggested that they were partially haemolysed.

Methods. Small droplets (<1 mm diameter) of the red cells were placed on the end of a bamboo splinter and plunged into supercooled Freon 22. The droplets were sectioned at −130 °C in a cryoultramicrotome and the ~100-nm-thick sections were placed on carbon support films and freeze-dried at $<10^{-5}$ torr. The freeze-dried cryosections were carbon-coated and analysed at −100 °C on the cold stage of a Philips 400T transmission electron microscope operated at 80 kV and equipped with a 30-mm^2 Kevex energy dispersive detector connected to a Kevex multichannel analyser and a PDP11/34 computer. The duration of individual analyses was 150–300 s, with a probe current of ~1 nA. The equipment configuration and its operating characteristics have been described elsewhere[20,21].

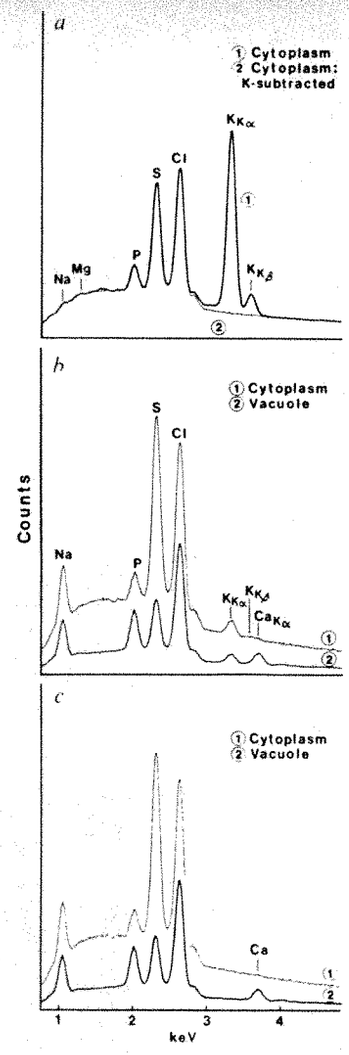


Fig. 3 Averaged X-ray spectra from normal (*a*) and sickle (*b*, *c*) red blood cells. *a*, Spectrum of normal red cells before (1) and after (2) subtraction of the potassium Kα and Kβ peaks, showing that the spectrum is flat in the calcium region. *b*, Averaged spectra (15 analyses obtained with focused 100-nm-diameter probes) for the cytoplasm (1) and (15 analyses) with probes focused over the vacuoles (2). CaKα indicates the position of the calcium Kα peak. *c*, The same spectrum as *b*, with the potassium Kα and Kρ peaks subtracted[27] to contrast the high calcium concentration within the vacuoles with the very low concentrations in the cytoplasm.

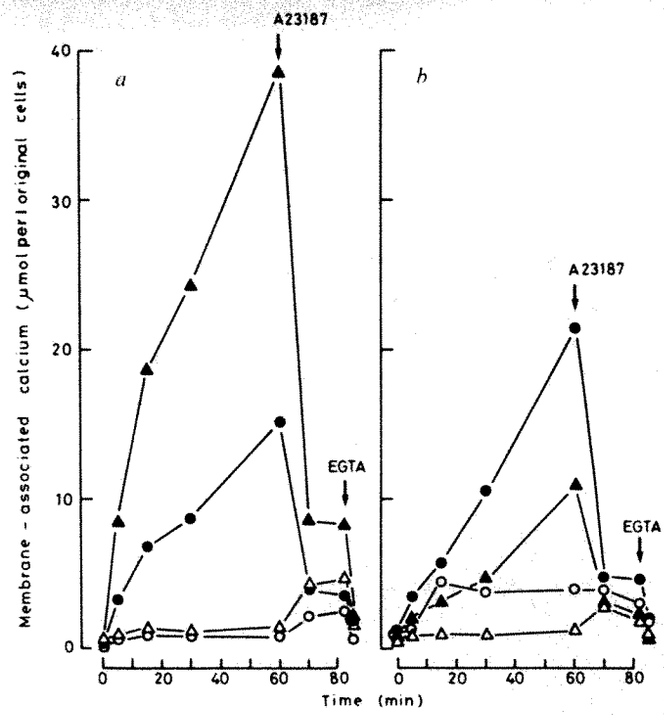


Fig. 4 Calcium uptake by sickle (*a*) and normal (*b*) disrupted red cell membranes in the presence and absence of ATP. Open symbols, ATP-free conditions; solid symbols, ATP added and maintained. ○, ●: Second-lightest cell fraction; △, ▲: densest cell fraction.

Methods. Red cells from normal and sickle-cell anaemic blood were separated on Stractan (arabinogalactan) density gradients[28] to obtain the second-lightest fractions (diskocytes free of white cells, platelets and most reticulocytes) and the densest fractions (~70% irreversibly sickled forms (ISCs) from the SS cells). After separation, the cells were washed twice in ice-cold medium A containing (in mM): NaCl, 75; KCl, 75; EGTA 0.1; Tris-Cl *p*H 7.5, 10, and lysed in 50 vol. ice-cold medium containing 2.5 mM K-HEPES *p*H 7.6 and 1 mM $MgCl_2$. These conditions are known to completely prevent spontaneous inside-out vesicle formation[22]. The membranes were centrifuged at 17,000 r.p.m. for 15 min in the cold, and the pellets were frozen and thawed once to ensure complete cell lysis. Each pellet was suspended in 1 ml ice-cold buffer containing 10 mM KCl, 50 mM Na-HEPES *p*H 7.5, 1 mM $MgCl_2$, 40 μM $^{45}CaCl_2$ and 3 μM calmodulin, and divided in half to receive either 1 mM Na-ATP with 5 mM creatine phosphate and 50 U ml^{-1} creatine phosphokinase (to maintain ATP), or 50 U ml^{-1} hexokinase and 25 mM glucose (to secure complete ATP depletion). After zero-time samples had been taken in the cold, the suspensions were incubated at 37 °C with stirring. Samples (50 μl) were delivered onto Millipore filters (type HA, 0.45 μM, prewashed with the suspension buffer containing, in addition, 2 mM $^{40}CaCl_2$) on a vacuum manifold, washed twice with the same ^{40}Ca-containing buffer, and the filters dissolved in scintillation fluid in counting vials. At the times indicated, ionophore A23187, at a moderately high concentration equivalent to 50 μmol per l of original cells, and subsequently 2 mM Tris-EGTA, were added to the incubation mixtures.

convoluted shapes. Enclosed vesicles were present in all the SS cells and ghosts. Relative to normal cells, SS vesicles were more numerous and much larger (particularly in the ISCs), showed marked variations in shape, size and numbers per cell, were often multi-lobed and contained electron-dense inclusions.

The compartmentalization of calcium in sickle cells was established directly by electron-probe X-ray microanalysis[20,21] of cryosections made from the dense, ISC-rich cell fraction. Figure 2 illustrates the X-ray spectra and Ca^{2+} peaks corresponding to representative vesicular images seen in frozen dried sections of ISCs. Figure 3 shows averaged X-ray spectra of normal red blood cells, and the vacuoles and adjacent cytoplasm of ISCs. Highly significant Ca^{2+} peaks were recorded only from the vacuoles that also contained higher concentrations of phosphorus than the adjacent cytoplasm. The P/Ca ratio within the vacuoles varied from 1.5 (the ratio in hydroxyapatite) to 7.6, suggesting that organic phosphates may participate in the formation of precipitates. The calcium content of the vacuoles averaged 35 mmol per kg dry wt. With about 0.3–0.4 kg dry wt per l of cells, and the range of vesicular volumes found in ghosts (see Fig. 1 legend), the measured intravesicular calcium concentration can easily account for mean total cell Ca^{2+} values in the range observed[10,12], that is, 40–100 μmol per l of cells.

If endocytic inside-out vesicles are to account for the observed calcium accumulation by SS cells during sickling, they must have ATP-dependent Ca^{2+} pumps capable of pumping Ca^{2+} inward, in competition with the Ca^{2+}-extrusion pumps of the outer cell membrane. The ATP-dependent, Ca^{2+}-accumulating capacity of any vesicles that might have resisted the procedures applied to disrupt the outer cell membrane, was investigated by the protocol described in Fig. 4 legend. The results of four similar experiments (see Fig. 4) demonstrate that disrupted

membranes from both SS and normal red cells are capable of substantial ATP-dependent Ca^{2+} accumulation in the presence of non-limiting concentrations of Ca^{2+}-pump substrates and cofactors. That accumulation of Ca^{2+} was uphill was demonstrated by the collapse of the Ca^{2+} gradient on addition of the calcium ionophore A23187 before the addition of excess Ca^{2+} chelator. In all instances, ATP-dependent Ca^{2+} uptake was maximal at the start of the incubation, without noticeable delay; this argues against uptake by newly formed inside-out vesicles as, even under the most favourable conditions for spontaneous endocytosis, more than 6–15 min of incubation at 37 °C are required to produce sealed inside-out vesicles[22]. Therefore, the observed uptake must represent the Ca^{2+}-accumulating capacity of those endocytic inside-out vesicles that either resisted lysis (possibly the same as Palek's ghost-associated ^{45}Ca fraction[4]), or which re-sealed spontaneously after lysis.

The results presented here explain the paradox of the silent calcium in SS cells and circumscribe the possible role of calcium in the generation of ISCs. Cytoplasmic Ca^{2+} concentrations must be increased transiently, at least, to lead to accumulation within endocytic inside-out vesicles. The possibility remains[1] that in some cells such Ca^{2+} transients could briefly activate K^+ channels and initiate the process of dehydration (thereby further promoting the sickling process), eventually resulting in ISC formation. Recent work lends some support to this possibility[23].

The endocytic inside-out vesicles present in red cells could record cumulatively any episodes of increased Ca^{2+} permeability as an increase in the calcium content of the cells. In view of the evidence for endocytic inside-out vesicles capable of Ca^{2+} accumulation in normal red cells (Fig. 4), the absence of a measurable pool of ionophore-mobilizable Ca^{2+} in these cells[10] argues against their having periods of substantially increased Ca^{2+} permeability, such as those proposed to occur with normal red cells during microcirculatory shear stress[24]. A normal spleen, however, might 'pinch-off' Ca^{2+}-loaded vesicles from traversing red cells[19], thus erasing such a record, whereas the functional asplenia of sickle cell anaemia patients[25] could account for the persistence of such vesicles.

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Reorganization of α-fodrin induced by stimulation in secretory cells

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Spectrin is an ubiquitous protein composed of heterodimers with α and β subunits. It was first described in erythrocyte cell membranes (see ref. 1 for review) and subsequently in brain, intestinal brush borders, kidney, liver and adrenals[2–8]. Brain spectrin (fodrin) α-subunit, responsible for actin binding, has a relative molecular mass (M_r) of 240,000, whereas the β-subunit, involved in membrane attachment, has an M_r of 235,000 (refs 1, 3, 9–13). The membrane of secretory granules from adrenal chromaffin cells increases the viscosity of F-actin solution[6,14], and spectrin-like protein is associated with storage granule and plasma membranes[6]. Here, we report the localization of fodrin in secretory cells using monospecific antibodies against the α-subunit of fodrin using indirect immunofluorescence. We find that the α-subunit forms an intensely stained continuous ring in the subplasmalemmal region of resting chromaffin cells. On stimulation of the cell with nicotine, high potassium or ionophores in the presence of calcium, fodrin forms patches. This aggregation is inhibited by trifluoperazine, hence the entrance of calcium into cells following cell depolarization seems to be the calmodulin-dependent stimulus initiating patch formation.

The chromaffin cell of the adrenal medulla is often used as a model to study the secretory process in neuroendocrine cells[15,16]. The recent development of primary cultures of adult chromaffin cells isolated from bovine adrenal glands[17–20] provides a system that can be used to probe the cellular mechanism underlying secretion.

To examine fodrin localization in cultured chromaffin cells we raised antibodies against the α- and β-subunits of bovine brain fodrin. Using immunoaffinity-purified antibody to bovine brain membrane β-fodrin no staining of chromaffin cells was observed, indicating that brain β-fodrin is immunologically distinct from that of paraneuronal cells (Fig. 1*a*). We confirmed these observations by immunoblotting experiments on chromaffin cell cytoskeletal proteins (data not shown). In contrast, immunoaffinity-purified antibody to the α-subunit of bovine brain stained the chromaffin cell periphery as an intense fluorescent ring (Fig. 1*b*). The α-fodrin antigens were apparently not accessible to the cell surface because no antibody staining occurred unless the cells were first permeabilized with Triton X-100. Preimmune serum or antiserum absorbed with fodrin did not decorate chromaffin cells. Thus, we conclude that α-fodrin antigens are concentrated in the subplasmalemmal region of cultured chromaffin cells.

In 85 ± 5% of cells from four different primary cultures, α-fodrin was visualized as a continuous ring. In the remaining 15% a different staining pattern was observed; α-fodrin antigens still occurred in the subplasmalemmal region but were aggregated in some places, producing a discontinuous labelling, suggesting that α-fodrin organization is related to secretory activity. Therefore, we stimulated cultured chromaffin cells with nicotine and then stained for α-fodrin antigens. In these experiments, antibody against dopamine β-hydroxylase (DBH), a marker for the inner surface of granule membranes, was also added during the stimulation period. This marker is known to be inserted into the plasma membrane during exocytosis[21] (Fig. 1*g*, *h*). Cells were examined, counted and classified as either showing a continuous subplasmalemmal fodrin ring or discontinuous labelling with patches. We found a close relationship between the number of cells displaying patchy fodrin distribution and

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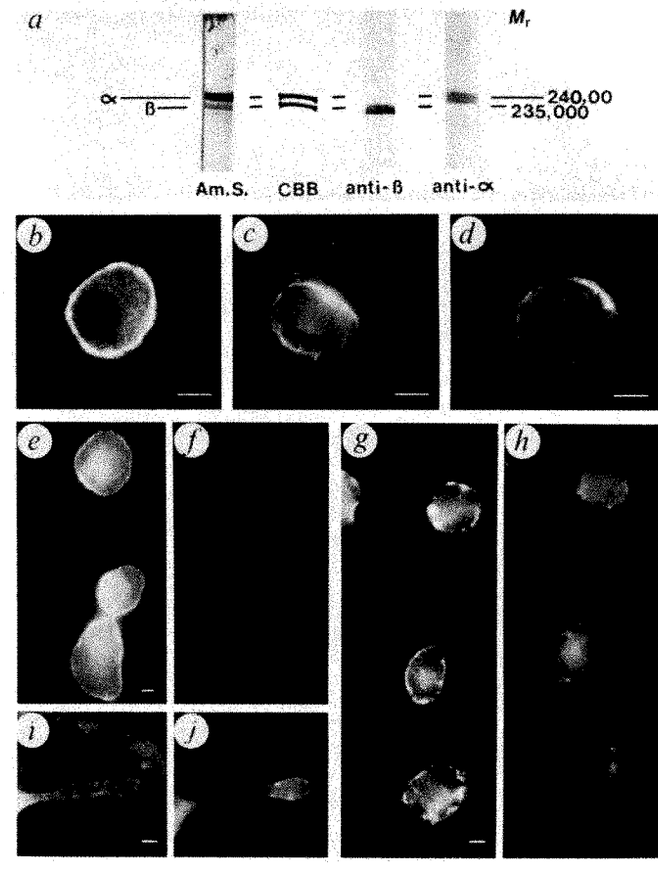


Fig. 1 Immunofluorescence localization of α-fodrin in cultured chromaffin cells. ***a***, Characterization of anti-α-fodrin and anti-β-fodrin antibodies. Bovine brain fodrin α- and β-subunits were separated by polyacrylamide slab gel electrophoresis and stained with Coomassie brilliant blue (lane CBB). After transfer to a nitrocellulose sheet, one lane was stained with Amido black (lane Am.S.), and the other two reacted with either anti-α-fodrin (anti-α) or anti-β-fodrin (anti-β) monospecific immunoaffinity-purified antibody. Bound immunoglobulins were then detected with goat peroxidase-conjugated anti-rabbit immunoglobulins (Nordic Immunologicals) and chloro-2-naphthol[30,31]. No cross-reaction of anti-α-fodrin with the β-subunit or anti-β-fodrin with the α-subunit was observed. ***b–d***, Localization of α-fodrin antigens in resting and stimulated chromaffin cells. Chromaffin cells in culture were incubated for 10 min at 22 °C with no secretagogue (*b*) or with 56 mM K^+ (*c*) or 25 μM X537A (*d*) in the presence of 2.2 mM Ca^{2+}. Cells were then processed for α-fodrin localization using immunofluorescence microscopy. ***e–j***, simultaneous localization of α-fodrin antigens (*e, g, i*) and exocytotic sites (*f, h, j*) in chromaffin cells in culture stimulated in the absence (pair *e, f*) or presence (pairs *g, h*; *i, j*) of external calcium. Antiserum to DBH was used as a marker for the inner surface of granule membrane as it is transiently becoming accessible during exocytosis. Chromaffin cells in culture were washed and incubated for 10 min at 22 °C in 2.2 mM Ca^{2+}-Locke's solution containing specific (ref. 6) rat anti DBH antiserum (dilution 1:100) with 10 μM nicotine (pair *g, h*) or 25 μM X537A (pair *i, j*); *e, f*, incubation performed in Ca^{2+}-free (5 mM EGTA) Locke's solution in the presence of 10 μM nicotine. Cells were then processed for immunofluorescence microscopy. Scale bars, 10 μm.

Methods. Anti-fodrin antibodies: Antibodies against fodrin purified from bovine brain membranes[32] were raised in rabbits. Brain fodrin was eluted from SDS electrophoresis[33] and injected into rabbits with Freund's complete adjuvant and 3 weeks later with Freund's incomplete adjuvant. To purify monospecific anti-α- and anti-β-fodrin antibodies, purified bovine brain fodrin was separated on 4–8% SDS/polyacrylamide slab gels and transferred to nitrocellulose sheet (30 V; 320 mA; 15 h; see ref. 34). After staining the proteins with Ponceau red, the strip corresponding to each fodrin subunit was cut out and incubated for 60 min at 37 °C in a solution of 3% bovine serum albumin in phosphate-buffered saline (PBS). Nitrocellulose strips were then incubated for 120 min at 20 °C in anti-fodrin antisera diluted 10-fold with PBS. After extensive washings with PBS, bound immunoglobulins were eluted with 0.2 M glycine-HCl (*p*H 2.8) for 4–5 min[35], and the eluate immediately neutralized with 1 M NaOH. The solution was then dialysed overnight against 0.2 M borate buffer (*p*H 7.8), analysed at 280 nm, divided into aliquots and stored at −70 °C. Chromaffin cells in culture: Chromaffin cells were isolated from fresh bovine adrenal glands by retrograde perfusion with collagenase and purification on self-generating Percoll gradients[17,31]. Cells were suspended at a concentration of 10^5 cells ml^{-1} of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum containing cytosine arabinoside (3 μg ml^{-1}), streptomycin (50 μg ml^{-1}) and penicillin (50 U ml^{-1}). Cells were seeded on rat-tail collagen-coated glass coverslips, which were placed into Falcon Petri dishes (35 mm diameter). Cells were cultured at 37 °C in an H_2O-saturated atmosphere of 95% air/5% CO_2 and used within 7 days after plating. Immunofluorescence microscopy: After stimulation (as indicated) cells were rapidly (1 min) rinsed in PBS, *p*H 7.4, and then fixed with 4% formaldehyde in PBS and permeabilized with acetone[31]. After a further wash, cells were treated with normal swine and goat immunoglobulins (2 mg ml^{-1}), then incubated with monospecific anti-α-fodrin immunoglobulins (1–2 μg on each coverslip) overnight at 4 °C. Finally, antibodies were detected with swine fluorescein-conjugated anti-rabbit immunoglobulins (Nordic Immunologicals; diluted 1:50 in PBS) and, when necessary, with goat rhodamine-conjugated anti-rat immunoglobulins (Nordic Immunologicals; diluted 1:60 in PBS). Coverslips were mounted in 1:1 mixture of 87.5% glycerol and PBS containing 50 mM Tris, 20 mM dithiothreitol at *p*H 8.1. Preparations were examined with a Zeiss Universal microscope using epi-illumination and a Zeiss Planapo 63×/1.4 oil immersion objective, with 450–490-nm and 515–560-nm filters for fluorescein and rhodamine fluorescence, respectively.

the external nicotine concentration (Fig. 2). At 10 μM nicotine, ~70 ± 10% of chromaffin cells showed a redistribution of fodrin antigens. There is a linear relationship between α-fodrin reorganization and catecholamines released at a given nicotine concentration (correlation coefficient 0.93, data not shown). Patches of α-fodrin antigens formed in 80% of cells 10 min and 94% of cells 25 min after nicotine stimulation, demonstrating the time-dependency of fodrin reassembly (Fig. 3).

To examine further the aggregation phenomenon of α-fodrin antigens, we show that 56 mM K^+ also induces reorganization of α-fodrin in the subplasmalemmal space similar to that provoked by nicotine (Fig. 1*c*). In addition, the formation of patches induced by high potassium and nicotine proceeded with the same time-course (Fig. 3), but fewer cells (55–60%) had K^+-induced patches. Note that even at relatively high depolarizing K^+ concentrations, catecholamine secretion is significantly less than with nicotine or acetylcholine[22]. Chromaffin cells stimulated with nicotine were also examined 60 min after stimulation, when α-fodrin labelling had returned to the level in resting cells even though anti-DBH staining of cell membranes was still visible (data not shown).

Thus, nicotine and high potassium produce similar effects on fodrin organization in secretory cells. Because the chromaffin cell requires external calcium in addition to a stimulus to secrete catecholamines and peptides, we next examined the effect of external calcium on fodrin movements on cells stimulated either with nicotine or high potassium. In the absence of external calcium ($<10^{-8}$ M), catecholamine secretion was blocked and the alteration of α-fodrin organization was no longer observed (Figs 1*e, f*; 2). The Ca^{2+}-dependence of fodrin reorganization was also examined by incubating cells in the presence of X537A, an ionophore provoking catecholamine release in the presence of calcium. In these experiments, incubation of cells with 25 μM X537A in the absence of calcium did not result in modification of fodrin localization, whereas in the presence of calcium there was a drastic rearrangement of fodrin (Fig. 1*d, i*). Treatment of cells with the more specific Ca^{2+} ionophore A23187 gave identical results (data not shown). These data indicate that the main stimulus activating α-fodrin movements results from the rise of intracellular calcium concentration following cell depolarization[23].

The α-subunit of brain fodrin is known to bind calmodulin[24]. Although α-fodrin from the adrenal medulla has not yet been shown to be calmodulin dependent, we tested the effect of trifluoperazine, a calmodulin antagonist, on α-fodrin organization in secretory cells. Nicotine-induced rearrangement of fodrin was abolished totally in 5×10^{-6} M TFP (Table 1). This concentration also inhibited 90% of the catecholamine release (ref. 25 and V. Ceña and D.A., unpublished results).

Because there is an exocytotic stage of secretion in chromaffin cells, it is possible that fodrin redistribution is the passive consequence of secretory vesicle fusion with the plasma membrane. Stimulation is followed by the addition of much of the vesicle membrane to the plasma membrane; therefore, some

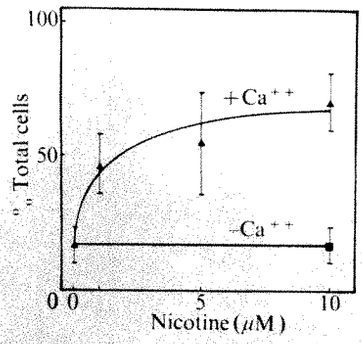


Fig. 2 Effect of nicotine concentration on the number of chromaffin cells showing discontinuous α-fodrin staining. Chromaffin cells in culture were washed with 2.2 mM Ca^{2+} or Ca^{2+}-free (5 mM EGTA) Locke's solution and stimulated for 10 min at 22 °C with nicotine as indicated. Cells were then rapidly washed, fixed and processed for α-fodrin localization as described in Fig. 1 legend. For each nicotine concentration, 150 cells were examined and classified. Experiments were repeated three times on three different cultures of chromaffin cells. Each value is the mean ± s.d.

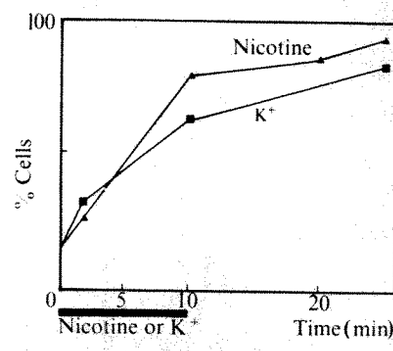


Fig. 3 Kinetics of α-fodrin patch formation in chromaffin cells. At indicated times after stimulation either with 10 μM nicotine or 56 mM K^+ at 22 °C in the presence of 2.2 mM Ca^{2+}, cells were fixed, stained for α-fodrin (see Fig. 1 legend) and counted. Stimulation with secretagogue was for a maximum of 10 min and then cells were washed with Locke's solution. Each value is the mean of two experiments.

clearing of plasma membrane proteins would be expected. In separate experiments (O. K. Langley, D.P. and D.A., unpublished results), resting and nicotine-stimulated chromaffin cells were labelled with anti-D2 antibody, D2 being a glycoprotein belonging to the family of cell-adhesion molecules and found on the surface of all chromaffin cells[26]. No alteration of D2 localization was found. In addition, examination of the paired cells in Fig. 1*g*, *h*, did not show any obvious correlation between fodrin patches and exocytotic sites; the latter has been visualized with the anti-DBH antiserum. Therefore, the possibility that fodrin movements result only from vesicle membrane integration into cell membrane is highly unlikely.

The redistribution of α-fodrin in the subplasmalemmal region of cultured secretory cells after stimulation of the cell-surface receptor suggests that the subplasmalemmal cytoskeletal organization is affected by interactions between surface receptors, internal calcium concentration, calmodulin and environmental stimuli. The organization of actin in stimulated cultured cells was also examined, but changes in the actin distribution were much more difficult to evaluate because its distribution[18] in resting cells is variable (that is, it is either localized as fibres or dots, diffuse in the cytoplasm or underlying the cell membrane).

The present study is the first demonstration that fodrin is redistributed when secretory cells are stimulated externally. Fodrin-related proteins have been localized previously in the cortical cytoplasm of many cells and found to redistribute during ligand-induced aggregation of receptors in fibroblasts and during capping on B and T lymphocytes[27,28]. 'Patching' has been described as a segregation of plasma membrane-associated macromolecules to certain domains of the membrane. Hence, fodrin seems to mediate surface receptor interactions with the cytoskeleton, and may act as a specific linker of certain membrane-associated proteins to the filamentous cytoskeleton.

Table 1 Effect of trifluoperazine (TFP) on α-fodrin distribution in resting and stimulated chromaffin cells in culture

Experimental conditions (in 2.2 mM Ca^{2+}-containing Locke's solution)	No. of cells showing α-fodrin rearrangement (%)
Control	5-10
Nicotine 10 μM, 10 min	80-90
TFP 5.10^{-7} M, 20 min + nicotine 10 μM, 10 min	60-65
TFP 5.10^{-6} M, 20 min + nicotine 10 μM, 10 min	5-10
TFP 5.10^{-6} M, 20 min	5-10

In addition, fodrin movements may also be involved with granule movement. The subcortical cytoplasm of eukaryotic cells where fodrin seems to be concentrated is characterized by a dense fibrillar array of interacting filaments and associated proteins. In resting cells the fodrin fluorescence ring probably reflects this subplasmalemmal dense network, which, when gelled, could act as a physical barrier preventing granules from moving towards the cell membrane and undergoing exocytosis. In stimulated cells, fodrin movements may cause the appearance of free spaces in the continuous ring resulting from a calcium-dependent solation and thereby allow granules to move. This solation process seems to be calmodulin dependent because trifluoperazine blocks both secretion[25] and fodrin movements. It is of interest to note that trifluoperazine-treated cells have been shown to contain an increased number of granules within 200 nm of the plasma membrane[29].

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Production of human α-interferon in silkworm using a baculovirus vector

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Microorganisms are generally used for mass production of foreign gene products, but multicellular organisms such as plants have been proposed as an economical alternative. The silkworm may be useful in this context as it can be cultured easily and at low cost. We have therefore developed a virus vector to introduce foreign genes, for example, the gene for human α-interferon (IFN-α), into silkworms. We used the baculovirus *Bombyx mori* nuclear polyhedrosis virus (BmNPV) which has a large (>100 kilobases, kb) double-stranded circular DNA genome within its rod-shaped capsid. Baculoviruses have been used previously as vectors for expression of β-interferon and β-galactosidase in established cell lines[1,2]. Although BmNPV has not been used previously as an expression vector, it has an advantage over the baculovirus *Autographa californica* NPV in that it has a narrower host range and will not grow in wild insect pests in the field. In the present study, the polyhedrin gene encoding the major inclusion body protein of BmNPV was identified by hybridization with complementary DNA and cloned in a plasmid. For insertion of foreign genes, we constructed a recombinant plasmid carrying a polylinker linked to the promoter of the polyhedrin gene, and inserted the IFN-α gene into this plasmid. The resulting plasmid and the BmNPV genomic DNA were co-transfected into BM-N cells, and stable recombinant viruses isolated by plaque assay on BM-N cells. The recombinant virus replicated in silkworm larvae, which synthesized as much as 5×10^7 units (~50 μg) of interferon in their haemolymph.

We used BmNPV T3 isolate, which has been plaque-purified on BM-N cells[3]. To identify the polyhedrin gene on the viral genome of the BmNPV T3 isolate, cDNA made from messenger RNA isolated from infected fat bodies was used as a probe. In *in vitro* translation experiments in reticulocyte lysates, over 90% of the total mRNA obtained from these fat bodies at a late stage of infection was found to encode the polyhedral protein. The labelled cDNA synthesized by reverse transcriptase was hybridized to viral DNA fragments separated on an agarose gel after cleavage with restriction endonucleases. An *Eco*RI 10.5-kb fragment or a *Hin*dIII 3.9-kb fragment hybridized specifically to the probe. The *Eco*RI 10.5-kb fragment was cloned into pBR322 at the *Eco*RI site. Figure 1 shows a physical map of the resulting plasmid (pBmE36). Further hybridization experiments revealed that a 1.8-kb *Hpa*I-*Hin*dIII fragment in pBmE36 hybridized best with the probe and contained the gene for the polyhedral protein gene.

The nucleotide sequence of 1,763 bases from the *Hpa*I-*Hin*dIII fragment was determined by the dideoxy chain termination procedure[4,5]; the inferred amino-acid sequence was compared with that of the polyhedrin protein of BmNPV[6]. The starting codon, ATG, of the polyhedrin protein was found 580 bases downstream from the *Hpa*I site and the stop codon TAA was found 448 bases upstream from the *Hin*dIII site. More than 85% of the deduced amino-acid sequence was homologous to that reported by others for *Autographa californica* NPV (AcNPV)[1,2]. The differences show the degree of evolutionary

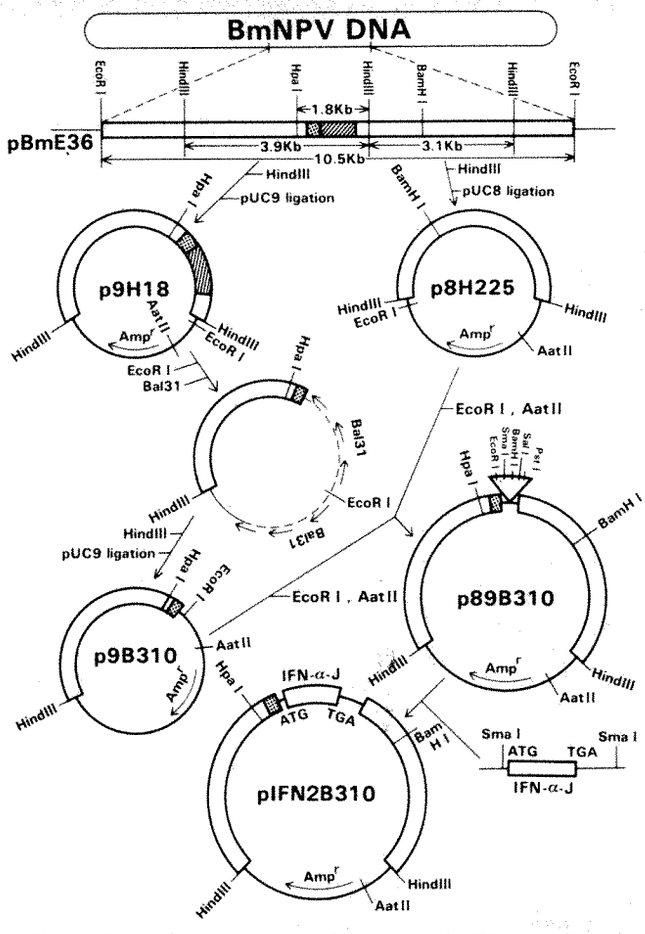


Fig. 1 Construction of vectors. The plasmid pBmE36 was cleaved with *Hin*dIII to obtain the 3.9-kb *Hin*dIII fragment containing the entire polyhedrin gene (represented by hatched blocks). This fragment was inserted into the *Hin*dIII site of pUC9. The resulting plasmid, p9H18 (50 μg), was cleaved with *Eco*RI, then incubated at 23 °C for 10 min with 50 U of *Bal*31 exonuclease. Samples (100 μl) were removed at 2-min intervals and the reaction stopped by adding 0.2 vol. 0.5 M EDTA. The samples were extracted with phenol and then chloroform, and precipitated with ethanol. The truncated p9H18 fragments were digested with *Hin*dIII, and 2.7-kb *Hin*dIII blunt-end fragments were isolated from the agarose gel. This fragment was ligated to the *Hin*dIII-*Sma*I site of pUC9 (p9B310). Sequencing of p9B310 revealed that the polyhedrin gene was digested as far as position −18 upstream of the first codon, ATG (see Fig. 2). A 3.1-kb *Hin*dIII fragment of pBmE36 was ligated to pUC8 and the resulting plasmid, p8H225, cleaved by *Eco*RI and *Aat*II to obtain the 3.5-kb fragment containing 3.1 kb of DNA downstream of the polyhedrin gene; this fragment was then ligated to the 4.7-kb *Eco*RI-*Aat*II fragment of p9B310. The resulting hybrid plasmid, p89B310, contains a polylinker (*Eco*RI, *Bam*HI, *Sma*I, *Sal*I and *Pst*I sites) just downstream of the promoter (represented by dotted blocks) of the polyhedrin gene. An IFN-α-J gene, which has *Sma*I sites just before the ATG start signal (. . . CCCGGGATG. . .), was ligated to p89B310 and cleaved by *Sma*I to construct pIFN2B310 (Fig. 2). In this plasmid, the IFN-α-J gene was oriented in the same 5′ to 3′ direction as the polyhedrin gene. The IFN-α-J gene used for this construct contained the signal peptide sequences, the coding sequences of mature IFN-α-J and the 3′-noncoding sequences (~670 bases from the stop signal, TAG) of interferon, including the signal for polyadenylation.

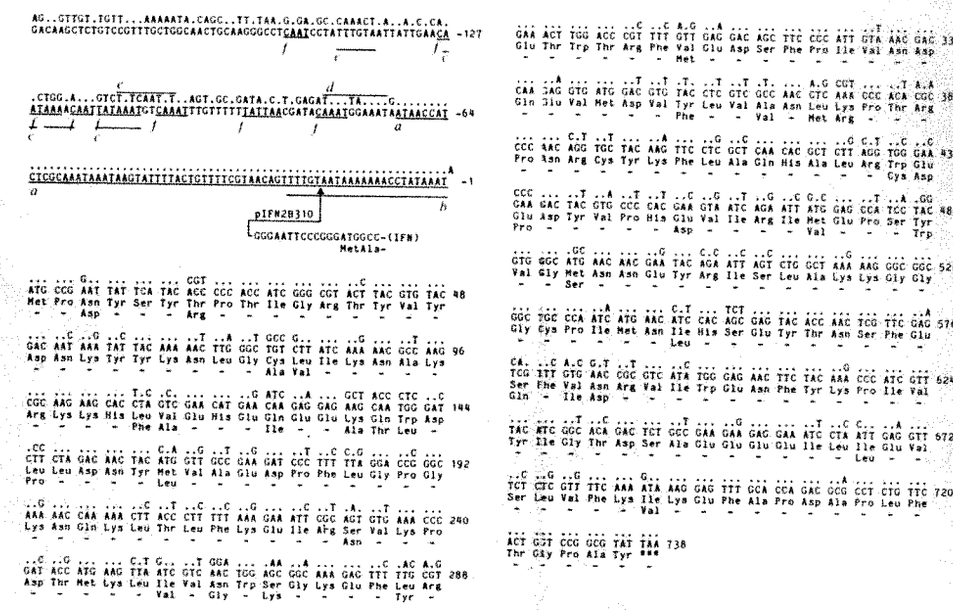


Fig. 2 Nucleotide sequence of the BmNPV polyhedrin gene and the flanking 5′-noncoding region, with the corresponding sequence of the AcNPV genome[1] shown above it. Nucleotides are numbered in the 5′ to 3′ direction beginning with the first residue of the ATG triplet encoding the initiating methionine. Dots indicate identity of the nucleotides. The deduced amino-acid sequence of BmNPV polyhedrin is shown beneath the nucleotide sequence of the BmNPV genome. The deduced amino-acid sequence of AcNPV polyhedrin is shown beneath that of PbNPV polyhedrin. Dashes indicate identical amino acids. The arrow indicates the connecting point of the polylinker in pIFN2B310.

distance between the AcNPV and BmNPV polyhedrin genes. The entire nucleotide sequence of the polyhedrin-coding region and 189 bases upstream from the translational start site are shown in Fig. 2 for both BmNPV and AcNPV[1]; 71 bases upstream of the translational start site are conserved (Fig. 2*a*). Twenty-four bases within this conserved region (Fig. 2*b*) represent an almost complete repetition of the region 110–143 bases upstream from the translational start site (Fig. 2*c*). This conserved sequence seems to be important for the high level of expression of the polyhedrin gene. There are several nucleotide changes in the coding region of the polyhedrin gene and many changes in the region upstream of the 71 conserved bases. Smith *et al.*[1] postulated that this upstream region was the promoter containing a TATA box (Fig. 2*d*) and a CAAT box (Fig. 2*e*); within the corresponding region on the BmNPV genome, we found sequences similar to TATA and CAAT boxes (Fig. 2*f*). Codon usages for the polyhedrin varied between BmNPV and AcNPV, while the amino-acid sequences of the two polyhedrins were well conserved.

A novel transfer vector carrying the 3-kb fragment containing the polyhedrin gene promoter, a polylinker derived from pUC8, and the 3.1-kb fragment downstream of the polyhedrin gene, was constructed as shown in Fig. 1. One of the recombinant plasmids used in the present study, p89B310, has the polylinker linked to 18 bases upstream from the translational start of the polyhedrin gene. A human IFN-α gene was cloned from a human gene library (provided by Dr D. G. Goeddel) using a synthetic 37-mer probe. The IFN-α gene, identified as class J[7] by sequence analysis, was fused with the plasmid p89B310 to construct the plasmid pIFN2B310 (Fig. 1).

Marker rescue was used for the insertion of the interferon gene as reported previously[1,2]. BM-N cells were co-transfected with pIFN2B310 and viral genomic DNA. Recombinant viruses, which did not produce polyhedral inclusion bodies, were isolated by plaque assay on BM-N cells from cell fluids obtained 5 days after transfection. To investigate whether the interferon gene was inserted in the expected region, DNA was extracted from a recombinant BmNPV, BmIFN2B310, and examined by hybridization analysis using labelled DNA from the interferon gene as a probe. The result confirmed that BmIFN2B310 contained the interferon gene at the expected site.

When BM-N cells were infected with the recombinant virus BmIFN2B310, the cells showed typical cytopathic effects 2 days after infection. The growth curve of BmIFN2B310 was similar to that of the wild-type isolate T3. The kinetics of interferon induction was examined in BM-N cells infected with BmIFN2B310: the titre of interferon increased to a maximum

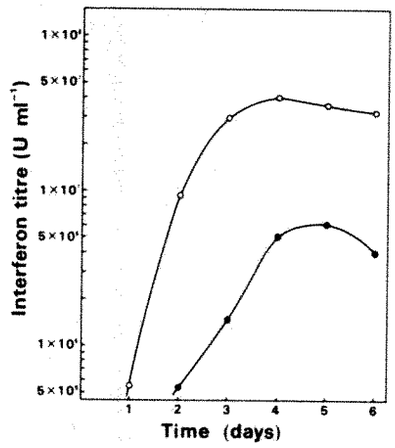


Fig. 3 Production of interferon in BM-N cells (●) and silkworm larvae (○). A solution comprising 2.5 ml of 50 mM HEPES buffer *p*H 7.1, 0.28 M NaCl, 0.7 mM Na_2CO_3 and 0.7 mM $NaHPO_4$ was added dropwise to the DNA mixture (2.5 ml of 0.25 M $CaCl_2$ containing 10 μg of BmNPV DNA and 65 μg of pIFN2B310) to form a fine precipitate. About 0.45 ml of this calcium precipitate was added to 3×10^6 cells seeded in a flask containing 4.5 ml of medium. After incubation for 12 h, the culture fluid was replaced with fresh medium and incubated for a further 4 days. A recombinant virus, BmIFN2B310, was isolated from the culture fluid and used for the expression studies. A monolayer of BM-N cells (3×10^6) was infected with BmIFN2B310 at a multiplicity of infection (MOI) of 2 plaque-forming units (PFU) per cell. 50 μl (3×10^5 PFU) of the viral solution was injected into the body cavity of silkworm larvae at an early stage of the fifth instar. At 24-h intervals after infection, the cell fluid or haemolymph was collected and assayed for interferon. The interferon activity was determined in FL cells challenged with vesicular stomatitis virus as described by Familletti *et al.*[10], and compared with that of an international standard.

of $\sim10^6$ units per ml of infected cell fluid 4 days post-infection (Fig. 3). Infected larval bodies near the abdominal appendage were pierced with a pin, squeezed slightly and the emerging haemolymph was collected in a small test tube and subjected to interferon assay. The kinetics of interferon production in the silkworm larvae was equivalent to that in infected BM-N cells; the relative activity, however, was much higher (Fig. 3). The interferon activity in both BM-N cells and larvae decreased slightly at 6 days post-infection. The activity in the haemolymph

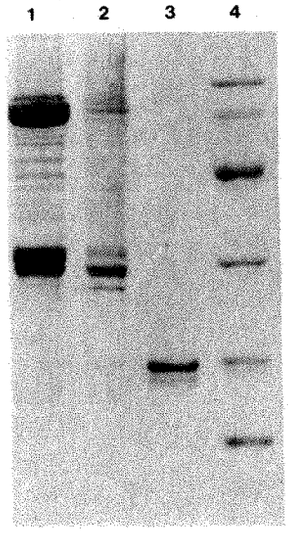


Fig. 4 Purification of the interferon produced in the silkworm. Lane 1, 5 μl of uninfected haemolymph; lane 2, 5 μl of BmIFN2B310-infected haemolymph; lane 3, 5 μl (20 μg protein) of the fraction eluted from the affinity chromatography column; lane 4, 5 μg of each relative molecular mass (M_r) marker (phosphorylase *b*, M_r 94,000 (94K); albumin, 67K; ovalbumin, 43K; carbonic anhydrase, 30K; trypsin inhibitor, 20.1K; α-lactalbumin, 14.4K).

Methods. About 10 ml of haemolymph from 15 silkworm larvae infected with BmIFN2B310 was collected as described in the text. The haemolymph was mixed with an equal volume of fresh medium for BM-N cells and centrifuged at 12,000 r.p.m. for 5 min. The supernatant was applied to an affinity Sepharose 4B column with monoclonal antibody against human IFN-α and washed several times with a solvent containing 0.2 M sodium acetate (*p*H 7.3), 0.5 M NaCl and 20% glycerol. The interferon was eluted from the column with a solvent containing 0.1 M citric acid (*p*H 2.2), 0.5 M NaCl and 20% glycerol, and concentrated ~10-fold. Samples were analysed by SDS-polyacrylamide gel electrophoresis on a 10% gel. The gel was stained with Coomassie brilliant blue R.

was at a maximum (4×10^7 U ml^{-1}) at day 4. The highest recorded level of human IFN-α produced in a eukaryotic system is 4×10^5 U ml^{-1} (for mouse cells with a bovine papillomavirus vector[8]).

The haemolymph from the infected silkworm was affinity-purified by column chromatography, then analysed by SDS-polyacrylamide gel electrophoresis. From 10 ml of infected haemolymph (250 mg protein) collected from 15 larvae, 0.3 mg of interferon was eluted. The specific activity (U mg^{-1} protein) increased from 2.1×10^5 to 1.0×10^8; the yield was higher than 50%. The eluted sample, separated on gels, was stained with Coomassie brilliant blue, which revealed a major band of relative molecular mass 19,500 (Fig. 4). The protein fraction corresponding to this band was extracted from the gel and showed interferon activity. An immune blot analysis indicated that labelled antibody to human IFN-α reacted exclusively with the major band. N-terminal amino-acid sequence analysis (11 amino-acid residues) of the major fraction established its identity to human IFN-α. These findings show that there is no difference between interferon produced in silkworm and that made in mammals, hence the signal peptide sequence can be removed in the same manner as in mammalian cells. Purification of IFN-α made in silkworm is just as easy as for that produced in mammalian cells.

The circulatory system in the silkworm is 'open', in contrast to the 'closed' system of vertebrates. About 30% of the total volume of the silkworm larva consists of the body cavity filled with haemolymph. The haemolymph also represents a storage tissue, for example, the major proteins of the silkmoth *Hyalophora cecropia*, called storage proteins, accumulate in the haemolymph at the late larval stage[9]. The interferon produced is probably deposited in the haemolymph in a similar way without degradation by endogenous enzymes and can be purified without apparent loss of activity.

The present study represents the first report of a high level of synthesis of a foreign gene product in a living animal. The silkworm has been improved for mass culture for several thousand years, and it is now possible to maintain unlimited amounts of silkworm under the control of computerized machines. The system described here, which uses these insects, thus offers an exciting alternative for mass production of foreign gene products.

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Retrovirus-induced *de novo* methylation of flanking host sequences correlates with gene inactivity

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The pattern of DNA methylation changes during development of eukaryotes, and hypomethylation frequently correlates with gene expression (for reviews see refs 1–4). A causal relationship between hypermethylation and gene inactivity has been established for retroviral genomes which are methylated *de novo* when inserted into the germ line of mice (ref. 5; for review, see ref. 6). The mutual interaction of the provirus with the host genome can influence virus expression[7] and can result in inactivation of the host gene by insertional mutagenesis[8]. We report here that the insertion of a provirus can change the methylation pattern of the host DNA. Sequences flanking the provirus become methylated *de novo* within 1 kilobase (kb) of the integration site. In Mov-13 mice, which carry a lethal mutation of the α1(I) collagen gene[9,10], *de novo* methylation of host DNA is associated with a change in chromatin conformation[11]. This suggests that virus-induced DNA methylation can alter DNA–protein interactions and thereby interfere with correct gene activation during embryonic development.

The Mov substrains of mice, each carrying a single and highly methylated Moloney murine leukaemia virus (M-MuLV) genome at a distinct mendelian locus[7], were used to study the interaction of a provirus with its flanking host sequences. DNAs from parental control mice and from Mov mice were compared to investigate whether insertion of virus affects the methylation state of its flanking sequences. Methylation patterns were analysed by digesting DNAs from different developmental stages with methylation-sensitive restriction enzymes, followed by hybridization with probes representing the viral preintegration site. Here we report the results obtained with Mov-7, Mov-9 and Mov-13 mice.

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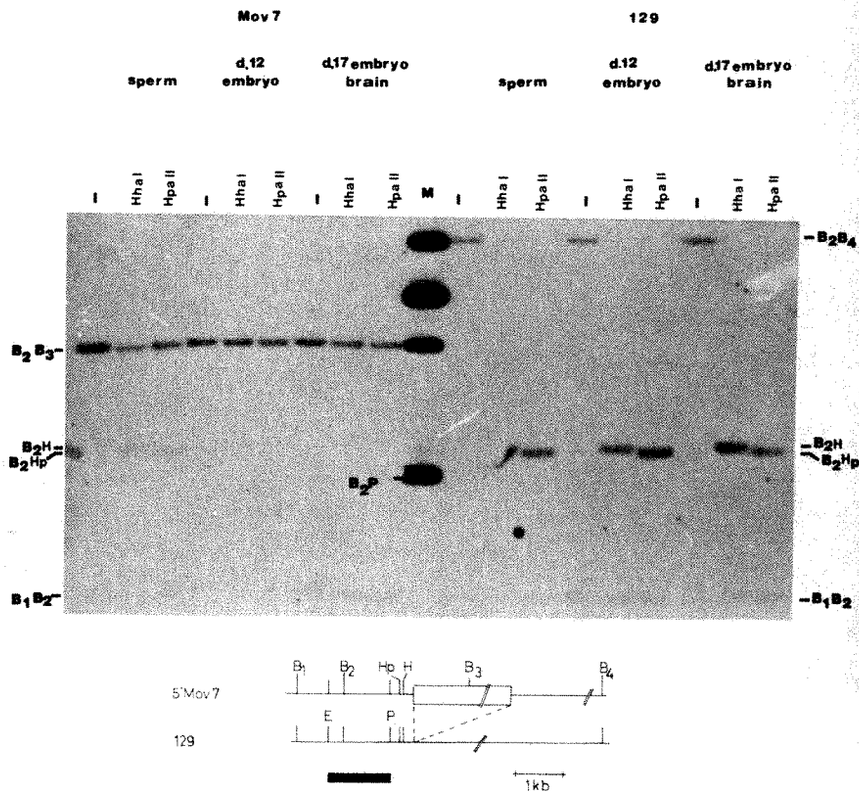


Fig. 1 Methylation of sequences 5′ of the integration site of the M-MuLV genome in Mov-7. DNAs were prepared from mature sperm, from pools of embryos and embryonic tissues, and analysed with restriction enzymes as described elsewhere[14]. Samples (20 μg) were digested with *Bst*EII (B) and in addition with either *Hha*I (H) or *HPA*II (Hp), fragments were separated on a 1.4% agarose gel, transferred to nylon membrane and hybridized with nick-translated[25] plasmid p7-1-1 which carries the *Pst*I(P)-*Eco*RI(E) fragment (solid bar in map at bottom) from the 5′-flanking region of the Mov-7 M-MuLV genome[26]. Cleavage products were mapped by comparison with the sizes of marker (M) bands derived from plasmid p7-2, which carries the Mov-7 provirus (open bar in map) within flanking cellular sequences. Digestion of p7-2 with *Bst*EII and *Bst*EII plus *Pst*I generated fragments B_2B_3 and B_2P, respectively, and, in addition, fragments of 8.6 and 4.7 kb, respectively, containing vector sequence. Partial methylation at sites H and Hp in Mov-7 sperm was indicated by the loss of intensity of band B_2B_3 and the appearance of weakly hybridizing bands migrating slightly above band B_2P (see map). The complete digestion of bands B_2B_4 (which were not transferred quantitatively to the nylon membrane), the high intensity of the cleavage products B_2H and B_2Hp, and the absence of other products, indicated lack of methylation at sites H and Hp in the DNAs from 129 mice.

Figure 1 shows *Bst*EII digests of DNA from sperm, and from day 12 and day 17 embryos of the Mov-7 and parental 129 mouse strains, which were hybridized to a probe representing 5′-flanking host sequences. This yielded the virus/cell junction fragment B_2B_3 in Mov-7 and fragment B_2B_4 spanning the integration site in 129 DNA. The junction fragments from day 12 and day 17 embryos of the Mov-7 strain were resistant to cleavage by *Hha*I and *Hpa*II, indicating complete methylation of the sites Hp and H in the 5′-flanking region (see the map in Fig. 1). A slightly lower level of methylation in sperm of Mov-7 mice resulted in digestion of the Mov-7-specific *Bst*I fragment and generated weakly hybridizing cleavage products. In contrast, both sites were completely unmethylated before virus integration, as demonstrated by the amount and the size of the *Hha*I and *Hpa*II cleavage products in 129 DNA (see Fig. 1 legend).

Similarly to Mov-7, the sequences flanking the provirus in Mov-13 mice were unmethylated before virus integration and became methylated after integration. Figure 2*a*, *b* shows the analysis of the 5′-flanking sequences and Fig. 2*c* that of the 3′-flanking sequence; the DNA bands shown are designated by letters corresponding to the respective restriction enzyme recognition sites indicated on the map. Because Mov-13 mice are heterozygous for the virus insertion[8], the probes recognize the virus-carrying and the normal allele in these mice and only the normal allele in the C57BL parental strain. Hybridization with probe A (see map in Fig. 2), which identifies sequences 5′ of M-MuLV integration site of the Mov-13 strain, revealed identical restriction patterns in *Bst*EII/*Ava*I- (Fig. 2*a*), *Bst*EII/*Hha*I- (Fig. 2*b*) and *Bst*EII/*Hpa*II (data not shown)-digested DNAs from sperm of both Mov-13 and C57BL/6 parental mice. The intensity and size of the digestion fragments (see Fig. 2 legend) indicated the absence of methylation at all seven sites within 1 kb of 5′ cellular sequences before and after provirus insertion (see Fig. 3). The same patterns as in sperm DNAs were present in the DNAs from day 12 embryos of C57BL/6 and Mov-13 mice (Fig. 2*a*, *b*). However, additional bands of higher relative molecular mass in Mov-13 embryo DNA indicated methylation at four sites present within 700 base pairs (bp) 5′ of the virus integration site (see Fig. 2 legend). An analysis of a 5′ virus/cell junction fragment, by *Kpn*I digestion of the same DNA, was used to distinguish between the two different chromosomes in the Mov-13 embryos, and localized the *de novo* methylated sites exclusively to the Mov-13 allele (Fig. 3).

The *Bam*HI-*Bgl*II cell virus junction fragment recognized by probe B served to determine the methylation of sequences 3′ of the provirus in the Mov-13 allele (Ba-Bg) and in the allele from C57BL/6 parental mice (Ba_1Bg; see Fig. 2*c*). *Ava*I and *Hpa*II cleaved these fragments completely in sperm DNA from Mov-13 and C57BL/6 mice, generating fragments that indicated non-methylation of the *Ava*I site and of two *Hpa*II sites within 300 bp 3′ of the provirus. The same three sites were completely methylated in the Mov-13 allele of day 12 embryos, as the virus/cell junction fragment was nearly resistant to cleavage. In contrast, the normal allele in both Mov-13 and C57BL/6 parental mice was not methylated at two sites, and only partially methylated at the one other site (see Fig. 2 legend; Fig. 3). The single *Hha*I site in this region was analysed similarly by *Sst*I digestion. The methylation at this site in sperm and day 12 embryos was partial in the C57BL/6 parental allele and complete near the Mov-13 provirus (Fig. 3).

The provirus in Mov-9 mice is flanked by cellular sequences which are methylated in day 12 embryos of the 129 parental mice as well as of the Mov-9 strain. Figure 4 shows that five CpG sites within 4 kb of cellular sequences at the 3′ end, and one site at the 5′ end, are fully methylated in embryonic DNA from the 129 parental strain. Provirus integration at the Mov-9 locus did not change the high level of methylation of the flanking host sequences, except for the *Hha*I site 5′ of the provirus, which is partially methylated in sperm of the Mov-9 strain and unmethylated in sperm of the parental 129 strain (Figs 3, 4).

Figure 3 summarizes our results. The cellular sequences at the respective preintegration sites of the Mov-7 and Mov-13 provirus were unmethylated in sperm as well as in somatic cells from day 12 and day 17 embryos and from adult mice of both the 129 and C57BL/6 parental strains. Analysis of somatic cells showed that provirus integration resulted in complete methylation of all tested sites in 1 kb of flanking sequences at both loci. The methylation status of sites that were farther than 1 kb away

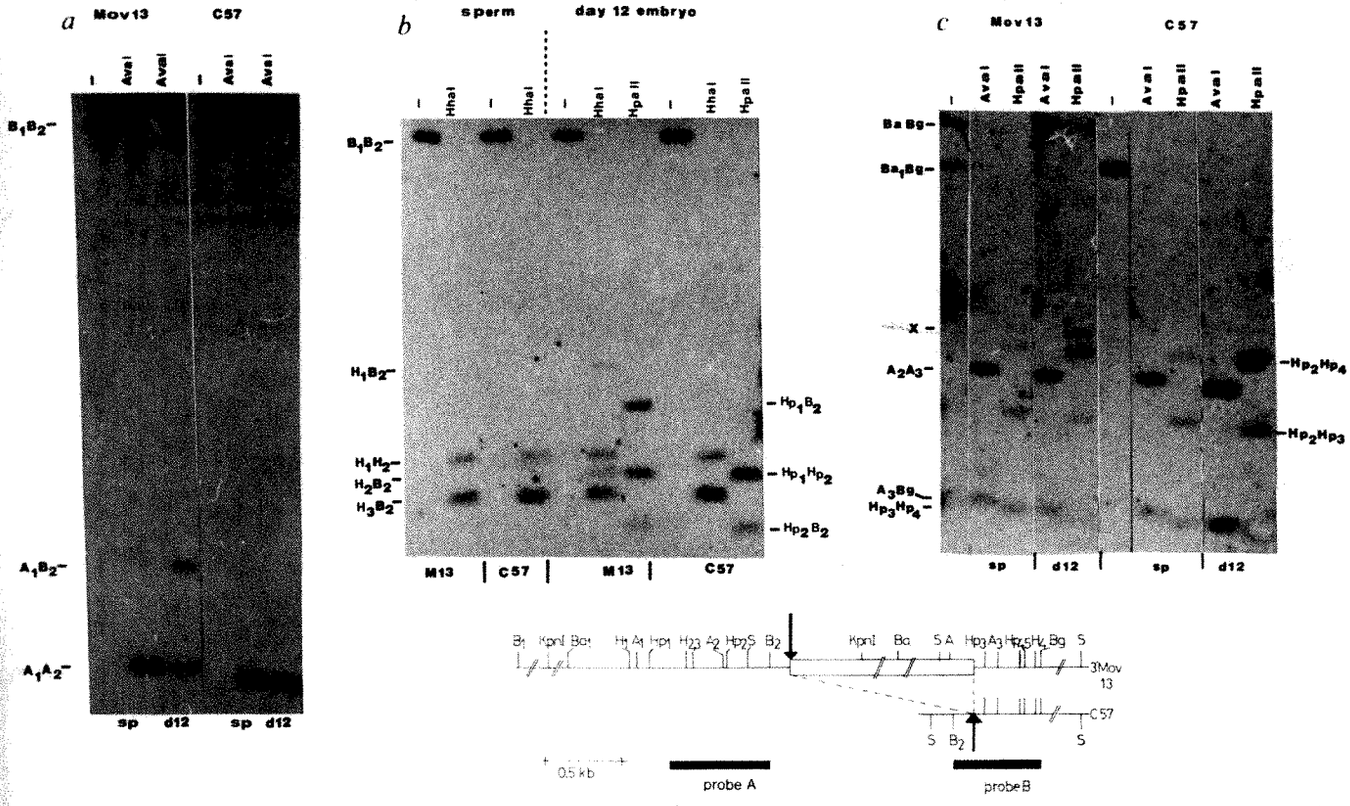


Fig. 2 Methylation of cellular sequences flanking the integration site of the Mov-13 provirus. DNAs from sperm and day 12 embryos of heterozygous Mov-13 × C57BL/6 mice[7] carrying a single M-MuLV provirus (open bar in map) and from uninfected C57BL/6 mice were prepared and analysed as described in Fig. 1 legend. The methylation of cellular sequences 5′ of the proviral integration site (vertical arrow) was determined in 20-μg samples of *Bst*EII (B)-digested DNAs with *Ava*I (A) (***a***), *Hha*I (H) and *Hpa*II (Hp) (***b***), using probe A (black bar) derived from cloned sequences of the Mov-13 locus[10]. The nucleotide sequence and map from this region[10] were used to deduce the recognition sites of the methylation-sensitive enzymes (see map) and cloned subfragments of the Mov-13 locus served as length markers (not shown) to map the cleavage sites of these enzymes in the mouse DNAs. Thus, the lengths of the *Ava*I cleavage products in *a* indicated the absence of methylation at sites A_1 and A_2 in C57BL/6 chromosomes generating band A_1/A_2, and methylation at site A_2 in the Mov-13 allele of day 12 embryos was indicated by band A_1B_2. Band A_2B_2 was too small to be detected in this analysis. Similarly, there was Mov-13-specific methylation at sites H_2, H_3 and Hp_2 but not at sites H_1 and Hp_1, as deduced from bands H_1B_2, H_2B_2 and Hp_1B_2, respectively, which appeared in embryonic DNA (*b*). Complete digestion of Mov-13 and C57BL/6 DNAs indicated the absence of methylation at all *Hha*I and *Hpa*II sites in sperm (*b*, *Hpa*II analysis not shown). ***c***, The 3′ cellular sequences were analysed with probe B (black bar), a *Bst*EII-*Bgl*II fragment from the *Mov-13* locus[10]. The *Bam*HI-*Bgl*II virus/cell junction fragment of 2.3 kb (Ba-Bg) and the corresponding cellular fragment of 1.7 kb (Ba_1Bg) were further digested with *Ava*I and *Hpa*II. The sizes of the cleavage products derived from DNAs of Mov-13 and C57BL/6 sperm indicated the absence of methylation at sites A_3, Hp_3 and Hp_4 in both alleles. The virus/cell fragments, generated from cleavage at the unmethylated 3′ viral sites A and Hp (see ref. 14) were not detectable due to their small stretch of homology with probe B (see map). In day 12 embryos, a fragment of 710 bp showed partial methylation at site Hp_3 in the C57BL/6 allele, and the resistance of fragment Ba-Bg to secondary digestion indicated methylation at all sites 3′ of the provirus. Fragment X was generated in DNA from Mov-13 embryos as a result of partial *Hpa*II cleavage at a site in the 3′ enhancer region of the provirus, which becomes preferentially demethylated during mouse development[14]. The methylation at site H_4 was determined in a similar manner by *SSt*I (S) and *Hha*I digestion using probe B (not shown; see Fig. 3).

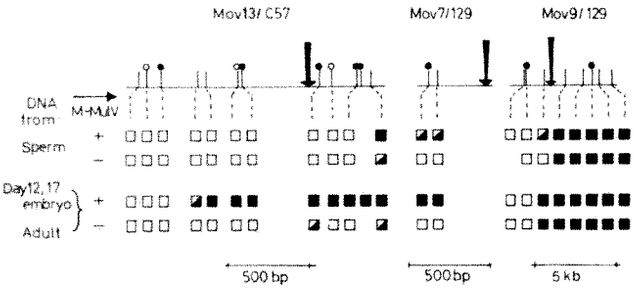


Fig. 3 The methylation at *Hha*I(|), *Ava*I(♀) and *Hpa*II(♀|) sites around M-MuLV integration sites (vertical arrows) was determined in Mov-7, -9 and -13 mice (+) and in homologous sequences of parental mouse strains (−) as described in the legends of Figs 1, 2 and 4. Filled, half-filled or open boxes indicate ≥80%, 20–80% or <20% methylation, respectively, of the individual sites. The horizontal arrow shows the direction of viral transcription from the 5′ to the 3′ end. The data for day 17 embryos and adult mice represent liver and brain or brain and kidney, respectively; the analysis of 5′ cellular sites from Mov-9 mice is described elsewhere[14].

from the integration site was not affected by provirus integration. The sequences flanking the Mov-13 provirus, which represent the 5′ end of the α1(I) collagen gene[10], were completely unmethylated in sperm, indicating that virus insertion induced *de novo* methylation of unique cellular sequences during embryogenesis. So far, *de novo* methylation has been observed only in repetitive sequences[12,13] or with proviral or cellular sequences introduced into transgenic mice[14,21]. The lower level of methylation in sperm than in somatic cells of Mov-13 mice may indicate either germline-specific lack of *de novo* methylation or specific demethylation at these sites during spermatogenesis. The relative hypomethylation of satellite DNA in extraembryonic as opposed to embryonic tissues[12] is consistent with the notion that *de novo* methylation occurs after the blastocyst stage, when these cell lineages have separated.

In contrast to Mov-7 and Mov-13, the provirus in Mov-9 mice has integrated into a hypermethylated region, the methylation status of which was not detectably affected by the insertion. A similar result was found for Mov-2 and Mov-3 (unpublished) and Mov-10 mice[14].

De novo methylation of retroviral sequences introduced into mouse zygotes, early mouse embryos or embryonic carcinoma

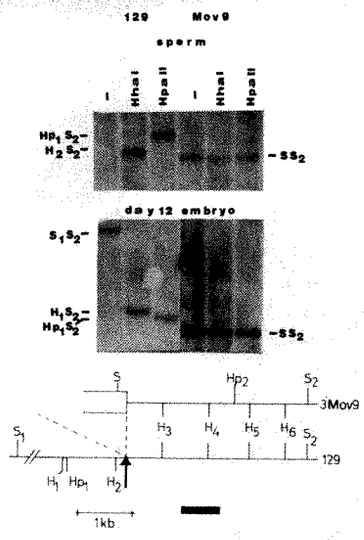


Fig. 4 Methylation of cellular sequences flanking the integration site of the Mov-9 provirus. DNAs from Mov-9 (ref. 7) and uninfected parental 129 mice were prepared and analysed with restriction enzymes as described in Fig. 1, legend, using plasmid p9-1-2, which carries sequences (black bar in map) 3′ of the integration site (vertical arrow) of the Mov-9 provirus (open bar; see ref. 26). DNA samples (20 μg) were digested with *Sst*I (S), generating the viral junction fragment SS_2 in Mov-9 DNAs and fragment S_1S_2 which spans the integration site in the 129 alleles. In 3′-flanking sequences the *Hha*I (H) and *Hpa*II (Hp) sites were methylated in Mov-9 mice, as seen by the resistance of band SS_2 to secondary digestion. The gel position of this fragment was used to localize, in 129 mice, the unmethylated sites Hp_1, H_2 or H_1 in sperm and day 12 embryos, respectively (see map).

cells correlates with transcriptional inactivity, whereas the absence of *de novo* methylation in somatic cells correlates with gene expression (refs 15–20; see ref. 3 for review). The present results show that a functional provirus becomes repressed even when integrated into a chromosomal region which, as in Mov-13 mice, has all the characteristics of active gene expression, that is, hypomethylation and the potential to develop an open chromatin configuration[11]. Thus, the integration of retroviruses may lead to altered chromatin structures that allow *de novo* methylation. The molecular basis for both provirus and host gene suppression may be the maintenance of these structures due to their methylation. Non-retroviral genes which have been introduced into the germ line of mice are often not expressed[21–24]; it remains to be seen whether this is correlated with similar changes in chromatin structure and methylation pattern as described here for Mov mice.

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A viral enhancer element specifically active in human haematopoietic cells

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One particular class of DNA regulatory elements, the enhancers or activators, can, relatively independently of distance and orientation, dramatically increase the transcriptional activity of homologous and heterologous promoters located in *cis* (see refs 1–3 for reviews, also refs 4–6). Sequence differences between various heterologous enhancers[7,8] may explain their apparent host- and/or tissue-specific action[9–15]. Furthermore, differences in the transcriptional control elements may contribute to viral tropism. At least for murine leukaemia virus isolates, thymotropism and leukaemogenicity have been attributed to alterations within the viral long terminal repeat[15–17], which harbours their enhancers and other transcriptional control elements. We report here the identification of a viral enhancer element possessing a very restricted tissue range. The enhancer is active in all human cells of the haematopoetic system tested, but not in cells of fibroblast or epithelial origin.

In the recently discovered lymphotropic papovavirus (LPV)[18], progeny production is restricted to continuously growing cells of primate B-lymphocyte origin[19–21], and sequence analysis[22] shows that the LPV genome has a structure typical of all other known polyomaviruses[23] (Fig. 1). In addition to early and late regions encoding virus-specific proteins, a noncoding region of ~700 base pairs (bp) is situated between the first early and late translational start codons and harbours several sets of repeated sequences. A large set of direct repeats (106 bp) presumably contains the origin of replication (Fig. 1)[22,23]. Because it is present twice on the cloned LPV DNA, this clone was probably produced from a natural variant carrying two origins of replication; this notion was confirmed experimentally by removing one of the direct repeats, for, despite this deletion, the LPV genome retains its viability (M.P., unpublished). A smaller (63 bp) sequence set lies to the late side of the 106-bp fragment. It is duplicated once flanking the origin proximal to the early region, and is present as a monomer flanking the distal origin (Fig. 1).

Several indications prompted us to search for an enhancer element in this smaller repeat. Its position on the genome is similar to that of all other known papovavirus enhancers[4,24–28], with the exception of papillomaviruses[29], and a sequence resembling the enhancer core sequence of simian virus 40 (SV40)[7] and the mouse immunoglobulin constant-region heavy-chain gene (C_μ)[10] is present within the 63-bp repeat. To test for enhancer function, a 234-bp *Hae*III fragment that included the 63-bp repeat (Fig. 1) was cloned into the pA10-CAT2 plasmid[9],

Table 1 CAT activity in cells of haematopoietic, epithelial and fibroblast origin after transfection with pSV2-CAT, pLPV-5′-CATs or pCμ-5′-CATs DNA

Cell line	Origin	CAT activity pSV2-CAT	pLPV5′-CATs	pCμ5′-CATs
Haematopoietic cells[37,43,44]				
K562	Chronic myeloid leukaemia	++	++	–
HL-60	Human acute promyelocytic leukaemia	(+)	(+)	–
KG-1	Human early myeoblast	+	+++	–
U937	Human myelosarcoma	+++	+	–
KM-3	Human non-B, non-T acute lymphoblastic leukaemia	++	++	–
LBL-1	Epstein-Barr Virus (EBV)-transformed cord blood lymphocytes	+++	+	ND
LBL-AK	EBV-transformed cord blood lymphocytes	+++	+	ND
BJAB	Human Burkitt's lymphoma, EBV-negative	+++	++	++
P3HR-1	Human Burkitt's lymphoma, EBV-positive	+++	++	ND
Daudi	Human Burkitt's lymphoma, EBV-positive	+++	+	ND
JM-1	Human acute T-cell lymphoblastic leukaemia	++	++	–
MOLT-4	Human acute T-cell lymphoblastic leukaemia	++	++	–
CEM-C7	Human acute T-cell lymphoblastic leukaemia	+	+	–
R-LICR LON/HMy 2	IgG-producing human myeloma	+++	+	ND
Peripheral blood	Human	(+)	(+)	(+)
Epithelial-like cells				
Hela	Human cervix carcinoma	+++	–	–
CV-1	Monkey kidney	+++	–	–
Fibroblast-like cells				
HFF	Human foreskin fibroblasts	+++	–	–
HE	Hamster embryonic cells	+++	–	–
LPV-HE[38]	Hamster embryonic cells, transformed by LPV	+++	++	+
F9	Mouse stem cells	++	–	ND
X63Ag8	Mouse plasmacytoma hybridoma	++	–	++
15E1	Mouse plasmacytoma hybridoma	++	–	++

The transfection procedures and CAT assays were done as described in Fig. 2*b* legend. After chromatography and autoradiography, the spots containing the acetylated (CM-Ac) and non-acetylated (CM) forms of ^{14}C-chloramphenicol were cut out, placed in scintillation liquid and counted. The percentage of CM-Ac radioactivity in the total (CM−Ac+CM) was determined. –, No enhancement; +, 5-fold enhancement; ++, 5–20-fold enhancement; +++, >20-fold enhancement. The percentage of acetylated chloramphenicol in cells transfected with pA10-CAT2 (enhancer minus constructions, see Fig. 2*a*) was taken as the denominator and an enhancement factor was calculated for cells transfected with constructions carrying SV40, LPV and C_μ enhancers, respectively. ND, not done.

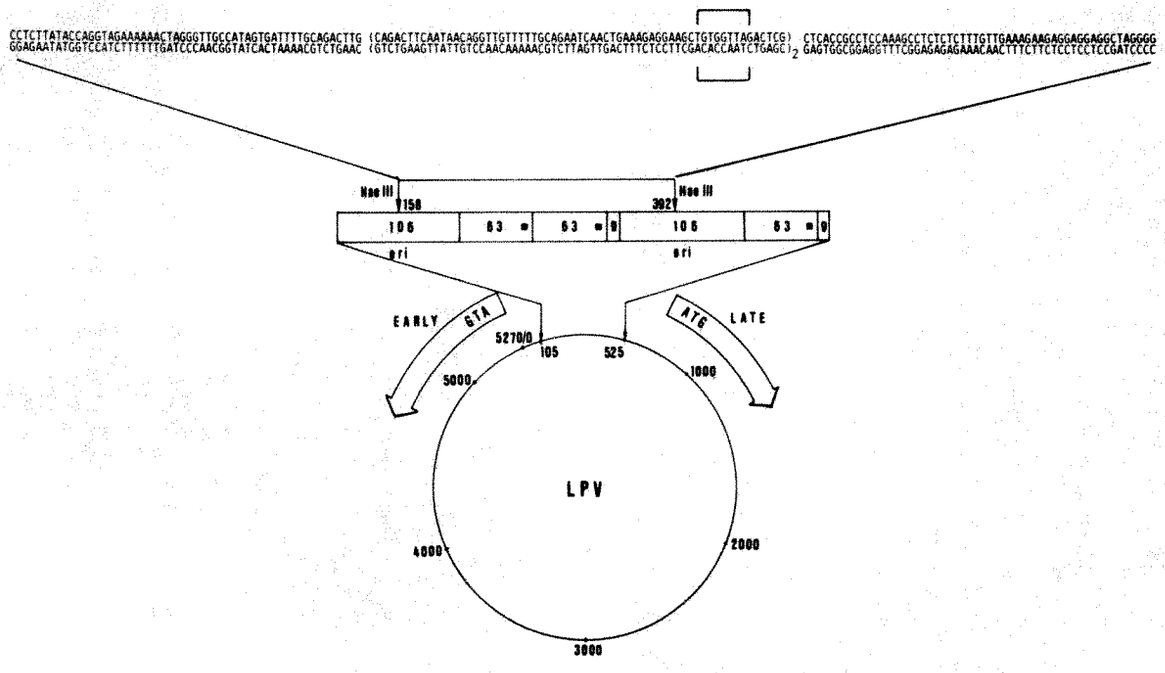
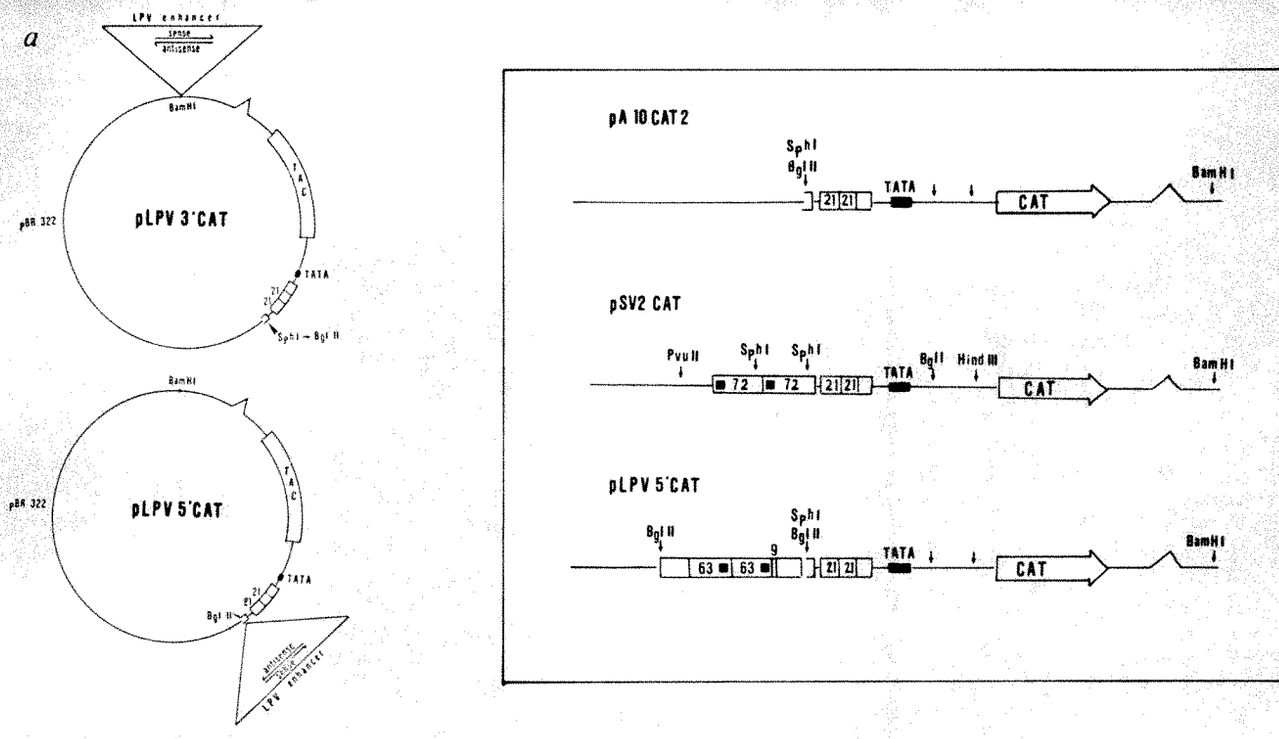


Fig. 1 Genomic organization of lymphotropic papovavirus (LPV). The early and late coding regions of LPV DNA are separated by a stretch of noncoding sequences of ~700 bp. This region contains several sets of direct repeats as represented by the open boxes (see text for further details). The numbers of base pairs which are homologous in each repeat are indicated by the numbers in the open boxes. A 234-bp *Hae*III fragment, containing the 63-bp repeat, was cloned into plasmid pA10-CAT2 (see Fig. 2). The sequences within the 63-bp repeat which resemble enhancer core nucleotides[7] are indicated by solid squares. The complete nucleotide sequence[22] of the *Hae*III fragment is given at the top, with the enhancer core nucleotides indicated by brackets.



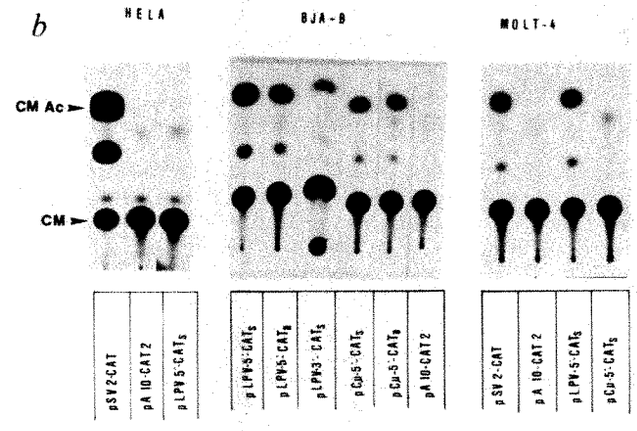


Fig. 2 *a*, Construction of pLPV-5′-CATs, pLPV-5′-CATa and pLPV-3′-CATs DNAs. Enhancer core nucleotides are indicated by closed boxes. *b*, CAT activity of BJA-B, MOLT-4 and HeLa cells after transfection with pLPV-CAT, pCμ-5′-CAT or pSV2-CAT DNA.

Methods. *a*, The 234-bp *Hae*III fragment (see Fig. 1) of LPV was excised, ligated to *Bgl*II linkers and cloned upstream of the CAT gene in the *Bgl*II site of pA10-CAT2 (ref. 9) in either orientation (sense or antisense). Another construction was made, containing the *Hae*III fragment downstream of the CAT gene in the *Bam*HI site of pA10-CAT2 (pLPV-3′-CATs). pA10-CAT2 and pSV2-CAT, which were also used for transfection experiments, have been described previously[9,30]. *b*, BJA-B cells[35] and MOLT-4 cells[33] were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS. Cell transfection was carried out by the DEAE-dextran method[33,34]. The cells (5×10^6) were washed twice with TBS[14] and subsequently incubated at room temperature for 30 min with a mixture containing 10 μg of plasmid DNA in 50 μl TE (15 mM Tris, 1 mM EDTA), *p*H 7.8, 250 μl TBS and 300 μl DEAE-dextran (1 mg ml^{-1}). After incubation, the cells were washed again with TBS, resuspended in fresh medium, incubated at 37 °C, 5% CO_2 and 48 h after transfection, collected to determine CAT activity. The CAT assay was performed as described by Gorman *et al.*[30] with a few minor modifications. Standard enzyme reactions were performed with 100 μl of a total of 150 μl extract and were incubated for 90 min at 37 °C. The assay mixture final volume (171.5 μl) contained 0.2 μCi ^{14}C-chloramphenicol ($\sim2\times10^5$ c.p.m.) and 4 mM acetyl-CoA in 0.25 M Tris-HCl buffer (*p*H 7.8). The reactions were stopped by extraction with 0.4 ml ethylacetate. The ^{14}C-chloramphenicol was dried and resuspended in 10 μl ethylacetate; its derivatives were separated by ascending TLC (0.25 mm silica gel) with a chloroform/methanol (95:5) solvent phase. The chromatography plates were then exposed to Kodak XAR-5 film for 12–24 h.

which can serve as an enhancer assay system as discussed previously[9] (see Fig. 2*a* legend for details). Thus, activation of this transcriptional unit can be estimated through the production of both RNA and chloramphenicol-acetyl-transferase (CAT) activity, the amount of which is dependent on the enhancer inserted[9,30]. The LPV fragment was cloned at the 5′ end of the CAT gene into a *Bgl*II site in opposite orientations (pLPV-5′-CATs, pLPV-5′-CATa (Fig. 2*a*). ('s' signifies sense, indicating that the orientation of the LPV fragment with the CAT gene is the same as it is with the early coding region of LPV; 'a' signifies the opposite, or antisense, orientation.) Similarly, the LPV fragment was inserted into a *Bam*HI site, located 3′ of the SV40 polyadenylation signal (pLPV-3′-CATs). As a control, similar plasmids were constructed carrying the known enhancer of C_μ. The *Xba* I-E fragment of the mouse C_μ gene[10] was excised and cloned in both orientations into an *Xba*I site of a test plasmid (pCAT-2 polylinker)[31,32]. No difference in enhancer activity was detected whether pA10-CAT2 or pCAT2-polylinker was used as a recipient for the same enhancer DNA fragments.

To test for the functional activities of the various plasmid constructions, purified DNA was introduced into eukaryotic cells using DEAE-dextran as facilitating agent[33,34]. As LPV was adapted to grow lytically in the human B-lymphoma line BJA-B (ref. 35), these cells were chosen as recipients for the first series of transfection experiments. Little or no CAT activity was detected in BJA-B cells transfected with pA10-CAT2 (Fig. 2*b*), but when the 234-bp LPV fragment was inserted at the 5′ end of the SV40 promoter region, the cells produced CAT, as demonstrated by the conversion of chloramphenicol (CM) into its acetylated forms (CM-Ac) (Fig. 2*b*). The conversion rate is independent of the orientation of the LPV fragment, as both the sense (pLPV-5′-CATs) and antisense (pLPV-5′-CATa) insertions gave similar conversion rates. Also, introduction of the 234-bp LPV fragment in the sense orientation at the 3′ end of the polyadenyl-

ation signal produced activation of the transcriptional unit, thus fulfilling the criteria described for most other viral enhancer sequences. The relative level of enhancement at the 3′ end (Fig. 2*b*; pLPV-3′-CATs), however, was three- to fourfold less than for the 5′ construction. We are investigating the reason for this reduction.

The relative strength of the transcriptional activation by the LPV enhancer was estimated by performing a control experiment to measure the activity mediated by the well-characterized C_μ enhancer[10]. Insertion of the *Xba* I-E fragment at the 5′ end of the SV40 promoter elements resulted in the production of CAT, independent of orientation (pCμ-5′-CATa, s), at levels comparable with those of the respective LPV-containing plasmids in BJA-B cells (Fig. 2*b*).

To demonstrate that the CAT results directly reflect the CAT transcriptions present, we performed S_1 nuclease analysis of the CAT RNA to map the 5′ ends. Although we found that the plasmid containing the LPV enhancer showed the same RNA start sites as the well-characterized plasmid pSV2-CAT that contains the SV40 enhancer (data not shown), the bands detected were very weak, preventing us from obtaining quantitative data. Therefore, the following experiments were carried out using the CAT assay, as this seemed more sensitive.

In investigating the host- and cell-type specificity of the LPV enhancer, because LPV seems to infect only cells of B-cell lineage, we used a human lymphoma cell line of T-cell origin (MOLT-4)[36] as a recipient for various plasmid constructions. Only the LPV 234-bp fragment and the SV40 enhancer[9,22,30] activated CAT production in MOLT-4 cells, and no enzyme production was detected after transfection of the plasmid carrying the C_μ enhancer (pCμ-5′-CATs) or the recipient plasmid without insert (pA10-CAT2) (Fig. 2*b*). A similar result was obtained after transfection of the plasmid constructions into two additional established human cell lines (JM-1, CEM-C7) with T-cell characteristics (Table 1). Thus, although the C_μ enhancer is active in cells of B-lymphoid origin, it does not seem to increase the transcription of transfected recombinant plasmids in the human T-cell lines used. On the other hand, the cell specificity of the LPV fragment seems less strict because LPV was also active in T cells and in several other non-B-non-T lymphoid cell lines (Table 1), whereas none of these cell types responded to the C_μ enhancer.

Because all the cell lines used in these studies have a transformed phenotype, it was essential to eliminate the possibility that transformation is required for LPV enhancer activity. We therefore transfected peripheral blood cells stimulated with concanavalin A and pokeweed mitogen and found that these cells recognize both LPV and C_μ enhancers (Table 1). Surprisingly, transfection of two mouse myeloma-derived cell lines, X63Ag8 (ref. 37) and 15E1 (B. Georg-Fries, personal communication), with pLPV-5′-CATs did not yield any CAT, although parallel transfections with pCμ-5′-CATa showed marked CAT activity (Table 1).

To determine whether the activity of the LPV activator is restricted to haematopoietic cells, we analysed the enhancer activities after transfection of test plasmids into several established and primary cell lines (Table 1). Using the HeLa cell line, which is of epithelial origin, we found that, in transfection experiments the LPV enhancer (pLPV-5′-CATs) did not stimulate activity in these cells, whereas SV40 enhancer (pSV2-CAT) yielded large amounts of CAT (Fig. 2*b*, Table 1). A similar difference between LPV and SV40 enhancers was found in secondary human foreskin fibroblasts, CV-1 cells and primary hamster embryonic cells. To our surprise, the LPV-transformed[38] hamster embryonic cell line responded to both LPV and C_μ enhancers. It is unclear whether this results from a specific cell type, selected by transformation with LPV, or whether it results from the presence of a *trans*-acting factor[39] provided directly or indirectly by LPV T-antigen.

Our results indicate that a 234-bp fragment from the LPV genome contains an enhancer element. This selected fragment contains a 63-bp repeat harbouring the putative enhancer core sequence[7]. Although probable, it remains to be shown that the transcriptional enhancement is mediated by this 63-bp repeat. Interestingly, this viral enhancer element exerts activity only in cells of human haematopoietic origin and in this respect differs from the mouse immunoglobulin heavy-chain gene enhancer[10] which, in our hands, is active only in B-lymphoma cells. However, recent experiments by others indicate that the mouse C_μ enhancer is active in some T-cell lines of mouse origin (W. Schaffner and D. Baltimore, personal communication). This result could be explained by host-range differences and it is interesting that the LPV enhancer seems to show a host-range effect in addition to the cell-type specificity (Table 1). Preliminary evidence indicates that the inability of LPV to produce progeny in T lymphoblasts or other haematopoietic cells results from a lack of adsorption and/or uncoating of LPV particles (M.P., unpublished). Taken together, our data are compatible with the previous observation that enhancers interact with cellular molecules (refs 40–42 and P. Sassone-Corsi and P. Chambon, personal communication). However, only the presence of more than one class of cellular factors could explain our data satisfactorily. Our present research is directed towards the identification of such factors.

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A novel immune system against bacteriophage infection using complementary RNA (micRNA)

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The 'operon' theory of gene regulation[1], in which protein repressor molecules bind to the operator site of a gene to prevent its transcription, is now well established. Recently, however, cases have been discovered in which gene expression is regulated by complementary RNA molecules that are able to bind to the transcripts of particular genes and consequently prevent their translation[2–5]. For example, the synthesis of OmpF protein (a major outer membrane protein) in *Escherichia coli* is regulated by a short RNA complementary to a region of *ompF* RNA encompassing the 'Shine–Dalgarno' sequence[6] and the translation initiation codon[2,3]. This RNA has been termed micRNA (messenger-RNA-interfering complementary RNA), and its discovery has prompted us to construct an artificial micRNA system designed to regulate gene expression in *E. coli*[7]. A given target gene can be repressed by artificially producing an RNA (micRNA) complementary to the mRNA encoded by that gene. A micRNA system has also been used successfully in tissue-cultured mammalian cells[8,9]. The use of artificial micRNAs to specifically regulate individual genes has great potential as a novel cellular immune system for blocking bacteriophage or virus infection. Here, we report that on induction of micRNAs directed against the coat protein and/or the replicase of the *E. coli* bacteriophage SP, phage proliferation was effectively prevented. We propose that the micRNA immune system provides an effective means of preventing viral infection as well as the expression of harmful genes in both prokaryotes and eukaryotes.

To design a micRNA immune system against phage infection in *E. coli*, we chose coliphage SP, a positive, single-stranded RNA, F-specific phage, which was isolated in Japan and classified in the group IV coliphages[10]. The entire phage genome consists of ~4.2 kilobases (kb) and its complementary DNA has been cloned (pSP219; Y.I. and A.H., in preparation). The nucleotide sequence of the entire genome reveals that the RNA phage codes for a maturation enzyme, coat protein, coat readthrough protein and replicase (see Fig. 1*a*) (Y.I. and A.H., in preparation).

Previous work has shown that micRNAs that are able to hybridize with the target mRNA over the region containing the Shine–Dalgarno sequence and the initiation codon are the most effective repressors of gene expression[7]. Therefore, fragments A and B in Fig. 1 were used to construct the micRNA immune system: fragment A consists of the 247-base-pair (bp) *Cla*I/*Aha*III fragment encompassing the Shine–Dalgarno sequence and the initiation codon of coat protein; fragment B consists of the 159-bp *Rsa*I/*Aat*I fragment encompassing the Shine–Dalgarno sequence and the initiation codon of replicase. In addition to these two fragments, a DNA fragment (fragment C in Fig. 1) from the 3′ end of the phage genome was also used; this fragment was derived from a *Bam*HI/*Pvu*I digestion of pSP219, a cDNA clone constructed from poly(A)-tailed phage RNA. Therefore, fragment C consists of ~690 bp: 411 bp from the C-terminal coding region of the replicase gene, 107 bp from the 3′-noncoding region, ~60 bp from the poly(A) tail, and 111 bp from the vector pBR322 (*Pvu*I–*Sca*I). These DNA fragments were inserted into the unique *Xba*I site of the micRNA cloning vector, pJDC406 (Fig. 1*b*), by blunt-end ligation. Thus, transcription of the inserted DNA is controlled by the strong *lpp* promoter and the *lac* promoter–operator, so that the production of a large amount of RNA transcript can be induced in the presence of a *lac* inducer, such as isopropyl-β-D-thiogalactoside (IPTG)[7]. The orientation of the inserted DNA fragments was determined by restriction site analysis, and those plasmids in which fragments A, B and C were inserted in the anti-sense transcription direction with respect to the *lpp* and *lac* promoters were designated pMIC-A, pMIC-B and pMIC-C, respectively. As clear gene dosage effects have been demonstrated previously[7,11], we also constructed the following plasmids carrying two or three micRNA genes: pMIC-A:A (2 × *micA*, that is, two *micA* genes in one plasmid), pMIC-A:B (*micA* plus *micB*), pMIC-C:C (2 × *micC*) and pMIC-A:B:C (*micA* plus *micB* plus *micC*).

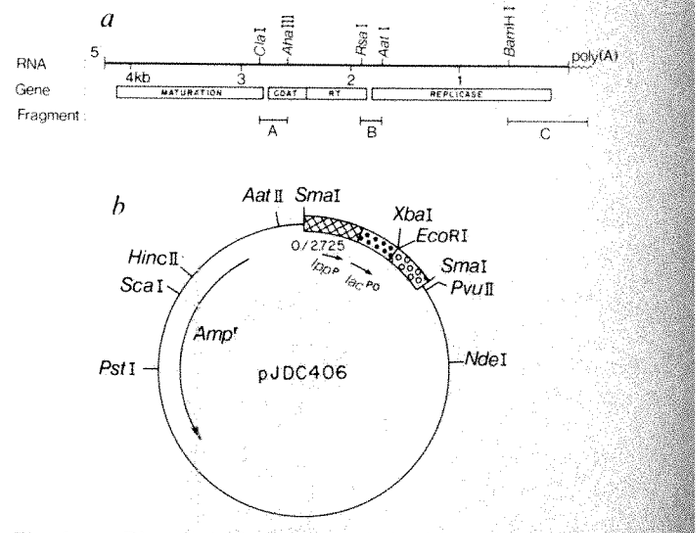


Fig. 1 *a*, Diagrammatic representation of bacteriophage SP. Numbers refer to the distance (in kilobases) from the 3′ terminus. Only the relevant restriction sites are shown. RT, coat readthrough protein. Fragments A, B and C were used to construct the micRNA plasmids pMIC-A, pMIC-B and pMIC-C respectively. *b*, Restriction map of micRNA vector pJDC406. Numbers refer to size in kb. Cross-hatching represents the *lpp* promoter, filled circles represent the *lac*UV5 promoter–operator, and the open circles represent the *lpp* ρ-independent transcription terminator. pJDC406 was constructed from pJDC402 (for description see ref. 7) by deleting 0.3 kb downstream of the *lpp* terminator (including the downstream *Eco*RI site) and changing both *Hin*fI sites flanking the micRNA transcription unit to *Sma*I sites with the use of *Sma*I linkers (CCCGGG). The micRNA plasmids were constructed by inserting the fragment of interest into the unique *Xba*I site after creating flush ends with DNA polymerase I Klenow fragment. To construct pMIC-C:C, the smaller *Sma*I fragment from pMIC-C was inserted into the *Pvu*II site of a second pMIC-C, resulting in two *micC* genes on one plasmid. pMIC-A:B and pMIC-A:A were constructed by inserting the smaller *Sma*I fragment from pMIC-A into the unique *Nde*I site of pMIC-B and pMIC-A, respectively. pMIC-A:B:C was constructed by inserting the smaller *Sma*I fragment from pMIC-C into the unique *Aat*II site of pMIC-A:B.

Figure 2*a* shows growth curves of *E. coli* JA221/F′LacI^q (*hsdR leuB6 lacY thi recA* Δ*trpE5*/F′ *lacI^q lac$^+$ proAB*)[12] carrying the construct pMIC-A:B:C. micRNA production was induced by adding 2 mM IPTG before phage infection. Phage infection was carried out at a multiplicity of infection of ~15. Control cells carrying the vector plasmid alone were lysed after infection whereas cells carrying pMIC-A:B:C did not lyse in the presence of IPTG, although growth stopped after phage infection (Fig. 2*a*). This result indicates that the induction of micRNAs inhibited the proliferation of phage SP. Partial inhibition was observed even without the addition of IPTG, a result of incomplete repression of micRNA gene transcription in pJDC406 (ref. 7). Plaque formation was also inhibited when the cells carrying pMIC-A:B:C were used as indicator bacteria in the presence of IPTG. Figure 3*a* shows plaque formation of phage SP on *E. coli* JA221/F′LacI^q carrying only the vector plasmid (pJDC406) in the absence and presence of IPTG. IPTG has little effect on either the number of plaques or the plaque morphology [Fig.

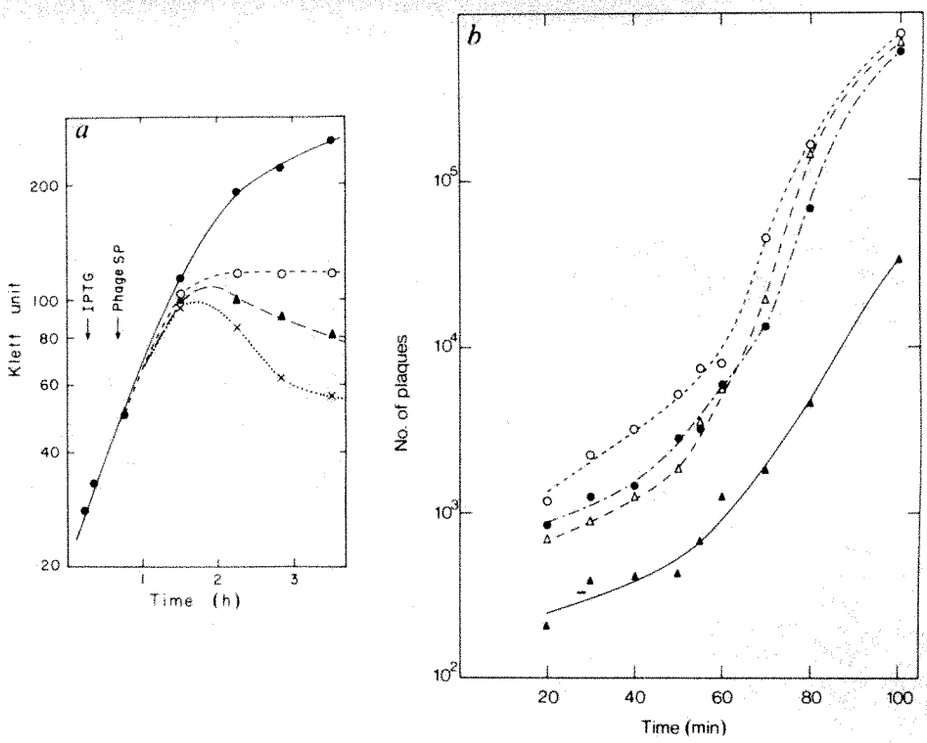


Fig. 2 *a*, Effect of phage SP on the growth curve of *E. coli* JA221/F'LacI^q containing pMIC-A:B:C or pJDC406. Cells were grown in L-broth[13] containing 5 mM $CaCl_2$ in the presence or absence of the *lac* inducer IPTG (2 mM). ●—●, Turbidity of cultures containing pMIC-A:B:C or pJDC406 without phage SP in the presence or absence of IPTG. ×···×, Turbidity of a culture containing pJDC406 plus phage SP in the presence or absence of IPTG. ○---○, Turbidity of a culture containing pMIC-A:B:C in the presence of both IPTG and phage SP. ▲--▲, Density of a culture containing pMIC-A:B:C in the absence of IPTG and in the presence of phage SP. The labelled arrows indicate the times of addition of IPTG (to induce the micRNA genes) and of phage SP. *b*, Phage titre of the cell culture at various times after addition of phage SP. Circles represent *E. coli* JA221/F'LacI^q harbouring pJDC406; triangles represent JA221/F'LacI^q harbouring pMIC-A:B:C. Open symbols represent cultures grown in the absence of the inducer IPTG; solid symbols represent cultures grown in the presence of IPTG (2 mM) for 1 h before phage infection. Cells were grown in L-broth containing 5 mM $CaCl_2$ to a Klett-Summerson reading of 10, at which point IPTG was added to half of each culture. The cultures were grown for a further hour to a Klett reading of 55, at which time phage SP was added at a multiplicity of infection of 0.1. After 10 min the cultures were diluted 10-fold to decrease the changes of re-infection. At each time point, the appropriate dilutions of each culture were immediately mixed with a large excess of a stationary culture of *E. coli* A/λ and plated on to plates of L-broth. Plaques were counted after 12 h of growth. The initial amount of free phage was subtracted from each time point; this number was determined by titering $CHCl_3$-resistant phage in the culture at 20 min. In all cases free phage numbered ~300.

3*a*(−) and (+)]. However, in the same *E. coli* strain carrying pMIC-A:B:C, the number of plaques was drastically decreased to about one-tenth of the control value (Fig. 3*a*) in the presence of IPTG. Note that 10 times more phage were plated out in Fig. 3*b*(+) than on all the others. In addition, the plaques in Fig. 3*b*(−) are much smaller than the control plaques. In the presence of IPTG [Fig. 3*b*(+)], the number of plaques decreased further, and the plaques were very small and difficult to see. These results are consistent with those shown in Fig. 2 and clearly demonstrate that micRNAs targeted against phage genes are able to effectively block formation of plaques and phage proliferation.

Figure 2*b* shows the phage titre of *E. coli* JA221/F'LacI^q carrying pMIC-A:B:C or the micRNA vector pJDC406 at various times after addition of phage SP. In the presence of IPTG, the number of infective centres decreased by about four-fold in cells carrying pMIC-A:B:C. At 100 min, the phage titre of the cells carrying pMIC-A:B:C in the presence of IPTG was ~0.05 of that observed for control cells (Fig. 2*b*). Note that *micA:B:C* had very little inhibitory effect in the absence of IPTG compared with cells containing the micRNA vector (pJDC406) alone. These results indicate that the inhibitory effect of *micA:B:C* is active throughout the phage infection. The poor yield of phage in the cells carrying pMIC-A:B:C in the presence of IPTG is probably a result of both direct inhibition of translation of the phage RNA and degradation of the phage RNA. Target mRNA degradation has been shown previously to occur after induction of micRNA[3,7].

To demonstrate that the initial effect of the micRNA is caused at least partially by the inhibition of phage protein production, *E. coli* JA221/F'LacI^q (containing either pMIC-A:B:C or the control plasmic pJDC406) infected with phage SP was labelled for 10 min with ^{3}H-leucine. (Infection with phage SP was for 15 min at a multiplicity of infection of 5, after 1 h induction of the micRNAs with 2 mM IPTG.) In the presence of *micA:B:C*,

Table 1 Effects of individual and multiple micRNA genes on SP plaque formation

	Absence of IPTG			Presence of IPTG		
Plasmid	Titre*	% Inhibition†	Plaque morphology‡	Titre*	% Inhibition†	Plaque morphology‡
pJDC406	3,650	0	Normal	3,320	0	Normal
pMIC-A:B:C	2,120	42	Small	140	96	Very small
pMIC-A	2,510	31	Normal	1,290	61	Normal
pMIC-B	2,670	27	Normal	1,190	64	Small
pMIC-C	3,570	2	Normal	2,060	38	Normal
pMIC-A:A	1,860	49	Normal	310	91	Small
pMIC-A:B	1,710	53	Normal	300	91	Small
pMIC-C:C	3,170	13	Normal	1,510	55	Small

* This value is an average of at least two determinations (s.d. < 30%).
† Calculated relative to the values obtained with pJDC406.
‡ Plaque morphologies are shown in Fig. 3: *a*(+) = normal: *b*(−) = small; *b*(+) = very small.

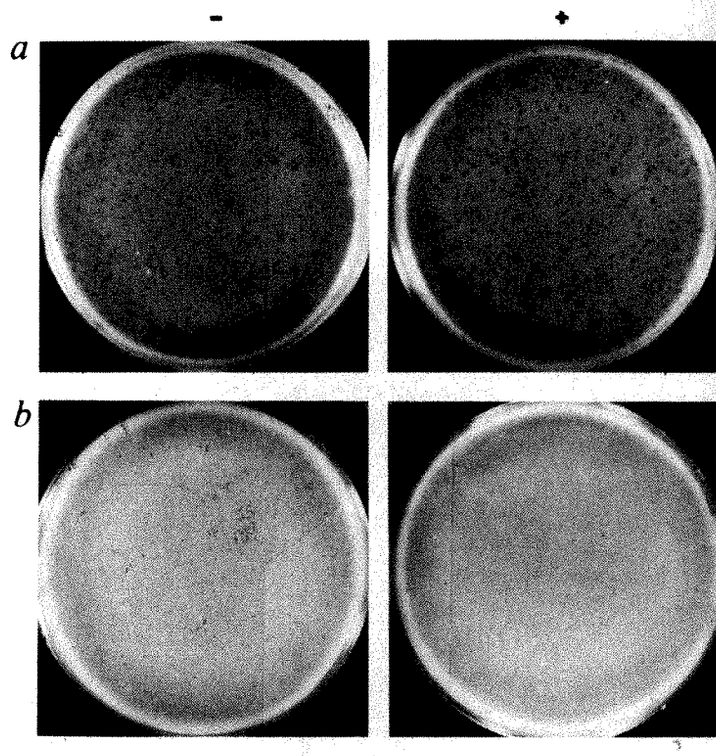


Fig. 3 SP plaque formation on a lawn of *E. coli* JA221/F'LacI^q containing pJDC406 (*a*) or pMIC-A:B:C (*b*) in the absence (−) or presence (+) of the micRNA gene inducer, IPTG. Cultures were grown to late log phase in L-broth[13], at which time IPTG was added (to a final concentration of 2 mM) to half of each culture, and growth continued for an additional 2 h. IPTG was added to the warm L-broth top agar just before plating. Phage and bacteria were mixed 1 min before plating. The concentration of phage plated is the same in each case, except for plate *b*(+) in which 10 times more phage were used.

synthesis of the phage SP coat protein was greatly reduced compared with cells carrying only the micRNA vector pJDC406 (data not shown); other proteins were unaffected.

The inhibitory effects of pMIC-A:B:C were not due to its nonspecific effects on the cells. Cells carrying pMIC-A:B:C are fully sensitive to coliphages Qβ (group III) and GA (group II), which are F-specific, positive, single-stranded RNA phages; the phage GA or Qβ titre remained the same when these phage were plated, with or without IPTG, on *E. coli* JA221/F'LacI^q carrying either pMIC-A:B:C or pJDC406 (data not shown). This result indicates that such cells are fully capable hosts for F-specific phage infection, and that the effects of pMIC-A:B:C are specific to phage SP.

To determine which micRNA genes make the largest contribution to the immunity conferred by pMIC-A:B:C, phage titres were measured for *E. coli* JA221/F'LacI^q carrying the various micRNA plasmids (Table 1). In the case of the single micRNA genes, *micB* and *micA* appear to be more inhibitory to SP in the presence of IPTG than *micC*. Clear gene dosage effects are observed in the presence of two *micA* genes, and when the *micA* gene plus the *micB* gene are present. The *micC* gene in either the single or double configuration also shows inhibition of phage production although to a lesser extent than *micA* or *micB*. It should be noted that the *micC* RNA does not encompass the Shine-Dalgarno sequence or the initiation codon of replicase mRNA. This micRNA may prevent replicase from transcribing the positive RNA strand to produce the negative RNA strand, by forming a hybrid at the 3′ end of the phage RNA.

We have demonstrated that micRNAs effectively block the proliferation of a specific virus. On the basis of our previous findings[7], the micRNAs block the expression of specific SP phage genes by inhibiting the translation of these genes. It is important to note that the micRNA immune system is highly specific; the cells harbouring the micRNA genes directed against phage SP are resistant to phage SP, but not to phage GA or Qβ. In particular, cells were not resistant to phage Qβ despite its extensive homology to phage SP (Y.I. and A.H., in preparation). The high specificity of the micRNA immune system is important for its application in preventing viral infection and the expression of harmful or toxic genes in eukaryotic cells.

Combined with existing technology, the present report provides a promising prospect for the application of the micRNA immune system to both plant and animal cells. It is now feasible to establish transgenic animals carrying a micRNA immune system(s) directed against a specific virus (such as poliovirus or foot-and-mouth disease virus, which are positive, single-stranded RNA viruses), by using micRNA genes that are stably integrated into the desired host genome. Similarly, micRNA immune systems directed against plant viruses can be introduced into the genomes of plant protoplasts which can subsequently be regenerated into mature fertile plants. Also, artificial micRNA repressors have great potential for use in the functional characterization of unknown genes, the repression of harmful genes such as oncogenes, and altered regulation of developmental genes.

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Note added in proof: Melton[14] has recently shown that complementary RNA injected into frog oocytes will block translation of the specific RNA *in vivo*. In addition, Rosenberg *et al.*[15] have recently shown that when RNA complementary to the *Krüppel* gene is injected into *Drosophila* embryos, a *Krüppel* phenocopy is produced. Furthermore, Izant and Weintraub[16] have demonstrated that a micRNA gene can be inducibly expressed to function in mouse L-cells. Also, Pestha *et al.*[17] have demonstrated in *E. coli* the production of β-galactosidase can be inhibited by the production of a complementary RNA.

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High-resolution structure of a DNA helix containing mismatched base pairs

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The concept of complementary base pairing, integral to the double-helical structure of DNA, provides an effective and elegant mechanism for the faithful transmission of genetic information[1,2]. Implicit in this model, however, is the potential for incorporating non-complementary base pairs (mismatches) during replication or subsequently, for example, during genetic recombination[3,4]. As such errors are usually damaging to the organism, they are generally detected and repaired. Occasionally, however, the propagation of erroneous copies of the genome confers a selective advantage, leading to genetic variation and evolutionary change. An understanding of the nature of base-pair mismatches at a molecular level, and the effect of incorporation of such errors on the secondary structure of DNA is thus of fundamental importance. We now report the first single-crystal X-ray analysis of a DNA fragment, d(GGGGCTCC), which contains two non-complementary G·T base pairs, and discuss the implications of the results for the *in vivo* recognition of base-pair mismatches.

Two principal schemes have been proposed for hydrogen bonding between non-complementary bases. One of these schemes[2] involves minor tautomeric forms of the bases (Fig. 1*a*, *b*); the resultant base pairs are stereochemically indistinguishable from complementary Watson–Crick base pairs (Fig. 1*c*, *d*). The estimated frequencies of minor tautomers correlate well with the observed frequencies of substitution mutations[5] but there is, to date, no direct structural evidence for such base pairs. In the second scheme[6], both bases are in the major tautomer form, but use different functional groups for hydrogen bonding than Watson–Crick base pairs (Fig. 1*e*, *f*). 'Wobble' base pairs of this type have been observed in various transfer RNA crystal structures[7–11] and in recent nuclear magnetic resonance solution studies of deoxyoligonucleotides[12,13] and polymers[14]. Mis-insertion of bases in their major tautomer form during replication is indicated by kinetic experiments with DNA polymerase I (ref. 15). The energetics of mismatched base pairs have been studied theoretically[16,17].

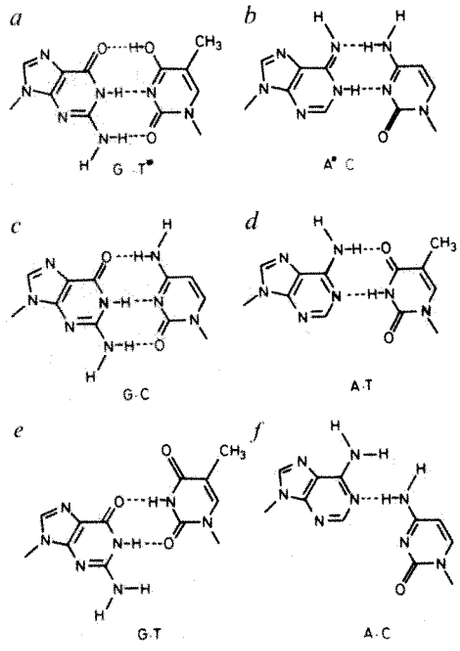


Fig. 1 Schematic diagram of various postulated base-pairing schemes. *a*, *b*, Tautomer pairs; *c*, *d*, Watson–Crick base pairs; *e*, *f*, wobble base pairs. Bases in their rare tautomer (imino/enol) forms are marked by asterisks.

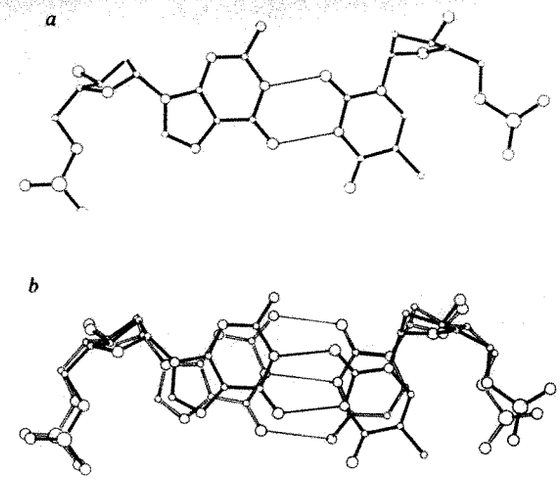


Fig. 2 *a*, The G·T wobble base pair observed in the crystal structure of d(GGGGCTCC). *b*, Superposition of the G·T wobble base pair observed in the crystal structure (dark bonds) on an ideal G·C pair. The figure was constructed by calculating the best molecular fit of the refined structure of d(GGGGCTCC) with a model of d(GGGGCCCC) derived from A-DNA fibre coordinates[24].

Detailed structural information about complementary base pairs is available from single-crystal X-ray diffraction studies of synthetic deoxyoligonucleotides[18–20]. We have now extended this technique to mismatched base pairs by modifying the sequences synthesized so as to include non-complementary bases. The sequence d(GGGGCTCC) is a variant on two other A-DNA type octamers already investigated, d(GGTATACC)[21] and d(GGGGCCCC)[22]. It was prepared by solid-phase triester methods[23] and crystallized from a buffered 3 mM solution of DNA (*p*H 6.5) with 28 mM $MgCl_2$, kept at 4 °C. The hexagonal crystals, space group $P6_1$, $Z = 1$ octamer duplex, had cell dimensions of $a = b = 45.20$ Å, $c = 42.97$ Å, close to those of the parent octamers. Diffractometer measurements at 2 °C yielded 2,414 unique reflections.

A molecular model of d(GGGGCTCC) was constructed, using fibre coordinates for A-DNA[24]. The G and T bases were related as in the minor tautomer pair of Fig. 1*a*. The coordinates of this model, after best molecular fit to d(GGGGCCCC), were the starting point for the refinement by constrained/restrained least-squares techniques. During the refinement no restraints were imposed on either the hydrogen bonding between the G and T bases or the sugar conformation. The present report describes the structure at the convergence of the CORELS[25] refinement at $R = 19.4\%$ for the 1,954 reflections with $F > 2\sigma(F_o)$. Further refinement by the Hendrickson–Konnert[26] technique reduced the R factor to 14.5% and allowed the localization of 56 water molecules, but did not result in any significant changes in the DNA structure. Full details will be reported elsewhere.

During the refinement the largest changes occurred at the positions of the G·T base pairs. The initial 2-fold molecular symmetry was, however, largely retained, as was the geometry of the six G·C base pairs. The bases in the mismatch pairs shifted substantially from their original positions, bringing the keto O-2 and the imino N-3 atoms of thymine within hydrogen-bonding distances of the imino N-1 and keto O-6 atoms of guanine. The refined structure shows unambiguously that both

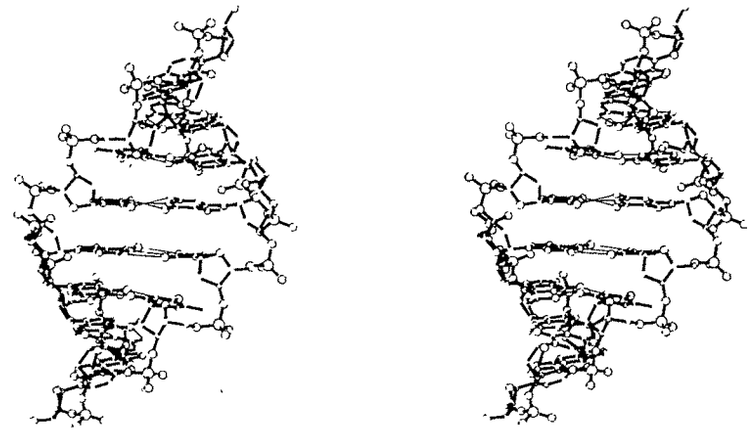


Fig. 3 Stereoscopic view of d(GGGGCTCC) looking into the minor groove. The G·T wobble pairs lie on either side of the two central G C pairs

mismatches are of the wobble type with the bases in the major tautomeric forms (Fig. 2*a*), and gives the first view of a base-pair mismatch in the crystal structure of a DNA fragment. The geometry of the hydrogen bonds linking the bases is good with N···O distances of 2.81 Å and 2.85 Å in the first, and 2.50 Å and 2.66 Å in the second wobble pair.

Comparison of the G·T wobble pair with a Watson-Crick G·C base pair (Fig. 2*b*) indicates that the mismatch can be accommodated in the double helix without appreciable perturbation of the backbone. The sugar conformation remains C-3′ *endo*. The C-1—C-1′ distance is 10.2 Å, close to that observed in G·C pairs.

The greater part of the lateral displacement of the bases is the movement of the thymine into the major groove with root mean square (r.m.s.) displacements of 0.74 Å (for G) and 1.25 Å (for T) (Fig. 2*b*). As a result, the angles between the glycosidic bonds and the C-1′—C-1′ vector, which are symmetrical in Watson-Crick pairs (54-58°)[27], assume values of 70° for T and 41° for G averaged over the two G·T pairs. The free keto O-4 atom of thymine now protrudes into the major groove and the N-2 amino group of guanine into the minor groove; these are available for hydrogen bonding to either solvent molecules or neighbouring double helices. The presence of the two G·T base pairs does not appreciably affect the overall conformation of the A-DNA helix (Fig. 3). The average helical parameters and torsion angles (Table 1) compare well with the corresponding values for the two isomorphous structures. The packing of the duplexes in the crystal structure is similar to that in other A-type structures[18].

Stacking interactions between adjacent base pairs have a major influence on the conformational stability of double-stranded DNA, and have been shown to give rise to sequence-dependent conformational effects[28,29]. Figure 4 compares the base-stacking overlaps in d(GGGGCTCC) with the equivalent steps of A-DNA from fibre data. The most striking change is the improvement of the stacking at the wobble pairs on the 5′ side of the thymine as a consequence of its displacement into the major groove (Fig. 4*c*). On the 3′ side of the thymine there is virtually no overlap between the pyrimidines (Fig. 4*b*). This trend has also been observed for G·U base pairs in tRNA[11]. The stacking of the purines on either side of the mismatch appears to be unaffected.

The structural results derived from the present investigation may have implications for the fidelity of replication and the enzymatic recognition of DNA containing a base pair mismatch. These results can be considered at four levels: the tautomeric form of the bases; local effects, such as base stacking in the neighbourhood of the mismatch; the influence of the base mispairing on the global conformation of the double helix; and the disposition of structural elements, particularly hydrogen donors and acceptors, in a mismatched base pair.

Fidelity of replication is maintained by an elaborate and as yet only partially understood mechanism involving specificity of base selection at the insertion step, proofreading (excision of incorrect bases) and post-replicative mismatch repair[30]. Biochemical experiments show that non-complementary bases in their major tautomer forms can be incorporated during replication. Fersht and co-workers have found this to be the case for purine-pyrimidine mismatches by kinetic analysis of DNA polymerase I mis-insertion rates[15]. The wobble base pair found in the crystal structure examined here may be a good model for G·T mis-insertions.

There is good evidence that mis-insertion frequencies *in vitro* are dependent on the DNA sequence and that this may be a consequence of base stacking effects at the site of the mismatch[31]. The present investigation, based as it is on experimental data from a specific sequence, gives precise information about local changes in base stacking; it shows in particular an improvement in pyrimidine-pyrimidine stacking on the 5′ side of the thymine base of the G·T wobble pair.

Table 1 Overall helical parameters and mean torsional angles for A-DNA structures

	d(GGGGCTCC)	d(GGGGCCCC)	d(GGTATACC)	Fibre model
Twist per b p.	33 4°	31.6°	32.3°	32 7°
Base pairs per turn	10.8	11 4	11.1	11.0
Rise per b.p (Å)	3 0	2.9	2.9	2 6
Base tilt	11°	13°	15°	19°
Propeller twist	15°	10°	13°	Variable
α	−81	−76	−62	−50
β	177	178	173	172
γ	65	62	52	41
δ	76	84	88	79
ε	−136	−156	−152	−146
ζ	−68	−70	−78	−78
χ	−163	−158	−160	−154

Helical parameters are as defined by Fratini *et al*[41]. bp, Base pair The glycosidic torsion angle is defined by the atoms O-1′—C-1′—N-1′—C-2 (pyrimidine)or O-1′—C-1′—N-9—C-4 (purine). Backbone torsional angles are defined by $P\overset{\alpha}{-}O\text{-}5'\overset{\beta}{-}C\text{-}5'\overset{\gamma}{-}C\text{-}4'\overset{\delta}{-}C\text{-}3'\overset{\varepsilon}{-}O\text{-}3'\overset{\zeta}{-}P$

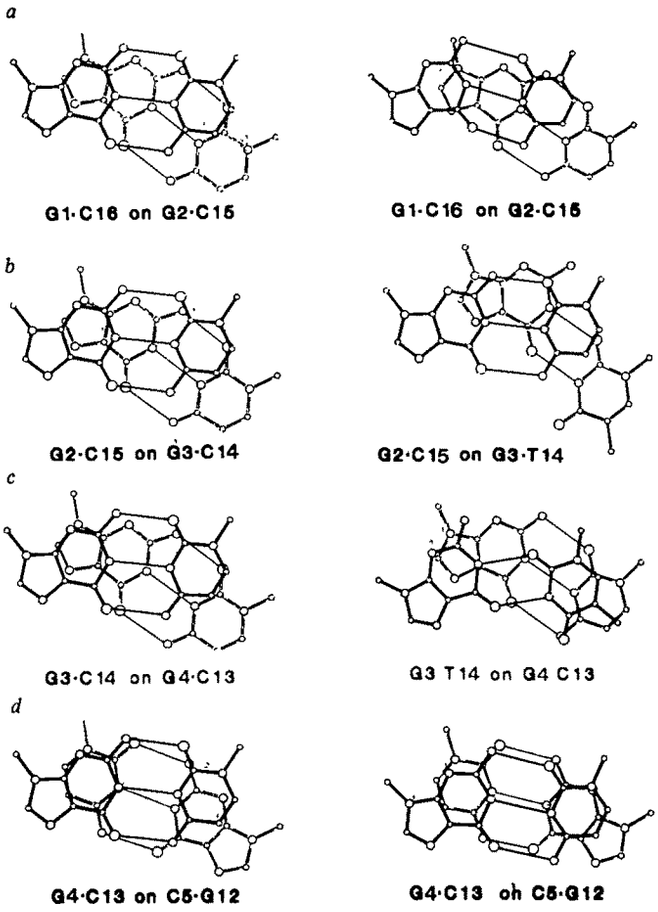


Fig. 4 Projected view of four steps of d(GGGGCTCC) (right) compared with equivalent steps derived from the A-DNA fibre model of poly(dG)·poly(dC) (left). The view is perpendicular to the mean plane through the upper base pairs and is in the 5′ to 3′ direction of strand (G-1-G-4) The upper and lower base pairs are differentiated by dark and light bonds, respectively. Only one half of the octamer is illustrated as the pattern of base stacking is symmetrical about the pseudo 2-fold axis. Note the similarity of the base stacking in the structures at the end step (*a*) and the centre step (*d*). At the site of the mismatch, the stacking of T on C is improved compared with C on C in the fibre (*c*) but the stacking of C on T (*b*) is unaffected.

Once the G·T wobble pair is incorporated in the double helix, it causes little perturbation to the global conformation. Nevertheless, it is discriminated against at various stages both during and after replication. The role of DNA polymerase in the recognition of incorrect base pairs is indicated by the variation of *in vitro* mutation frequencies with the nature of the polymerase[32–35].

DNA polymerase is thought to have only one active site for synthesis, which is able to accommodate all four Watson-Crick base pairs[36]. These base pairs have, as a characteristic structural feature, a pseudo 2-fold axis in the plane of the base pair perpendicular to the C-1′—C-1′ vector, resulting in symmetrical disposition of the glycosidic bonds (Fig. 1*c*, *d*). As a consequence of this symmetry, the positions of the N-3 of guanine, the O-2 of cytosine, the N-3 of adenine, and the O-2 of thymine are approximately equivalent. These atoms are all potential hydrogen acceptors.

The X-ray analysis shows that G·T wobble base pairs in d(GGGGCTCC) lack diad symmetry. The guanine N-3 and thymine O-2 atoms are displaced from their positions in standard Watson-Crick base pairs and are no longer equivalent. The location of these functional groups may be an important factor in enzymatic recognition. The positions of other atoms in the G·T wobble pair also change. The movement of the O-4 and the 5-methyl groups of thymine into the major groove may result in steric hindrance at the active site. Despite these differences, some major tautomer mismatches escape detection during *in vitro* DNA replication, possibly because of the lack of base specificity of DNA polymerase. However, at the post-replicative stage there may be a more specific recognition system with separate enzymes or active sites for the different mismatched pairs[37].

The present investigation gives high-resolution structural information about a G·T mismatch in a DNA sequence which crystallizes as an A-type helix. There is a need to examine base pair mismatches in all known DNA helices as there is accumulating evidence for conformational flexibility of *in vivo* stretches of DNA[38–41]. We have recently solved, and are currently refining, other crystal structures and find that the G·T wobble pair, with the same hydrogen-bonding pattern, also occurs in a Z-DNA fragment d(TGCGCG) ($R = 24\%$), in a B-DNA dodecamer d(CGCGAATTTGCG ($R = 23\%$), and in another A-type octamer, d(GGGGTCCC) ($R = 15\%$). The results of these studies will be reported elsewhere. Our current investigations establish the feasibility of introducing errors into DNA sequences known to crystallize as A, B or Z helices, opening up the possibility of studying a variety of base pair mismatches and modified bases in different environments. This approach may lead to a better understanding, at the molecular level, of departures from complementary base pairing in the double helix.

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The future lies ahead

Gunther S. Stent

Solid Clues: Quantum Physics, Molecular Biology, and the Future of Science.
By Gerald Feinberg.
Simon & Schuster: 1985. Pp.287. $17.95. To be published in Britain later this year by Heinemann.

IN THE Introduction to *Solid Clues*, Gerald Feinberg observes that 40 years ago no one predicted the enormous expansion of science then imminent. This unpredicted expansion included also the rise of "futurology", the very discipline dedicated to correcting our generally myopic view of the future. In contrast to old-time, apocalyptic or millennarian prophecy, which foretold the future on the basis of inspired visions in terms of value-laden, prescriptive proclamations, the predictions of futurology are phrased as rational arguments, based on some theory of the dynamics of history, and take the form of value-free and descriptive propositions.

Feinberg's avowed purpose is to predict the changes that will take place over the next few decades in the content of science and the lives of scientists. He concentrates his attention on physics and biology, the two branches of contemporary science which he finds to be progressing most rapidly and likely to produce the most important discoveries. Fortunately, as a professor of physics at Columbia University and having read some books and articles on contemporary biology, Feinberg is able to assess the present status of these two vanguard disciplines. Unfortunately, he seems to lack the grounding in the philosophies of science and history needed by the would-be futurologist, as well as an appreciation of what counts as a rational argument in the social sciences (rather than a mere assertion) and as a significant prediction (rather than a futurological platitude).

Feinberg will surprise few readers with his disclosure that one of "the main engines of change in science, the forces that act to change science from within and from the outside" is the development and application of new experimental, observational and mathematical techniques. Equally unsurprising is his revelation of the public attitude to science, reflected by the provision (or withholding) of financial support and the encouragement (or proscription) of certain topics of research, as another such main engine (or brake). So, how, being driven by these engines, is science going to advance in the future? Well, it will answer some old questions and discard others, with some of the answers to old questions in turn raising new questions. Is science going to progress indefinitely? Yes and no: "in some areas of science, new answers may bring us close to exhausting fundamental discoveries in the field . . . [whereas] other fields, such as cosmology, are more open-ended". Feinberg lists some of the currently unsolved problems of cosmology and suggests novel observational techniques whose development would open up entirely new cosmological vistas. But he does not present any explicit argument which shows why cosmology is open-ended.

For the future of biology, Feinberg seems safe in predicting that its "main work is to find answers to many of the open questions about living things". He believes that the origin of life, metazoan development — from zygote to cadaver — and the nervous system loom large among such questions. He predicts, moreover, that the incipient development of X-ray laser holography will advance the frontiers of biology as far as did the invention of the optical microscope in the seventeenth century. Whether there are any open-ended areas of biology, however, Feinberg does not say, nor does he trouble to justify his rather doubtful prediction that "biology will become more like the physical sciences, reasoning from general principles to specific cases".

Feinberg thinks that "of all the changes that have taken place in recent years in how science is done, none is more fundamental and sweeping than the use of computers . . . computers are now launching science into a new age". This phenomenon is attributable not merely to the capacity of computers to manage vast amounts of data, such as those which accumulate in particle physics experiments carried out in high-energy accelerators or in molecular biological analyses of DNA and protein sequences. No, we are on our way to a new age because "one of the most intriguing possibilities is that computers will someday be developed that equal or even surpass human capabilities for creating new scientific ideas". Feinberg cites Herbert Simon's "Bacon" program of scientific induction as a first stage on the trip to the new age, apparently unaware that the inductive Baconian model of scientific discovery failed, not because, as he claims, "there was no straightforward method of finding pattern in the data", but because of the need to make an intuitive preselection of the kind of data in which a significant pattern is to be sought in the first place. (In the case of Simon's program, the computer is provided with such preselected data as paired values of force and acceleration and, finding that they vary in direct proportion, the computer "discovers" Newton's Law.) The well-known epistemological critique of claims on behalf of creatively "intelligent" machines notwithstanding, Feinberg imagines "a future in which in the early training of a scientist the computer will act as a teacher, and later as a research collaborator".

What about technological advances? Feinberg points to "nanoengineering", or direct physical manipulation of molecules or atoms, which will allow "the construction of objects of a previously unimagined complexity" and the production of novel materials whose strength exceeds that of presently available materials by factors of many thousands. And then there is, of course, the new "biotechnology", including genetic engineering, for which Feinberg is not alone in anticipating profound effects on the human condition, especially in so far as modifying the natural process of ageing is concerned.

Feinberg concludes his essay with a

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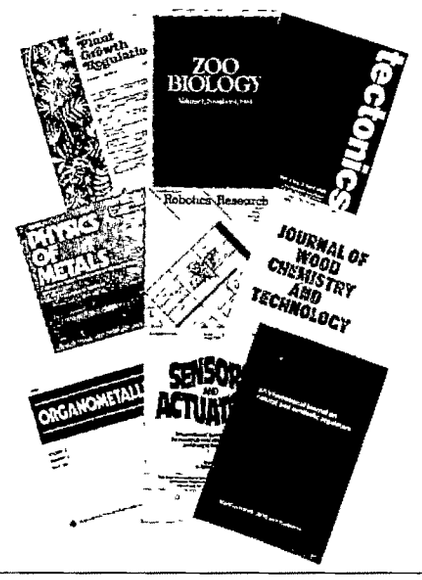
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(ii) it is published at least three times a year;

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discussion of two philosophical issues. One is the place of science in the world; here, more prophet than futurologist, Feinberg strikes a righteous pose. He offers dogmatic pronouncements about a wide range of religious, ethical, political and sociological topics, which reflect mainly the unsophisticated views of the typical scientist-in-the-street. The other issue concerns the change in the nature of scientific explanation and the possible limits to understanding. Thanks to his professional background in modern physics, Feinberg has a much better grasp of this matter. He shows how the rise of the quantum theory has eroded some of the basic conceptual ingredients of explanation — foremost among them cause and effect. Moreover, many of the unsolved problems in both physics and biology pertain to highly complex systems in which tiny fluctuations in one variable have huge effects on other variables. The "chaotic" behaviour of such systems limits the degree to which we can hope to fathom them. Thus, as Feinberg points out to his regret, "future science may see more restrictions on what we are able to understand and predict". Also, because of "the immense expansion of scientific information . . . scientists can learn an ever smaller part of what is known and can concern themselves with an ever narrower segment of it . . . . Science is thus in some danger of losing its character as a cumulative body of knowledge". Despite his regret over these limits, Feinberg ends on an upbeat note, befitting his smiling, *simpatico* portrait shown on the dust jacket: "Present and future discoveries in science can transform human life. All of these represent bright prospects of future science".

It may be mean to cavil at this well-meaning essay by a scientist whose interests and concerns clearly transcend his research specialization. But, all the same, it would be negligent for a reviewer not to point out that, just as in the "hard" natural sciences, there are also professional standards in the "soft" social sciences, and that, just as in particle physics, in futurology triviality is even more grievous than error. □

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At the interface

D.H. Everett

Adsorption and the Gibbs Surface Excess.
By D. K. Chattoraj and K. S. Birdi.
Plenum: 1985. Pp.471.
$71.40, £56.53.

IN THIS book Chattoraj and Birdi have set out to demonstrate the central role played by the Gibbs surface excess in describing adsorption phenomena in systems involving liquid–vapour, liquid–liquid, solid–vapour and solid–liquid interfaces, and in biological structures. This attempt to present a unified approach to a wide range of phenomena is to be welcomed. Unfortunately, the treatment leaves much to be desired.

The arguments employed often lack the rigour and self-consistency essential in discussions of thermodynamics. Thus, in the introductory chapter, the surface tension is introduced simply as "surface energy", while in considering the equilibrium between bulk and surface phases the role of surface tension is ignored. Later, in Chapters 3 and 5, correct derivations of the equilibrium condition are given and the surface tension term is now included. In the earlier treatment the surface tension term is apparently hidden in the surface activity coefficient, as perhaps implied in the unhelpful comment that "the standard scale for the surface activity coefficient is uncertain". Elsewhere different definitions of surface activity coefficients are introduced: that they are different quantities is suggested by the comment that one must "use the appropriate reference scale". Which? Also puzzling is this statement:

> In the derivation of the Gibbs adsorption equation the contribution of the β-phase to the interfacial phase is implicitly neglected. The surface activity coefficients thus evaluated *in all probability* [my italics] include this contribution.

Words such as "in all probability" are unacceptable in a thermodynamic argument.

Description of the Gibbs method is, surprisingly, deferred until Chapter 3: it should have been in Chapter 1. The formal definition is correct, but its status is obscured by the comment that "this approach is extrathermodynamic but no mathematical error is involved in the postulate". There is nothing extra-thermodynamic in Gibbs's treatment: what it does is to provide a means of quantifying the macroscopic stoichiometric effect of the inhomogeneous distribution of matter in the interface. At no stage did Gibbs "imagine" that the "moles of solvent present in the interfacial region is zero". His definition is independent of any molecular model, a feature which gives it its power, usefulness and wide applicability.

The book is illustrated by a substantial body of experimental material drawn from a variety of systems, much of it originating from the authors' laboratories. Overall, one has considerable sympathy with Chattoraj and Birdi's objectives, which are to some extent achieved. But it is unfortunate that to get the best out of the book the reader has to be constantly on the alert to avoid being misled. □

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Breeding resources

A.D. Bradshaw

Crop Genetic Resources: Conservation and Evaluation.
Edited by J.H.W. Holden and J.T. Williams.
George Allen & Unwin: 1984. Pp.296. Hbk £25, $40; pbk £9.95, $14.95.

THE key to this book is on p.212. In a well-documented review amongst a group of similar papers, J.R. Harlan assesses the value of wild relatives of crop plants in modern plant breeding. If anybody thought that we could do without all this wild material, here is the corrective — a sober account of the improvements in ecological tolerance, disease and pest resistance, quality, yield and even modes of reproduction which have come from relatives of present crop plants. But at the same time Harlan illustrates the consequent dilemma: because this potentially valuable material is scattered through innumerable species around the world, it is almost impossible to know where the next important source will be.

This is of course only half the story. The enormous losses of natural and semi-natural environments and the increasingly widespread use of new crop varieties mean that this wealth of genetic material is rapidly being depleted. Landmarks in the growth of concern were two FAO/IBP conferences (1968 and 1973) on the conservation of crop genetic resources. Also in 1973, the International Board for Plant Genetic Resources was formed to bring some international organization into the many efforts which were then starting, including the international network of regional centres for crop research and genetic conservation.

Ten years have now passed. In the book there are 24 papers reviewing the progress that has been made, each written by people who are eminent in their field. It is their undoubted experience which leaves a feeling of great unease about the magnitude of the task ahead.

It is easy to define some of the difficulties. In the first part of the book, on seed storage, Roberts and Ellis show elegantly the progress that has been made in quantifying deterioration by predictive equations, and Hanson how we are becoming able to store even some recalcitrant seeds. Ellis and Roberts discuss programmes for monitoring deterioration, advocating important new systems of sequential probability ratio tests. But while the International Rice Research Institute had 25,600 accessions in 1974, it now has more than 50,000. Despite improvements, the problem remains enormous.

The second group of papers deals with the capture and retention of variability. Gale and Lawrence consider how to overcome the problems of the decay of variability in conserved material, while

Chapman discusses the size of seed banks. This should be a crucial paper but few people will be convinced that the problem can be resolved on the basis that the variability in which plant breeders are interested is distributed at random; indeed several of the other contributions indicate that it is not, particularly because of the local origin of variation and the impact of local selection. The assessment by Singh and Williams is much more pragmatic.

Progress in *in vitro* preservation is covered by Withers; the problems of preservation of wild species *in situ* by various forms of nature reserves are assessed judiciously by Ingram and Williams; and there is a series of excellent papers on the evaluation of what has been collected. Apart from Harlan's review, we are left with the overwhelming picture that we can conserve anything if we have the money, but that we are drowning in the twin problems of what to conserve and, once we have conserved it, how to use it. Papers on the fashionable new technique of some clonal variation and recombinant DNA suggest that this is where the future lies. I doubt it.

In his final paper, Holden argues that orthodoxy allied to critical commonsense is the real way forward. We shall need further collections, but now they must be only of threatened material and to cover particular deficiencies, and must be on an international basis: a very sensible end to a valuable and provocative book. □

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Mixed selection

Richard G. Klein

Hominid Evolution and Community Ecology: Prehistoric Human Adaptation in Biological Perspective.
Edited by Robert Foley.
Academic: 1984. Pp.296. $37.50, £24.

WHILE the layman may be fascinated simply by the great age of ancient bones and artefacts, palaeoanthropologists value them mainly as data by which to follow the course of human biological and cultural evolution. Such objects are often ambiguous in their implications, and using them to construct a truly compelling and comprehensive story remains a difficult task. In large part, the problem is the actual quantity and quality of bones, artefacts and other real data, which are often distressingly sparse. In part, it is also a matter of the need for more powerful analytical frameworks, based largely on present processes that must also have operated in the past.

One framework that nearly all palaeoanthropologists use, at least implicitly, centres on the concept of natural selection and its ramifications, that is evolutionary theory. Robert Foley, the editor of this book, believes that palaeoanthropologists should apply evolutionary theory more explicitly, especially the idea of coevolution, according to which members of an ecological community evolve at least partly in response to evolutionary changes in other members. Thus, for example, as predators become fleeter of foot, natural selection will tend to favour fleeter prey, and fleeter prey will in turn lead to selection for fleeter predators.

To explore the implications of ecological thinking for palaeoanthropology, in 1981 Foley organized a symposium from which the present book grew. Six of the contributors attended the symposium and four did not, but each was asked to write a chapter illustrating the role of ecological concepts in understanding human evolution. Perhaps not surprisingly, Foley's own contributions come closest to the mark. In the first of his two chapters, he succinctly outlines those aspects of evolutionary theory he thinks palaeoanthropologists will find most pertinent. In the second he discusses the variables — mode of locomotion, body size, population density, home range size, group size, diet, social organization, environment and so forth — that are clearly crucial for reconstructing the ecology of very early people. Unfortunately, most of the variables are very poorly controlled with real data, and Foley's ecological reconstruction is necessarily vague and inconclusive.

Among the other authors, perhaps the one who took Foley's brief most seriously was Garrard, who suggests that the extinction of some Pleistocene mammals in south-west Asia may have been caused by "human interspecific competition", by which he means competition between people and animals for the same resources. This is an interesting possibility, but Garrard presents no real supporting evidence, and his treatment of the broader issue of extinctions is very weak, despite its obvious relevance to the book's main purpose.

Two other contributors who deal reasonably closely with Foley's stated theme are Turner and Gamble. In an essay that is stimulating and thoughtful, but ultimately unconvincing, Turner argues that the Middle and Upper Pleistocene dispersal of human beings across Eurasia and into the Americas can be illuminated by an analysis of the dispersal of other large mammals, especially predators. In a stylistically similar essay dominated by highly readable argumentation rather than empirical demonstration, Gamble suggests that differences in available energy can be used to explain regional variation in the Palaeolithic archaeological record of Europe.

Some of the remaining chapters are closer to the book's theme than others, but none deal with it directly. Potts concludes that early people probably accumulated most of the bones at some sites in Olduvai Bed I, though the sites need not have been "home bases" in the sense of modern hunter-gatherer camp sites. To support his conclusion, Potts cites the presence of numerous stone artefacts, as well as features of the bone assemblages that are probably due to people rather than to carnivores or other "natural" agencies.

Scott deals clearly and succinctly with the evidence for human occupation of Britain in the second half of the Last Glacial period. Conditions were obviously extremely harsh and, compared to the situation in many parts of western Europe, people appear to have been rare. Hill touches on the question of how we might distinguish bone accumulations created by hyenas from those created by people, but he focuses mainly on the scientific-philosophical nature of explanation or interpretation in palaeoanthropology. Although it might not have been Hill's intention, it could be concluded that he believes the interpretation of Plio-Pleistocene bone assemblages is something we can write about but not actually do.

Gowlett sees stone artefacts, humanly butchered animal bones and other archaeological traces as reflections of the existence of human mental abilities from at least two million years ago. He may also believe that natural selection sponsored progressive change through time in these abilities, but from his presentation one cannot be sure.

Finally, Roberts provides a clear, up-to-date and comprehensive overview of Pleistocene climatic change, particularly as it is known from deep-sea cores, and Stringer surveys the human fossil record, stressing broad issues such as the mode of human evolution (whether gradual or punctuated) and the possible importance of climate as a selective factor on body form in fossil people. As independent contributions in their respective fields, Roberts's and Stringer's chapters are among the best in the book, but they are also among the furthest from the central theme.

Sadly the book does not demonstrate the value of specific ecological concepts for understanding human evolution, if only because most of the authors fail to address the issue directly. Beyond lacking a common focus, the chapters also vary considerably in quality, and I think it can reasonably be asked whether they should have been published together. There is a great deal in the book to interest any palaeoanthropologist, but there is also much that would have been winnowed out or improved if the papers had been subjected to the refereeing process used by most specialist journals. □

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Recipes for success in the laboratory

Richard Perham

Methods in Molecular Biology, Vols 1 and 2.
Edited by John M. Walker.
Humana/Wiley: 1984/1985. Each volume pp.370, $45, £41.

A CYNICAL older colleague once advised me that any successful academic would turn out to be one-armed, for only then would he be prevented from saying "On the one hand, this; on the other hand, that". By the same token, the author of a book on methods is confronted with a comparable problem; do I tell my readers about all the different ways to carry out this or that technique or do I limit myself to one which, from personal experience, I know works? Any practising molecular biologist will be aware that the same experimental technique is often carried out differently in different laboratories. The differences may be major, they may be minor: in some instances they will actually matter.

The contributors to these two volumes have, for the most part, opted for the second course. Each method selected for description receives an account replete with experimental detail, taken presumably from the author's laboratory notebooks. There are few caveats, even fewer descriptions of variations or alternatives. There are, of course, further references given for those interested in pursuing them and sometimes a range of useful hints on trouble-shooting. Volume 1 deals with proteins, Vol. 2 with nucleic acids. I cannot speak with authority on every method listed — the net has been cast very wide — but I could find no serious fault with the recipes for those methods I know well.

I do have some reservations. For example, Vol. 1 has 38 chapters covering techniques from the Lowry method of protein estimation to manual microsequencing, from polyacrylamide gel electrophoresis to hybridoma production. Some room might have been found for the chemical modification of proteins and the methods, enzymic and chemical, of peptide bond cleavage. Even something as basic as the *S*-carboxymethylation of proteins is tucked away in a description of peptide separation by hplc. On the other hand, automated sequencing is wisely eschewed, given the requirement for unusually sophisticated and expensive apparatus.

The second volume is, if anything, more comprehensive, the 53 chapters starting with DNA assay and isolation and ending with DNA sequencing. In between it covers mRNA, cell-free translation, plasmids, cloning in bacteriophage lambda and a host of other things. It was therefore disappointing to find, for example, no description of nucleic acid hybridization directly in dried agarose gels or of the chemical synthesis of oligonucleotide probes.

But these are quibbles. Molecular biology now permeates so much of present research that a working knowledge of its methods can be essential; it is at once the queen and handmaiden of the biological sciences. The editor tells us that the intention was to collect descriptions of methods that would definitely work, particularly in the hands of novices, and I fancy that these books will rightly be welcomed in such quarters. They will not replace the fuller, more discursive treatments given by the originators of these techniques in, say, *Methods in Enzymology*. Nor perhaps will they have the impact of Cold Spring Harbor's celebrated *Molecular Cloning: A Laboratory Manual* by Maniatis *et al.* (now sadly getting out of date) which informed so many beginners in their first fumbling steps in recombinant DNA work some three years ago. However, if, as intended, these books enable those of stout heart but limited experience to gain entry to the growing battery of molecular biological methods, they will have served a useful purpose. □

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Fields of physics

Graham G. Ross

Quantum Field Theory.
By F. Mandl and G. Shaw.
Wiley: 1984. Pp.354. Hbk £38, $34.95; pbk £19, $24.95.

CONSIDERING the importance of the subject, there has been a remarkable shortage of textbooks available for the student of field theory. For many years there were no significant texts to follow the pioneering, and excellent, accounts of Bjorken and Drell, and Schweber, and a very nice short book by Mandl. The situation has improved lately with the encyclopaedic work by Itzykson and Zuber, a very readable text by Ramond covering the path integral formalism and many others. It is in this context that Mandl and Shaw's *Quantum Field Theory* appears.

A pleasing feature of the book is that it is accessible to the new student, starting immediately with the Lagrangian formalism, and giving a detailed and careful treatment of quantum electrodynamics (QED). I liked the emphasis on performing explicit perturbative calculations and the implementation of the renormalization programme, although I was disappointed that the renormalization group is not discussed. Throughout, the text is augmented with worked examples and problems. It thus provides a welcome update of earlier textbooks on electrodynamics; it is more straightforward than Bjorken and Drell, more detailed than another recent introduction to the subject by Halzen and Martin, and should give both the experimental and theoretical student a good working knowledge of QED.

In its treatment of developments beyond QED the book has some shortcomings. There is no discussion of QCD, the non-Abelian gauge field theory for the strong interactions, and only a relatively short account of the gauge theory for the electroweak interactions. Indeed the concept of gauge invariance, central to modern developments in field theory, is only developed in Chapter 12 and the path integral formalism, which simplifies the analysis of spontaneously broken theories, is not covered. As a result the discussion of the electroweak theory is disappointing, particularly as it does not present a calculation in a spontaneously broken theory beyond the leading order, nor cover the renormalization in such theories. This renders the book less useful than it might have been for it is in this area that much progress has been made which is not yet adequately covered for the novice graduate student.

Mandl and Shaw have produced a sound introduction to the subject of Abelian gauge field theories which should also be useful as a reference text for perturbative calculations. However it provides only part of a modern introductory course and should be read together with another text, for example that of Ramond, which deals with the advances beyond QED. □

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● After writing this review I received a second text on the same subject, *Quantum Field Theory* by Lewis H. Ryder (Cambridge University Press; £40, $74.50). The similarity to Mandl and Shaw ends with the title, for Ryder provides an introduction to many of the recent advances in field theory, using the path integral formalism to derive Feynman rules for spontaneously broken non-Abelian gauge theories and including a discussion of some of the topological properties and solutions of these theories. The approach differs too, with emphasis on the more formal aspects, no problems and few worked examples. The book is quite readable and is a good introductory text suitable for a theory student, but is probably best read after Ramond's book. In many ways it complements Mandl and Shaw, although the lack of worked examples will be hindrance to the student wanting a full understanding of the subject, and again there is no explicit calculation presented for a spontaneously broken gauge theory. G.G.R.

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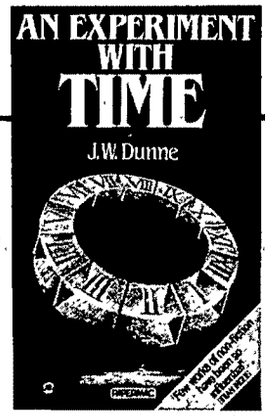
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For the technically minded

Disk 1 contains the boot programs which. (1) modify resident Applesoft BASIC to accept STATSTREAM commands The commands include procedure calls (USE NAME will invoke the named procedure, automatically overlaying it from disk if necessary), IF–THEN–ELSE–ENDIF, REPEAT–UNTIL, etc (2) Permit up to 5 text windows The machine code snaps windows open and shut The window command can be invoked from your own BASIC STATSTREAM program (3) Shape tables to assist graphics.

Plus 32 example programs and the Statpack which is based on STATSTREAM commands which, in this mode, are each called with a single keystroke

Disk 2 contains the library of STATSTREAM commands When a command is used, the appropriate procedure is automatically loaded into memory, executed and forgotten Commands can be executed immediately or included in BASIC programs For example all that is required to retrieve a STATSTREAM data file is

```
]USE MOVE,1,1,1,1
```

The parameters specify the left-most and right-most columns and top-most and bottom-most rows In this case they are dummies because the data file itself will dictate the size of the data table and the data array will be dimensioned automatically Array sizes are checked and altered if necessary The MOVE command is typical of STATSTREAM commands It opens a window, displays a menu, allows the user to interact (choose filename, display data, etc) and finally snaps the window closed

Once the data are in memory any other command can be used in immediate mode, e.g

```
]USE MEANRE,1 – finds the mean of column 1
]USE ADRW,1,2,10,1, "DEMO", "X-AXIS", "Y-AXIS"
        – draws a graph of 10 rows of column 2 against column 1 using a line and annotates it
```

A simple program which allows 2 columns of data, each of 10 rows to be input, computes the logarithm of column 2, graphs it, calculates linear regression, graphs line with residuals and errors would consist of

```
]10 DIM RE(2,20) .REM sample BASIC program utilizing STATSTREAM commands
]20 USE KEYINQ,1,2,1,10
]30 FOR I=1 TO 10 ·RE(1,2)=LOG(RE(1,2)) ·NEXT I
]40 USE RESFIT,1,2,0
]30 END
```

It can be seen that STATSTREAM has a uniform syntax at both command and program level, and since the commands interact through transient windows they do not interfere with your own screen layout

STATSTREAM includes the following command procedures for statistical methods, and more can easily be added:

Mean, standard deviation, standard error and confidence interval
Student's *t* test, ANOVA one way, ANOVA two way, F test
Linear regression, correlation
χ^2 (chi-squared) test, contingency tables
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Signtest, Wilcoxon signed rank test, Mann-Whitney test
Kruskal Wallis, Friedman t-test, F test
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STATSTREAM can be used to analyse data in pre-existing Apple data files and it also has a routine for using Viscalc files.

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Computer applications

These pages feature computer hardware and software for the automated laboratory, from a 'metabolic computer' to a Digital Paintbrush graphics package.

● BALANCE, a new statistical software package from Elsevier Scientific, makes it possible to compare two series of measurements. BALANCE determines whether the means of two series of measurements are significantly different. The package can be used, for example, in comparing the results of two different medical treatments, investigating the effect of a drug, or of two analytical methods. This kind of comparison is probably the most frequently used statistical technique in industrial, clinical, chemical and quality control laboratories. BALANCE guides the operator automatically to the correct statistical test; it does not require knowledge of the statistical background by the user. BALANCE incorporates the following tests: tests for small numbers and for large numbers of observations; independent and related (paired) tests; one- and two-tailed (sided) tests; Student's *t*- and Cochran's tests (parametric tests); and Wilcoxon's matched-pairs signed-rank test and Mann-Whitney's *U*-test (non-parametric tests).
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Stat-Attack, an anti-static computer cleaning system.

● Zenith's Electronics' Service, Parts and Accessories Division has introduced Stat-Attack, an anti-static computer cleaning system packaged in a convenient tray that doubles as a desk/shelf organizer. The Stat-Attack system contains an anti-static spray that protects equipment from static discharge. It can be sprayed directly onto printers and other static-generating computer equipment. When fitted with a special extension, it can be lightly sprayed onto carpets or countertops. It converts lint-free wipers (included in the system kit) into static neutralizing cloths.
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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



A microcomputer, the CompuPro 816/C from Viasyn Corporation of California, is used to maintain a complete database on the behaviour and physical condition of Ling-Ling, the Giant Panda in the US National Zoological Park. This assists in determining her oestrous period, and hence the best time for breeding. (Picture courtesy of the Zoological Society of London.)
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● The IBM PC microcomputer can now be used as a sophisticated data acquisition and control system for laboratory instruments using the ADALAB-PC from Interactive Microware Inc. A wide range of scientific instruments — including chromatographs, spectrometers, strip chart recorders, temperature controllers and *p*H meters — may be controlled by the ADALAB-PC using the specially designed ADAPT software which includes a menu-driven data acquisition program. The system provides accurately-timed collection of multiple data values; fast analog-to-digital (A/D) voltage sampling with direct memory access; data storage and retrieval from an extended RAM memory up to 640K; and a scrolling strip chart data display. A complete self-test checking procedure is also featured. ADALAB-PC consists of a single plug-in card, enabling the computer to interface easily with the lab instrument. The IBM-compatible system joins the established ADALAB products for use with the Apple II micro.
Reader Service No. 102.

● Packard Instrument has developed an advanced network of gas chromatography instruments. A GC network of up to 12 satellite or conventional GCs and their accessories can be programmed and monitored from one central control station. The control station can be either the stand-alone Model 830 network manager or Packard's top-of-the-line Model 439 GC. The model 830 is a light and compact desk-top unit. Control software for a number of GC accessories, including Packard's One-Shot Samplers, is built into the control stations. Four models of satellite GCs are available, covering the complete range of analytical requirements.
Reader Service No. 103.

● The Oxymax-85 metabolic computer from Columbus Instruments simultaneously measures: oxygen consumption, CO_2 production, respiratory quotient, respiration rate, relative tidal volume and temperature in one to four animal cages. The IBM-PC personal computer is used for data collection as well as a main system controller. An electronic laminar flowmeter is used at the input of each animal cage to ensure accurate measurement of air supply, and an electronic barometer, thermometer and hygrometer allow presentation of results in STP units, standardized to 760 mmHg, zero humidity and zero (°C) temperature. Results of measurements are printed at 1-min intervals, and Oxymax 85 is designed for 24-hour uninterrupted operation.
Reader Service No. 104.

● A new option has been introduced for ChemGraf, a chemical modelling package based on the DEC VAX minicomputer. The new option is ChemGuide, an artificial intelligence program designed principally for novice and casual users of molecular modelling techniques. It helps the user to start using ChemGraf, select methodology, use specialist techniques, and tailor techniques to specific applications. Previously, only consultancy support could provide this assistance; support which is necessarily limited. Initially ChemGuide presents the user with a series of options. Depending on the option selected, ChemGraf commands are automatically executed, the user is asked for information, or is presented with a series of further options. ChemGraf systems, and the new ChemGuide program, are produced by Chemical Design Ltd.
Reader Service No. 105.



Computer-controlled spectrophotometry from Beckman Instruments.

● Latest in the Beckman Instruments, Inc. range of software is DATA CAPTURE, which interfaces the DU-50 series spectrophotometer with the IBM Personal Computer. The software is part of a PC software library that interfaces a wide variety of Beckman instruments to personal computers. Through the direct integration of spectral measurement and processing capability, data can be acquired, analysed, displayed, archived and reported on a single economical computer.
Reader Service No. 106.

● Epsilon Consultancy Ltd. is introducing a comprehensive software package to enable the Tablet Digitizer to be interfaced to a small computer so that the coordinate information produced by the Digitizer can be handled in a meaningful way. The true purpose of the software is to enable the user to make measurements of the material which has been digitized. The interface between digitizer and computer has been written to make use of the RS232 interface, because this interface is the most widely used one. By cabling the digitizer to the RS232 port in the computer, the user can start the software programs going and initiate a filing system by which the digitizations are stored in a manner which will make it easy, later on, to operate sorting methods on the files; calculations, tabulations and measurements can be done. The software can be expanded to handle special requirements, such as statistical comparisons between measurements.
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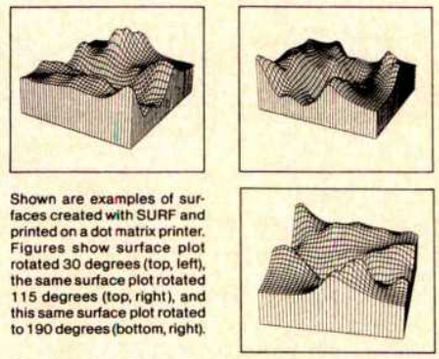


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● Two graphics packages designed specifically for scientists and engineers are now available on the IBM PC from Heyden Datasystems — Scientific Plotter-PC and Curve Fitter-PC. The Scientific Plotter-PC package plots graphs from data entered from disk, via the keyboard, or calculated by a user-defined equation. Axes are scaled according to the user's requirements, in either linear or logarithmic form, so that data are presented in the most suitable proportions. Plots may be superimposed in different colours; and there are 20 different plotting symbols in four size ranges to choose from. Variable-length error bars may be superimposed on the symbols. Curve Fitter-PC simplifies the fitting of a wide variety of curves to data which are input from disk or keyboard. The program automatically scales the plots and data, which may then be averaged and smoothed to reduce the number of points or average out background noise. Scientific Plotter-PC and Curve Fitter-PC are mutually compatible. Both packages are available in Apple II versions as well as the new versions on the IBM PC.
Reader Service No. 108.



Shown are examples of surfaces created with SURF and printed on a dot matrix printer. Figures show surface plot rotated 30 degrees (top, left), the same surface plot rotated 115 degrees (top, right), and this same surface plot rotated to 190 degrees (bottom, right).

Golden Graphics plotter output.

● Golden Graphics System is an integrated package that produces contour maps and three-dimensional surface plots, plus a variety of two-dimensional charts and graphs. Produced by Golden Software, Inc., the new package is designed to allow the user to visualize the implications of multivariate data. The Golden Graphics System consists of five applications: TOPO which creates contour maps; SURF which creates three-dimensional surface drawings, also known as block diagrams or fishnet plots; GRAFIT which creates *xy* graphs, bar charts, pie charts, line graphs, and scatter charts; QGRID which creates a regularly spaced grid from irregularly spaced data; and PLOTCALL which creates plots from plotting commands that have been placed in a data file. The Golden Graphics System may be used with either a monochrome display card or a graphics card. If a graphics card is installed, output may be previewed on the screen. The Golden Graphics System runs on the IBM AT, IBM PC, and compatibles and requires 128K bytes of memory and one disk drive. Over forty different dot matrix printers are supported.
Reader Service No. 109.

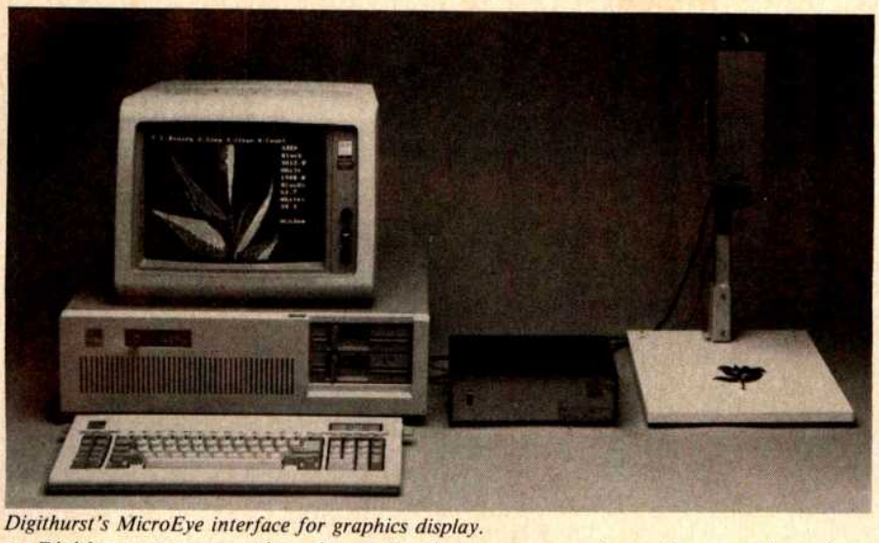


Photomail, from Chorus Data Systems.

● PhotoMail, available from Chorus Data Systems, allows PC users to capture images with a standard video camera or VCR and transmit them to a remote PC via ordinary phone lines. Still-frame pictures are sent at a resolution of up to 640 × 400 × 16 colours or levels of grey. PhotoMail operates with IBM PC, XT, AT and compatible computers. PhotoMail is icon-driven and supports voice communications so users can converse while an image is being displayed. By using a 'mouse' or the keyboard, each party can simultaneously point to a specific area of the picture and have a cursor displayed on both screens. By drawing with the mouse, users can designate specific areas of interest on the display. Images can also be annotated with text to allow users to label areas of interest, type messages and create image descriptions or specifications.
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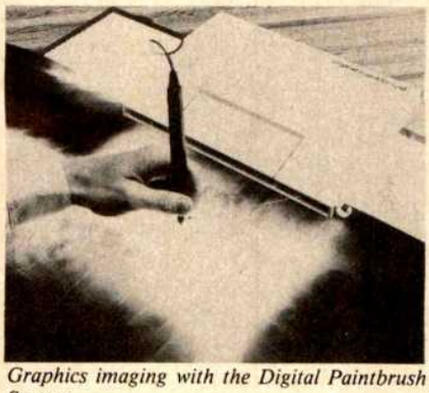
● Walonick Associates has introduced a new version of the statistical analysis program StatPac. The new package (V5.2) incorporates enhancements suggested by hundreds of StatPac users. Owners of the package will receive this update at no cost. The new features in StatPac include banner crosstabs, probit regression, principal components analysis with multicolinearity diagnostics, and new graphics. Walonick Associates is a computer software publishing firm that specializes in vertical application software. In addition to StatPac, the company offers Forecast Plus, a graphics time-series analysis package, and Mach, a chemical-dependency assessment program.
Reader Service No. 111.

● The Microlink modular interface has been enhanced to provide a comprehensive hardware and software solution in data acquisition and control. With the addition of 15 new modules, Microlink, from Biodata, offers a choice of more than 40 modules including analog and digital inputs/outputs, transducer inputs, timers and counters. The new modules include programmable gain amplifiers, transient capture devices, *p*H and RTD inputs, frequency/event counters and alarms. With the availability of new software for use with the HP150 and BBC, Microlink hardware can now be used with most popular micros — including IBM PC, Apricot, HP 80 series, HP 9816, Sirius and Apple. Sample programs are provided in BASIC for each of these computers.
Reader Service No. 112.



Digithurst's MicroEye interface for graphics display.

● Digithurst announce the release of a version of MicroSight I which will produce images on the Tecmar Graphics Card. The images are read into the IBM via the Digithurst's MicroEye interface and are held in random access memory as a 256 × 256 pixel file at 255 grey levels. Using the Tecmar Graphics Card, it is possible to display 16 of these levels at any one time. Although the increase in the display quality will primarily benefit users in the graphics field, it is expected that the ability to display more grey scales will also be of benefit to those using Digithurst's MicroScale packages for computerized image analysis.
Reader Service No. 113.



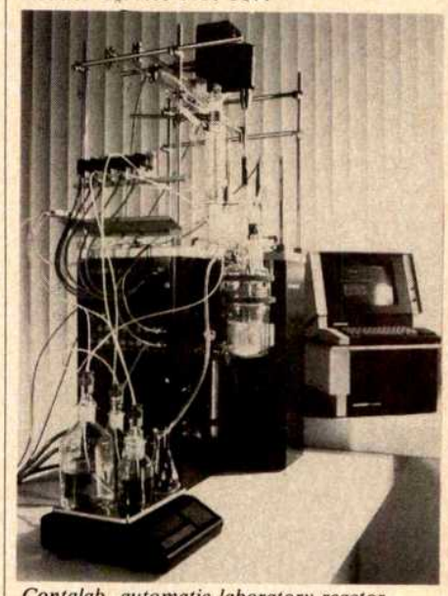
Graphics imaging with the Digital Paintbrush System.

● The Computer Colorworks, a San Francisco-based company, has introduced an IBM PC version of its Digital Paintbrush System, a complete hardware/software colour graphics package designed for the manipulation of graphical data. A more powerful, advanced version of the already popular Apple product, the new system is designed for scientific and professional users of the IBM PC, XT, AT, and compatibles. Graphic images made with the Digital Paintbrush can be shown like slides on a colour monitor or TV screen, printed as hard copy or as transparencies for overhead projection, and photographed with various hood cameras and picture processors (including the Polaroid Palette) to make 35-mm slides.
Reader Service No. 114.

● ALLOCATE is a software package from the UK division of Elsevier, designed for the organizers of animal experiments or clinical trials. ALLOCATE rapidly assigns clinical or experimental subjects to treatment groups. The random allocation, on the basis of a chosen parameter such as body weight, is repeated (with statistical tests to assess uniformity of groups performed automatically after each iteration) until criteria set at the outset are met, or the 'best allocation so far' is accepted. Full details of groups and statistical results can be displayed on-screen, and printed out. Unequal group sizes are catered for, and an optional cull facility enables outliers to be deleted. Manual and disk are available for the IBM PC, DOS 2.0. ILEUM is a package for the accurate simulation of the effects of drugs on the *in vitro* guinea pig ileum. Drugs available for the ILEUM simulation include a dozen familiar agonists (which will contract the muscle) together with blockers of various sorts. Experiments to identify an 'unknown compound' can be carried out, for which the computer selects, at random, one of 20 'unknowns'. Random elements are incorporated into ILEUM to simulate the biological variability of response to the same dose of agonist. It is available for Apple II, IBM PC and BBC B computers.
Reader Service No. 115.

● As an accessory to the Hewlett Packard 8451A UV/visible diode-array spectrophotometer, the new HP 89075A multicell transport automatically handles the concurrent measurement of up to seven samples over any length of time. The new transport provides accurate serial or random access to cells and is programmable, providing flexibility in method development. For applications such as kinetics, where multiple measurements must be taken and where data are acquired over long periods of time, the new product will deliver increased throughput and automated accuracy. The system mounts in the sample compartment of the HP 8451A spectrophotometer and uses standard 1-cm-square cuvettes and/or flow cells.
Reader Service No. 116.

● Northwest Analytical, Inc. has introduced a new statistical quality control charting package, called QUALITY ANALYST, which provides the software for both process capability studies and process monitoring. The package is available for the IBM and compatibles, MS-DOS computers, Burroughs B20, CP/M and Apple's Macintosh. QUALITY ANALYST provides an effective alternative to time-share and minicomputer-based quality control systems. QUALITY ANALYST is a complete package and can be used effectively as a stand-alone system. It can interface with other software packages and be used as the analytical core of a workstation using other software such as databases and spreadsheets. QUALITY ANALYST works well with standard communications packages and can process information transmitted from a mainframe database.
Reader Service No. 117.



Contalab, automatic laboratory reactor.

● Contalab, recently introduced by Contraves, is a computer-controlled automatic laboratory reactor that can operate for 24 hours a day, completely unsupervised. Complete reactions can be performed and monitored automatically and a simple question-and-answer dialogue guides the operator through all data entry procedures. The computer controls the experiment, compiles all the data, and produces a record of all results. The system automatically assigns alarm limits to enable 24-h operation. Contalab is especially suited to experiments which must be repeated many times or performed consecutively with minor changes (serial tests and optimization for example). Contalab consists of a reactor assembly with balance (for use in a laboratory fume cupboard), control unit with dual disk drive, peripheral equipment for data input and output, and software package (for controlling system operation and processing sequences). The reactor assembly is equipped as standard with a 1-litre reaction vessel, a reflux condenser and a stirrer.
Reader Service No. 118.

● Kratos Analytical's new DS 650 allows real-time data acquisition and system control for chromatography and data management. The DS 650 hardware is based on Data General's model 10/SP desk-top microcomputer and includes a 15 MB Winchester hard disk, one 368 KB diskette drive, 256 KB of main memory, a 12-inch green monochrome bit-mapped monitor, a graphics printer, and a multiplexed A/D converter. This converter is capable of accepting data simultaneously from up to eight detectors, using four separate times bases. The computer's menu-driven software guides the user.
Reader Service No. 119.

● Nelson Analytical Inc. of Cupertino, California, has developed an advanced chromatography workstation designed to meet the data acquisition, analysis and file management needs of individual chemists or small laboratories. The workstation comprises the Hewlett Packard 9000 series 200 computer, a Winchester disk drive and the HP ThinkJet printer, together with Nelson's XtraChrom software and one of four intelligent A/D interfaces. Up to ten instruments, each interfaced to up to two detectors, may be controlled by this HP3362A or B chromatography data system. As well as standard gas and liquid chromatography applications, the system can be used for capillary GC, ion chromatography and amino-acid analysis.
Reader Service No. 120.



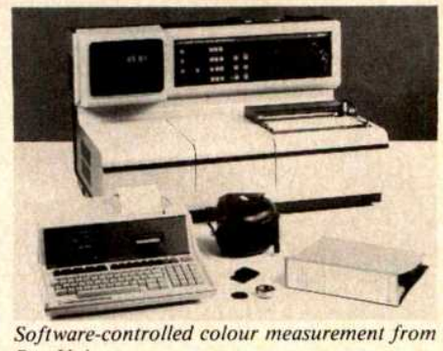
Waters Expert software for chromatography.

● The Waters Expert multi-instrument chromatography software gives the Waters 840 data and chromatography control station simultaneous HPLC, GPC, GC and ion chromatography data reduction, together with facilities for multi-instrument control. Expert software provides chromatography data acquisition from up to four chromatographs simultaneously, with each system acquiring data from up to four detectors. In addition, up to four Waters HPLC systems can be controlled from the keyboard of the Waters 840 data and chromatography control station. The efficient multi-tasking operating system of the Waters 840 allows the monitoring of a separation from up to four different detectors simultaneously on the CRT screen. In addition, the Waters 840 provides virtually unlimited networking capabilities, including ETHERNET compatibility for high-speed data transfer. An IEEE, RS-232 interface allows communication with additional 840 systems or other computers.
Reader Service No. 121.

● The Consort II computer interface system, designed and developed by ICI (Australia), forms the basis of a comprehensive Apple computer-based data station for chromatography. The instrument provides a three-in-one system for accurate data collection on two channels, dual pump control for HPLC and control of peripheral devices, such as autosamplers and fraction collectors. Consort II operates with either an Apple II+ or IIe computer in conjunction with a comprehensive software system called Appligration II designed by Dynamic Solutions Corporation of California. The system can be configured by the user to cope with a wide range of chromatographic data including capillary GC, with sampling rates adjustable from 0.05 Hz up to 200 Hz. A special package for gel permeation chromatography is included. The Consort II computer interface system is marketed in the UK by Biotech Instruments.
Reader Service No. 122.

● Designed for use with the ACT Apricot range of microcomputers, Dynatech's new ABO/Rhesus blood-grouping programs interpret results from the microtitre plate, whilst offering flexibility of plate format, accumulative records of groups performed, and storage space for full data on up to 750 full plates (7,500 patients per data disk). The program also allows for alteration of interpretation parameters which may be used to re-interpret raw data from memory without the need to re-read the plate. This new program, developed in conjunction with Sanguin Computing, operates on the Apricot PC and Xi microcomputers and also on the Sirius microcomputer. Also new from Dynatech is its MicroBank System for the full automation of the entire blood-grouping operation in microtitre plates. Composed of three different work stations, the MicroBank has been designed to maintain patient integrity and avoid incorrect grouping due to operator error.
Reader Service No. 123.

● The latest version of LABTECH NOTEBOOK, a sophisticated menu-driven software program for real-time data acquisition, control and analysis, now supports hardware devices from Action Instruments, Cyborg, Datel, IBM, Interactive Microware, Keithley DAS, and Tecmar. Equipment from Acrosystems, Burr Brown, Data Translation, Metrabyte and Taurus is already supported by this software. NOTEBOOK delivers full hardware functionality of every data acquisition device it supports. The latest version, LABTECH NOTEBOOK 2.5, provides support for real-time thermocouple linearization and compensation. Data may be collected as temperature points directly from standard thermocouple devices, without requiring linearizing data conditioners. LABTECH NOTEBOOK supports the IBM AT, and it can also be integrated with spreadsheets like Lotus 1-2-3.
Reader Service No. 124.



Software-controlled colour measurement from Pye Unicam.

● The latest software-controlled colour measurement system from Pye Unicam is based on the PU 8800 high-performance UV/visible spectrophotometer. The new system uses an integrating spheroid and a fast, powerful software package for the HP 85 computer and is the only non-dedicated system that completely conforms to the requirements of the CIE, the governing body of colour science. The PU 8800 range includes a video display, built-in recorder and printer options and a choice of side or end-window photomultipliers. Available on tape and disk, the software provides measurements of colour or colour difference in up to three modes — diffuse or total reflectance and transmission. Illuminants A, C, D_{65} and D_{75} are offered, together with an additional two illuminants of the user's choice.
Reader Service No. 125.



Mettler software kit for thermal analysis.

● The performance of the Mettler FP800 thermal analysis system can be substantially enhanced by connection to a computer. For this purpose Mettler offers a software kit, which has been specially designed for the Epson HX20 hand-held computer. In addition to controlling the unit, it also permits various standard applications to be called up, results to be calculated and data stored for later processing. At very little extra cost, the FP800 can also be adapted for special applications, so that it operates automatically in accordance with national and international standards (such as melting point of plastics according to ASTM, clear melting point of fats according to AOCS). The program is written in MICROSOFT BASIC, so that it can be adapted easily to the individual requirements of the user.
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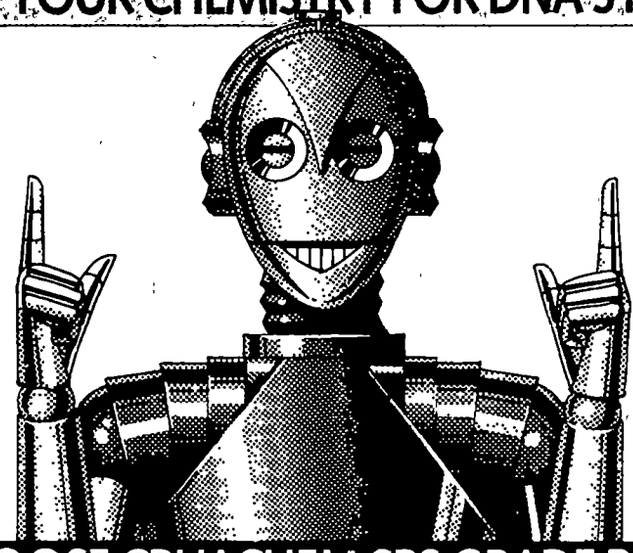
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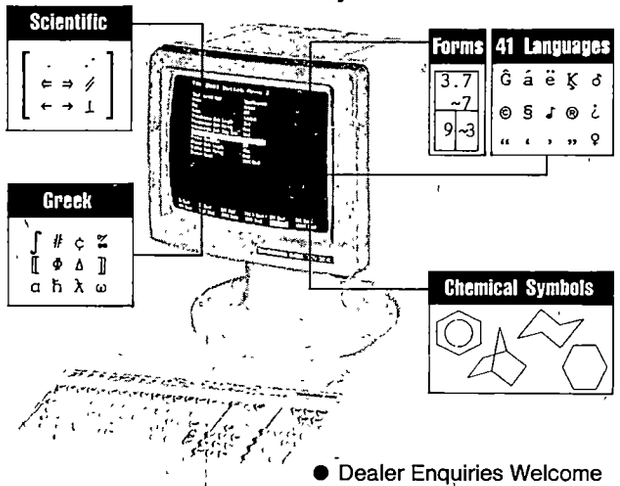
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ITC is concerned with international scientific education, research and consulting in aerial photography and teledetection, photogrammetry, aerospace survey application in landresources studies, agriculture, earth sciences, human settlements, geo-data processing and cartography, in particular directed towards the needs of developing countries.

The Institute undertakes scientific training at B.Sc., M.Sc. and Ph.D. levels in survey techniques especially for students from developing countries.

Most instruction at the Institute is given in the English language and includes both introductory and advanced courses in Soil Survey using Aerial Photography and other Remote Sensing techniques.

Applications are invited for the post of

professor in soil survey

charged with education and research in soil survey and its applications.
Capable of liaising with other earth- and agricultural sciences as well as with disciplines specializing in physical aspects of geo-data processing.
Applicants will preferably have a Ph.D. degree based on research in Soil Science, proven research ability. Experience in post graduate teaching and in Soil Survey, with emphasis on Soil- and landscape relationships and including the application of image interpretation, is required. Experience in developing countries is required. Leadership qualities are required.

(Further details about profile and task description will be sent to candidates in the form of the Structural Report on the Professorship in Soil Survey).

For further information you may contact Prof.dr.ir.K.J. Beek, Rector, telephone 053-320330, ext. 214.

Salary will be in accordance with the Netherlands Government scales. The gross salary ranges from *f* 6.364,- (minimum) to *f* 9.005,- (maximum) per month exclusive of allowances.

Written application and detailed curriculum vitae should be sent as soon as possible to the Head of Personnel Dept. of ITC, P.O. Box 6, 7500 AA Enschede (the Netherlands).

Information from third persons regarding suitable candidates will be considered.

(W1937)A

FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF UTRECHT

The Faculty of Veterinary Medicine of the University of Utrecht, The Netherlands, invites applications for the position of

Full Professor in Cell Biology and Histology

Applicants are expected to meet the following requirements:
- expertise in cell biological and experimental histological research and research management;
- experience in teaching;
- willingness to collaborate with the other disciplines within the faculty;
- willingness to serve on faculty boards and committees.

Candidates should:
- possess a Ph.D. or equivalent academic degree;
- be specilized in cell biology and histology as evidenced by publications of high standard;
- be willing to learn Dutch within a reasonable period of time.

Further information is available on request.

The salary will be determined according to the experience of the applicant.

Applications, accompanied by a full curriculum vitae and a list of publications, or suggestions of suitable candidates, should be (confidentially) sent to:

J.J. van der Kaaden, Secretary of the Appointment Committee Professorship Veterinary Cell Biology and Histology, Faculty of Veterinary Medicine, P.O. Box 80.163, 3508 TD Utrecht, The Netherlands. no. 170.173.

(W1944)A

DEAN

College of Earth and Mineral Sciences
The Pennsylvania State University

Applications and nominations are invited for the position of Dean of the College of Earth and Mineral Sciences Candidates must possess an earned doctorate, scholarly/professional qualifications for a tenure professorship, and administrative experience and inter-personal skills needed to provide academic and organizational leadership.

The College of Earth and Mineral Sciences offers B S., M.S. and Ph.D. degree programs in six departments (geosciences, geography, mineral economics, meteorology, materials science and engineering, and mineral engineering), and has an outstanding reputation for both teaching and research

The Search Committee will begin to review résumés on July 1, 1985 and will continue to receive them until a candidate is selected.

Applications and nominations, accompanied by résumés and other supporting material, should be submitted to: Arnulf Muan, Chairman, EMS Dean Search Committee, 201 Old Main, University Park, PA 16802.

(The Pennsylvania State University is an Equal Opportunity/ Affirmative Action Employer). (NW1863)A

IMMUNOLOGY

The University of Pennsylvania has a tenure track position available in the field of viral immunology. This position is assigned to the Section of Viral Oncology, Department of Clinical Studies, New Bolton Center, the rural campus near Kennett Square

Duties will include research, teaching and service. Emphasis will be placed on the development of an active research program on the immunobiology of the bovine leukemia virus (BLV). This program may include studies on mechanisms of BLV-induced leukemogenesis at the host, cellular and molecular levels, including identification and characterization of the target cells for neoplastic transformation. Service responsibilities will include consulting in diagnostic immunology. Teaching will relate to graduate students as well as students of veterinary medicine. The appointee will be expected to assume a leadership role in the field of immunology on this campus.

Qualifications are a PhD degree or the equivalent and at least two years postgraduate experience in research. A DVM or MD degree would be an advantage. Experience in viral immunology and monoclonal antibody techniques would also be an advantage. Rank and salary will depend on qualification and experience. **Applications should include a curriculum vitae and the names of three referees. They should be addressed to Dr. Jorge F. Ferrer, Chief, Section of Viral Oncology, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348.** *The University of Pennsylvania is an equal opportunity employer. Women and minorities are encouraged to apply.*

(NW1851)A

MEMBRANE TRANSPORT

Postdoctoral and advanced research assistant positions are available immediately for individuals interested in pursuing studies on the structure and mechanism of ion-translocating ATPases. The ATPase in the plasma membrane of *Neurospora* is an electrogenic proton pump that operates via a phosphoryl-enzyme intermediate identical to those of the animal cell transport ATPases. Research will involve investigations of the biochemistry and molecular biology of this ATPase with the goal of elucidating its molecular mechanism. Salaries are commensurate with experience. Send curriculum vitae and names of three references to. **Dr. Gene A. Scarborough, Department of Pharmacology, School of Medicine. University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514.**

Equal Opportunity Employer (NW1866)A

UNIVERSITY OF STOCKHOLM
Department of Zoology

ZOOECOLOGIST

Applications are invited for a position as "eo docent" (roughly equivalent to US assistant professor) primarily intended for a person with several years of post-doctoral research experience.

The position is open provided certain formal decisions are taken and it is advertised for a period of three years, but is usually renewed for another three year period. Starting salary L 18 (Swedish crowns 120 000 per annum = roughly 13 300 US$).

Docent is a research and teaching position, and requires a minimum of 75 hours of lecturing per annum or equivalent supervision of graduate student research, some administrative duties, and a vigorous research programme Applicants are selected primarily on the basis of their scientific publications, but teaching experience is also required.

Research is presently conducted mainly in the fields of evolutionary ecology (study objects insects and mammals) and marine and brackish water ecosystems and population ecology.

For further information contact: Professor H Ackefors, Department of Zoology, University of Stockholm, 106 91 Stockholm, Sweden (Telephone 00946-8164020).

Applications including Curriculum vitae plus one copy of published accepted and submitted papers, a short summary of the applicants research and teaching experience, names and addresses of three references and any other documents the applicant wishes to submit should be addressed to The Board of Stockholms University, Fredrik Bloms Hus, University of Stockholm, S-106 91 Stockholm, Sweden, and received no later than August 15, 1985. Quote reference number 338/85

(W1941)A

MOLECULAR BIOLOGIST

The Biology Department at the University of California, San Diego invites applications for a tenure-track position at the assistant professor level, salary range $25,500-$32,200. Applicants must have Ph.D. and should have a minimum of two years postdoctoral experience. The department is interested in applicants who have a commitment to applying molecular techniques to problems of the nervous system. The applicant will be expected to interact with an active group of neurobiologists and molecular biologists in the Biology Department. **Applicants should forward their Curriculum vitae with names of three references and selected reprints by July 15, 1985 to: Dr. A.I. Sleverston, Chairman, Search Committee, Dept. of Biology, B-022, University of California, San Diego, La Jolla, CA 92093.**

The University of California, San Diego is an Equal Opportunity/Affirmative Action Employer.

(NW1858)A

Faculty Positions in Developmental Biology

The Molecular Biology and Virology Program at the Sloan-Kettering Institute and the Sloan-Kettering Division of the Cornell University Graduate School of Medical Sciences is seeking to fill several tenure track positions in the area of prokaryotic and eukaryotic developmental biology. Individuals will be expected to establish independent research programs that utilize genetic and molecular approaches to the analysis of model pro- and eukaryotic organisms. **Applicants should submit their Curriculum Vitae, a brief statement of research accomplishments and goals and the names of three references to Dr. Ora M. Rosen, Sloan-Kettering Institute, 1275 York Avenue, New York, N.Y. 10021.** (NW1847)A

MOLECULAR VIROLOGIST

The University of Pennsylvania has a faculty position available in molecular virology. The position is in the Section of Viral Oncology, Department of Clinical Studies, New Bolton Center, the rural campus near Kennett Square.

Duties will include research, teaching and service. Emphasis will be placed on the development of a research program on the molecular biology of the bovine leukemia virus (BLV). The precise area of this program will be complementary to ongoing studies of the molecular mechanism of BLV-induced leukemogenesis and the regulation of viral transcription in BLV-infected and leukemic cells. Service responsibilities will include consulting in diagnostic virology. Teaching will relate to graduate students as well as students of veterinary medicine.

Qualifications are a PhD degree and at least two years postgraduate experience in research on molecular aspects of RNA tumor viruses. Further experience with recombinant DNA technology, gene cloning and sequencing, and gene expression in prokaryotic and eukaryotic systems would be an advantage. Rank and salary will depend on qualifications and experience. **Applications should include a curriculum vitae and the names of three referees. They should be addressed to Dr. Jorge F. Ferrer, Chief, Section of Viral Oncology, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348.** *The University of Pennsylvania is an equal opportunity employer. Women and minorities are encouraged to apply.* (NW1852)A

CSIRO
AUSTRALIA
RESEARCH FELLOW BIOCHEMISTRY/ENZYMOLOGY
$A26,001 - $A38,243
DIVISION OF PROTEIN CHEMISTRY PARKVILLE VIC

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Protein Chemistry is a member of the Institute of Industrial Technology

GENERAL: The Division of Protein Chemistry, with a staff of 118, conducts scientific research on the structure and function of proteins and their production via biotechnology and synthetic routes. The Division is well equipped and has a wide range of facilities for biochemical and biotechnological research.

Located in Parkville, the Division is situated close to a major university and several related research facilities. Collaboration with scientists from other CSIRO laboratories will be encouraged.

The appointee will be a member of a program which has 12 professional scientists with expertise in the fields of protein chemistry and wool technology.

This position is funded by a two year grant from the Wool Research Trust Fund with the possibility of renewal for a further two years.

DUTIES: In collaboration with research scientists from this Division and in close association with scientists from the Divisions of Textile Industry and Chemical and Wood Technology, the appointee will undertake research into the potential use of enzymes in wool processing and effluent control. It is envisaged that enzymes will be isolated from various sources to carry out specific hydrolysis reactions of sterol esters and non-glyceride fatty acid esters for the hydrolysis of wool wax and for effluent treatment from scouring works. A parallel study will require the isolation of cellulose and lignin degrading enzymes suitable for the removal of vegetable matter contamination from processed wool.

In the event of suitable enzymes being isolated for either of the end uses, it is proposed that the genetic engineering facilities of this laboratory will be used to develop economic methods of production of the required enzymes.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant field and demonstrated research ability. Post-doctoral experience in enzymology or related fields would be an advantage.

TENURE: A term of 2 years, with the possibility of a further term of 2 years. Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No Y8789, should be directed to:

The Chief
CSIRO Division of Protein Chemistry
343 Royal Parade
PARVILLE VIC 3052
AUSTRALIA

by 4 weeks from date of publication

CISIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1947)A

FACULTY POSITIONS IN MOLECULAR BIOLOGY

The Molecular Biology and Virology Program at the Sloan-Kettering Institute and the Sloan-Kettering Division of the Cornell University Graduate School of Medical Sciences is seeking applications from molecular biologists for several tenure track faculty positions. The potential recruit should be using modern genetic, molecular biological and biochemical techniques to study problems in the general area of prokaryotic and eukaryotic cellular or viral gene expression and gene replication. The specific rank of appointment will be commensurate with the candidate's experience and achievements. This is a rapidly expanding program consisting of 20 independent faculty members with diverse research interests who are committed to basic research and the training of graduate students.

Please submit your Curriculum Vitae, present and future research plans and names of at least three referees to: Dr. Robert Krug or Dr. Jerard Hurwitz, Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021. (NW1846)A

CSIRO

AUSTRALIA

APPLIED/ COMPUTATIONAL MATHEMATICIAN

$A26,001 - $A38,243

DIVISION OF MATHEMATICS AND STATISTICS ADELAIDE SA Australia

The Division of Mathematics and Statistics has a staff of 97, including 75 professional scientists. The Division's Headquarters are at Canberra, and there are Regional Offices located at Melbourne, Sydney, Adelaide, Brisbane and Perth Scientists of the Division provide advice on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, as well as conducting basic research in statistics and mathematics.

The Division has an active Applied and Computational Mathematics Program working on collaborative research projects with CSIRO Divisions in a variety of areas including manufacturing technology, oceanography, energy technology and applied physics. Members of the group are currently located in Canberra, Hobart, Melbourne and Sydney.

The successful applicant will be located in Adelaide and in addition to working on collaborative projects with Adelaide Divisions (including Soils and Manufacturing Technology) will be expected to contribute to the basis research of some aspect of applied and computational mathematics.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant branch of mathematics, together with a proven record of research and collaboration in relevant areas of mathematics. Expertise in the numerical solution of partial differential equations, numerical linear algebra and computational problem solving is particularly relevant. Familiarity with industrial applied mathematics and the mathematical modelling of the thermal processes is also desirable.

TENURE: The position is available for 3 years with the possibility of a further term. Australian Government superannuation benefits available

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two referees and quoting reference No A3499, should be directed to

The Chief
CSIRO Division of Mathematics and Statistics
GPO Box 1965
CANBERRA ACT 2601
AUSTRALIA

one month from date of publication

CISIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1948)A

The **Department of Biology, University of Saskatchewan** invites applications for a tenure-stream position at the **Assistant Professor** level in the area of the molecular genetics of plants.

Candidates should have a Ph.D. degree and, preferably, additional research experience in recombinant DNA techniques. Capability in the field of control of gene expression and plant genetic transformation is essential.

Send curriculum vitae and the names of at least three referees to **Dr. John King, Head, Department of Biology, University of Saskatchewan, Saskatoon, Sask., S7N 0WO, Canada by August 1, 1985.**

In accordance with Canadian immigration requirements this advertisement is directed to Canadian citizens and permanent residents. (NW1850)A

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

is seeking a

Principal Training Officer

at ICRISAT Sahelian Center, Niamey, Niger

To organize and conduct several levels of training in agriculturally-related subjects in West Africa for the benefit of scientists and technical support personnel of the National Agricultural Research Systems (NARS) of the region and related extension and action agencies. The Principal Training Officer must also evaluate on a recurring basis, the training and agriculturally-related education needs of West African NARS; aid in the identification of trainees and students for assignment to Training Programs conducted in West Africa and at ICRISAT Center, India; facilitate matters relating to the designation, assignment and travel, of all students and trainees from the West African region; and have responsibility for the housing, personal welfare and financial arrangements for all trainees and students assigned to ICRISAT in West Africa, develop over time an inventory of human resources available for agricultural research in the region and follow-up on trained personnel to measure impact of training program. The incumbent must also organize workshops, conferences and symposia and aid the research staff in the conduct of these activities.

Qualifications: Candidates must have an advanced degree (preferably PhD) in an agricultural discipline with formal training in educational subjects, five years experience in teaching agricultural subjects at various levels of student educational preparation, and some evidence of research accomplishment. A competence in French and English is essential. Most teaching and course materials will be in French The position will require an ability to travel frequently in the region. An initial orientation period of about 4 months at ICRISAT Center, India will be required. Salary for the position is at the principal staff level, according to qualifications and experience. The position is on a three year contractual basis with possibilities of further extension. The post is located at the ICRISAT Sahelian Center near Niamey, Niger, which is regional headquarters for ICRISAT's West African programs. Niamey, the capital city, has good living conditions, international airlines connections, and educational facilities for children in French and English.

Send résumé and names and addresses of three references within 30 days of the date of advertisement to Director General, ICRISAT, Patancheru P.O. Andhra Pradesh 502 324, India. (W1934)A

Sunderland & Teeside Polytechnics

NORTH EAST BIOTECHNOLOGY CENTRE

The North East Biotechnology Centre (NEBC) is a National Centre for Biotechnology based at Sunderland and Teesside Polytechnics The Centre has the following vacancies for research staff.

RESEARCH ASSISTANT

Applications are invited from honours graduates, or final year students expecting to graduate, in Microbiology/Biochemistry or related subjects to undertake research into the flocculation behaviour of *Bacillus* cells in liquid culture. The investigation will involve the use of chemostat cultures and also biochemical fractionation techniques for isolating and purifying cell surface polyelectrolytes. The research supervisor will be Dr Tony Abbott.

POSTDOCTORAL RESEARCH FELLOW

This post is open to candidates with a Ph.D. in microbial physiology or a related discipline, and those about to complete their Ph.D The research will be concerned with microbial synthesis of chiral compounds of industrial importance and will involve fermentation in addition to enzyme purification and characterisation techniques. The principal research supervisor will be Dr John Colby.

An application form and further particulars may be obtained from the Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland, SR2 7EE or telephone (0783) 76231 Ext 11.

Closing date: 27 June 1985 (6792)A

BRISTOL POLYTECHNIC

Science Department

Postgraduate Research in Biological Sciences

Applications are invited for the following posts:

Researcher (Ref No R/50
To work on "Transport and Compatibility in Downy Mildew Infections" with Dr P Spencer-Phillips.

SERC Quota Studentship tenable in one of the following areas

Biochemistry and Pharmacology of Insect 5-HT Receptors (Dr K Cattell & Dr R Osborne) **Ref R/51a**
Novel approaches in the Study of Growth and Killing of Skin Organisms (Dr J Greenman) **Ref R/51b**
Molecular Analysis of Race Structure in Bacterial Plant Pathogens (Dr A Vivian) **Ref R/51c**
Histopathology and Biochemistry of Dietary Cadmiam in the Shore Crab (Dr G Carney & Dr C Phillpotts) **Ref R/51d**

Applicants should indicate the post(s) they require further information about.
Applicants for the Researcher A should hold or expect to obtain a good Honours Degree in Biological Sciences, and for the Studentships, at least a 2ii, class degree.
For further details and an application form, to be returned by 24 June 1985, please contact the Personnel Office, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol or ring Bristol 656261, Ext 216 or 217.
Please quote appropriate Reference Numbers in all communications. (6784)A

Development Scientist — Histology

New Technologies — Research Products

£8,500-£14,000 **South Bucks**

Amersham International, a highly successful British Company, is a market leader producing a wide range of high technology products for life science research, medicine and industry.

Our New Technologies Department is playing an increasingly important role in the development of the Division's business strategy through the innovation and development of immunological and molecular biology techniques at the very forefront of this fast moving area.

The department is currently addressing the need for systems and reagents for DNA cloning, gene expression, nick translation, non-radioactive labelling, hybridisation, the requirements of biotechnology and the production of anti-bodies and enzyme systems for cell biology and Pathology.

As a result of continuing expansion we now have an exciting career opportunity for a development scientist to evaluate and develop a broad range of tissue processing and staining techniques for localisation of biological molecules.

Qualified to at least first degree level in a relevant discipline, you should have both practical and theoretical knowledge of tissue processing and staining. The aptitude/ability to run projects with the minimum of supervision and work to tight deadlines is essential. Your flexibility will allow you to work with scientists of several different disciplines and to apply your knowledge in a number of new fields.

Salary in the range indicated will reflect qualifications and experience and will be enhanced by a benefits package including a generous pension scheme, 25 days' annual holiday and assistance with relocation, where appropriate.

Please write enclosing a CV, detailing your career to date, to Simon Voake at the address below.

Alternatively, please telephone Little Chalfont (02404) 4488, ext. 3569 for an application form. (6829)A

Amersham International plc
Amersham Laboratories
White Lion Road, Amersham
Buckinghamshire

Amersham

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW,
MIDDLESEX, HA1 3UJ

CHIEF TECHNICIAN

A chief technician is required to co-ordinate and supervise our growing central molecular biology facilities. Duties will combine both administrative and technical responsibilities. The successful candidate will be expected to play an active role in the experinmental work of the molecular medicine research group.

Applicants should have at least ten years laboratory experience of which at least five should have been spent in the molecular biology field. Previous administrative experience is essential.

Salary will be within the range £9,987 — £11,489 inclusive, depending on experience. Informal enquiries may be made to Dr James Scott, Head of the Molecular Medicine Research Group — Telephone 01-864 5311 extn. 2479. Application forms and job description may be obtained by writing to Miss B.A. Shaw, Personnel Dept. Quoting Reference 111/2/4275.

Closing date for applications 4 July 1985. (6781)A

PLANT GENE TECHNOLOGY and DNA RESEARCH

POSTDOCTORAL POSITION

Candidates with a background in plant physiology and/or DNA research are invited to join an industry-funded independent research group at the Institute of Molecular Biology of the Austrian Academy of Sciences in Salzburg.

We offer a salary and benefits commensurate with experience according to Austrian Academy of Sciences scheme. The initial appointment will be for one year with possible extention up to four years. **Résumé, list of publications and three letters of reference should be submitted to CHEMIE LINZ AG, Personalwirtschaft, POB 296, A 4021 Linz, Austria.**

CHEMIE LINZ AG (W1939)A

DRUG DESIGN SCIENTIST

G.D. Searle is a major international pharmaceutical company whose Research & Development Division at High Wycombe is involved with the discovery of new chemical and biological entities.

The Division is expanding its Drug Design and Computer Graphics expertise in line with other developments in its Medicinal Chemistry Department. There is now a vacancy for a scientist with interests and experience which lie in one or more of the following areas:-

- **Computational Chemistry and its application to drug design problems.**
- **Analysis of Structure-Activity Relationships using regression, classification and multivariate techniques.**
- **Physical Chemistry measurements (FT-nmr, FT-ir, pKa, logP) and their use in drug design.**
- **Software development in computational chemistry.**

The Drug Design group contributes in a major way to Medicinal Chemistry research programmes and also supports Protein Engineering and Molecular Genetics research. Software currently in use includes Chemlab and Chemgraf running on a VAX cluster

The Company offers competitive salaries, 23 days holiday, contributory pension fund, health insurance scheme, subsidised cafeteria, recreation club and modern offices in a very pleasant location. Generous relocation assistance will be available in appropriate cases

Please apply to the Personnel Manager, Searle Research & Development, Lane End Road, Sands, High Wycombe, Bucks. (Tel: High Wycombe 21124, Ext. 3372). Please quote reference CH157.

(6770)A

SEARLE

CHAIRPERSON

Department of Physiology and Biophysics

THE UNIVERSITY OF TEXAS MEDICAL BRANCH AT GALVESTON

Applications and nominations are invited for the position of Chairperson of the Department of Physiology and Biophysics. Candidates must be a highly qualified individual with nationally recognized credentials in research and senior academic accomplishments with administrative experience or potential. The position offers excellent opportunity to develop an internationally leading adademic department due to outstanding resources, scientific environment and faculty positions.

Application or nomination should include a curriculum vitae. Address correspondence to: James C. Thompson, M.D., Chairman, Department of Surgery, The University of Texas Medical Branch, Galveston, Texas 77550.

The University of Texas Medical Branch is an Affirmative Action/Equal Opportunity employer. (NW1856)A

Several positions are available at Geo. J. Ball, a major horticultural company in northern Illinois. The positions and qualifications are:

1 Plant Tissue Culture: 4 positions; PhD, MS or BS with single or combined background and experience in any of the following areas:

a. *in vitro* micropropagation
b. acclimation of tissue cultured plants *in vivo*
c. product system development in tissue culture
d. plant pathology
e. plant physiology
f. cell culture

2. Greenhouse Manager: BS preferred with experience in laboratory micropropagation.

Send résumé to:

Ball

Dr. Irwin Y.E. Chu
Vice President -
Research & New Project Development
Geo. J. Ball, Inc.
P.O. Box 335
West Chicago, IL 60185

EQUAL OPPORTUNITY EMPLOYER

(NW1865)A

Postdoctoral Positions in

MOLECULAR VIROLOGY

Postdoctoral positions available in July to study molecular details of replication and gene expression in plant viruses and to construct gene vectors. Molecular biology and/or recombinant DNA experience desirable.

Send curriculum vitae and three letters of reference to: **Dr. Paul Ahlquist, Biophysics Laboratory, University of Wisconsin, Madison, WI 53706 USA.** An equal opportunity employer.

(NW1838)A

FACULTY POSITION - ENVIRONMENTAL TOXICOLOGY

The Department of Environmental Health Sciences of the School of Public Health, University of Alabama at Birmingham (UAB) is seeking an assistant or associate professor in environmental toxicology. This is a tenure-track position with responsibilities that include teaching of graduate level toxicology courses and initiation of independent and collaborative research. A doctorate in toxicology and an interest in carcinogenesis neurotoxicology, or inhalation toxicology, will be shown preference. Salary is negotiable, depending on qualifications. Curriculum vitae and names of three references should be sent before 30 June 1985 to: **Dr. Robert G. Meeks, Chairman, Search Committee, Department of Environmental Health Sciences, School of Public Health, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294.** *An Equal Opportunity/Affirmative Action Employer.*

(NW1859)A

WASHINGTON, DC

Immediate and Fall Post-Doctoral Research Positions. Past Interest and knowledge of the Molecular Biology of the nucleus desirable. To combine gene cloning and chromatin biochemistry to study Poly (ADP-ribosylation) involving replication, gene expression and nuclear oncogene proteins. Collaboration with leading labs at NIH.

Send curriculum vitae, reprints and names of three references to: Dr. Mark Smulson, Dept. of Biochemistry, Georgetown Univ. Schl. of Medicine, Washington, DC 20007.

(NW1853)A

RESEARCH ASSISTANT

Immediately available in protein chemistry. Qualified applicants should have BS or MS in chemistry or biochemistry. Experience with protein purfication and analytical instrumentation (HPLC, protein sequencer is preferred.

Send cv and 3 letters of reference to Personnel Manager, W. Alton Jones, Cell Science Center, 10 Old Barn Road, Lake Placid, New York 12946 refer to job order — CR-8506. (NW1845)A

POSTDOCTORAL POSITIONS

Two postdoctoral positions available August 1, 1985 for studies of the mechanisms of senescence of differentiated cells in culture and mechanisms of immortalization in neoplastic transformation. PhD or MD required with training in cell and molecular biology

Send curriculum vitae and the names of two references to Dr. Peter J. Hornsby, Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912. EOE/AAP J2900. (NW1842)A

POSTDOCTORAL POSITION

Recently formed multidisciplinary group with stable long-term funding seeks PhD or MD to participate in studies of the role of DNA topoisomerase in tumor biology and cancer chemotherapy. Investigations at the molecular/cellular level will be coordinated with those examining the basis for tumor responsiveness to established and newly synthesized topoisomerase-mediated anticancer drugs. Salary negotiable.

Send curriculum vitae, three reference, and brief statement of research interests and experience to: Warren Ross, MD, or Tom Rowe, PhD Box J-267, JHMHC, University of Florida, Gainesville, Fla. 32610.

(NW1843)A

Boehringer Ingelheim

Research Opportunities in the Field of CNS Pharmacology

for a PHYSICIAN, BIOLOGIST or BIOCHEMIST in WEST GERMANY

The Boehringer Ingelheim Company is an internationally oriented pharmaceutical company with a range of pharmaceuticals originating largely from its own research and development.

For our C N S Research Group we are looking for an enthusiastic scientist with a strong experimental background in physiology, biochemistry or pharmacology.

It is our aim to identify new therapeutic principles. We are seeking to achieve this goal through interdisciplinary research in chemistry, biochemistry, molecular biology and genetic engineering.

Special knowledge in the areas of biochemical psychopharmacology or electrophysiology would be of considerable benefit.

For further information please contact Professor Jennewein Tel. 06132/77-2135.

Applicants should send their full C.V. to

Boehringer Ingelheim KG, Personalabteilung
D-6507 Ingelheim am Rhein/West Germany (W1950)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals, Houndmills, Basingstoke, Hants. RG21 2XS, UK
or
Paul Siman, Bio/Technology, 65 Bleecker St., New York, NY 10012.

PHILIPPS-UNIVERSITÄT MARBURG

Im Fachbereich Humanmedizin am Institut für Normale und Pathologische Physiologie ist eine

Professur (C4)

für Physiologie

zu besetzen. Der Bewerber soll das Gesamtfach Physiologie in der Lehre vertreten Können und wissenschaftlich international ausgewiesen sein.

Fur die Ernennung zum Professor sind grundsatzlich padagogische Eignung und — als Nachweis der Befähigung zu wissenshaftlicher Arbeit — neben der Promotion die Habilitation oder gleichwertige wissenschaftliche Leistungen erforderlich. Im übringen gelten die Einstellungsvoraussetzungen des § 39 a Hug.

Bewerbungen mit den ublichen Unterlagen (das Schriftenverzeichnis ist nach Originalarbeiten, Übersichtsartikel und Fallstudien zu gliedern. Bei gemeinschaftlichen Veroffentlichungen soll der Bewerber ausführen, worin seine Miturheberschaft besteht) sind bis zum *19.7.1985* zu richten, an den *Präsidenten der Philipps-Universität Marburg, Biegenstraße 10, D 3550 Marburg.* (W1930)A

CSIRO
AUSTRALIA
RESEARCH FELLOW
$A26,001 - $A38,243
DIVISION OF ATMOSPHERIC RESEARCH
ASPENDALE VIC

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists The Organization's research activities are grouped into five Institutes Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Atmospheric Research is a Member of the Institute of Physical Sciences.

GENERAL: The Division of Atmospheric Research has a staff of 130 including 70 professional scientists and undertakes research into physical and chemical aspects of atmospheric phenomena and related areas. The Clouds and Radiation Program in the Division is concerned with aircraft, lidar and satellite observation of various atmospheric and surface characteristics as well as related theoretical studies of clouds (in particular) as they control the energy input to the earth's atmosphere system Particular emphasis at this time is on satellite observations using the new CSIDA facility for satellite reception and data analysis

DUTIES. Although there will be considerable freedom to pursue the appointee's own ideas, he/she will conduct research in one or both of two related areas.

(a) use of satellite radiance data to abstract real information on atmospheric and surface phenomena and

(b) use of various lidar techniques for analysis of upper level clouds and aerosols

The appointee will be expected to relate the work to the general problem of climate modelling and address such questions as the radiative control of deserts and drought

QUALIFICATIONS· A PhD degree or equivalent qualifications in a related field and strong evidence of independent research ability

TENURE. A term of three years, with the possibility of a further term of 2 years Australian Government superannuation benefits available

APPLICATIONS. Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A1827, should be directed to·

The Chief
CSIRO Division of Atmospheric Research
Private Bag No 1
MORDIALLOC VIC 3195
AUSTRALIA

by 4 weeks from date of publication

CISIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1945)A

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW,
MIDDLESEX, HA1 3UJ

POST DOCTORAL FELLOW IN MOLECULAR BIOLOGY

Applications are invited from science graduates for a 3 year postdoctoral position in the Molecular Medicine Research Group to work on the molecular basis of atherosclerosis. The work will be particularly concerned with growth factor and apolipoprotein genes A minimum of 3 years experience in molecular biology is essential.

Salary scales are related to those for university non-clinical academic staff and salary on appointment will depend on age and experience Informal enquiries may be made to Dr James Scott (Head of Group) on 01-864 5311 Extn 2257.

Application forms and further details may be obtained by writing to Miss B.A. Shaw, Personnel Section quoting reference 111/1/4267.

Closing date for application 4th July 1985. (6780)A

UNIVERSITY OF ZIMBABWE
DEPARTMENT OF PHYSICS
(2 posts)

LECTURESHIPS/SENIOR LECTURESHIPS/ ASSOCIATE PROFESSORSHIP

Post A: Applicants should have a BSc Honours degree in Physics but preferably a PhD. The successful candidate is expected to teach at both undergraduate and postgraduate levels, as well as supervise MPhil and DPhil students in relevant research area

Post B. Applicants should have a BSc Honours degree in Physics but preferably PhD The successful candidate must be competent to teach Electronics to BSc undergraduates as well as supervise postgraduate research in Electronics Preference will be given to a candidate with some proven industrial experience.

Salary scales: Lecturer: Z$11,880 -Z$20,076, Senior Lecturer. Z$20,580 - Z$23,400, Associate Professor: Z$21,084 - Z$23,904 (including allowance).

Appointment on the above scales according to qualifications and experience.

Conditions of Service: Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on short-term contract basis with an initial period of two years Short-term contracts may, in exceptional cases, be extended.

Six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees should be addressed to the Director, Appointments and Personnel, University of Zimbabwe, P O Box MP 167, Mount Pleasant, Harare, Zimbabwe (Telex: 4-152 ZW). Candidates in the UK should also send an additional copy to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may be obtained

Closing date for receipt of applications is 12 July 1985.

(W1943)A

POSTDOCTORAL RESEARCH ASSOCIATE

A 2-year position is offered to study the effects of recombinant tumour necrosis factor in vivo and in vitro.

Potential areas of research include therapy in murine models, regulation of tumour cell sensitivity and regulation of tumour necrosis factor activity. Applicants should have a Ph D with experience in cellular immunology and biochemistry.

Send curriculum vitae and brief summary of research experience to German Cancer Research Centre (DKFZ), Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG. (W1932)A

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth
DEPARTMENT OF SURGERY

RESEARCH OFFICER/RESEARCH FELLOW

Applications are invited fro scientists who have completed a Phl degree in experimental anatomy c pathology, for a post-doctora fellowship in experimental opthalm pathology, in particular related to th neuro-anatomy of the macula Funding will be guaranteed for thre years in the first instance bu applicants should consider it as a opportunity to establish their ow laboratory and develop a long-ter career as a principal investigator Th Opthalmic Research Unit, Depar ment of Surgery, University c Western Australia is currentl relocating into the Lions Ey Institute, Queen Elizabeth II Medica Centre This facility consists of 6 modernised laboratories and office which will provide a unique mix c patient care, animal studies and basi research laboratories all in the sam geographic area. The unit ha extensive interests in retinal an vitreous disease and has active labor atories based on neurophysiology biochemistry and cell culture. Th successful applicant will collaborat closely with the Head of the Uni Professor Ian Constable, on anima and human studies of the macula an also with Associate Professor Joh Papadimitriou, Head of Departmen of Pathology, University of Wester Australia on new technology relevan to electron microscopic analysis o macular disease. Enquiries fo further information should b directed to Professor Constable a (09) 325:0101, extension 2822.

Current salary ranges: Researcl Officer — $A20,003 - $A28,306 pe annum; Research Fellow - $A26,236 $A34,467 per annum. Benefit include fares to Perth for self an dependent spouse Conditions o appointment will be specified in an offer of appointment which may b made as a result of this advertise ment

Applications in duplicate statin full personal particulars, qualifi cations and experience and the name and addresses of three referee should reach the Staffing Officer University of Western Australia Nedlands, Western Australia 6009 by 31st July 1985 (W1929)A

CHEMICAL ENGINEER

Conduct research of Asiatic herbs demonstrated to be beneficial to the prevention & treatment of metabolic disorders using English & Chinese lang. publications; perform chem analysis of herbs to identify active ingredients, use lab. eqpmt. & chem. extraction techniques incl. solvent & centrifuge extraction & steam distillation to process herbs to high purity compounds or active components. Req. PhD in Chemical Engr 4 yrs exp. in job offered & proficiency in Chinese $32,500 yr., job in San Francisco Send this ad & résumé to job no 4516, PO Box 865, Sacramento, Ca 95804 not later than June 28, 1985.

(NW1841)A

FERMENTATION RESEARCH

John & E Sturge Limited, a member of the RTZ Chemicals Group, wish to appoint a person to participate in an ongoing programme of fermentation research for processes for the production of organic acids, enzymes and other products. The work will be conducted in our Research Department, on the site containing our large scale fermentation facilities in Selby, Yorkshire, where citric acid and enzymes are currently produced.

The successful applicant will have obtained at least a 2 i) Honours Degree in microbiology or a related discipline, and would preferably have academic or industrial post-graduate experience in microbial physiology. The person appointed will be paid a salary commensurate with his/her qualifications and the responsibilities of the post. Assistance with relocation will be given in appropriate cases.

Sturge Biochemicals a member of the RTZ Group

Please send a detailed c.v. to Dr. W.J.E. Gardner, John and E. Sturge Ltd., Denison Road, SELBY, North Yorkshire, YO8 8EF. Previous applicants need not re-apply. 6698)A

The W. Alton Jones Cell Science Center
invites applications for the position of

Assistant Scientist

in the protein chemistry laboratory. Candidates should be willing to collaborate closely with the recombinant DNA laboratory and with several tissue culture laboratories that are engaged in the isolation, characterization and modification of bioeffectors produced by various differentiated cell types.

Expertise in modern microtechniques of protein sequencing is expected. Synthetic peptides will be used for structure-function studies in cell cultures and for raising antibodies.

Applicants should send curriculum vitae and references to: Personnel Manager. W. Alton, Jones Cell Science Center, 10 Old Barn Road, Lake Placid, New York 12946. (NW1844)A

The Waksman Institute of Microbiology, the Department of Chemistry, and the Department of Biochemical Engineering of Rutgers, The State University of New Jersey, invite applications and nominations for senior professorial positions in

PROTEIN ENGINEERING
PROTEIN CHEMISTRY
MACROMOLECULAR CRYSTALLOGRAPHY

at the Institute on the New Brunswick/Piscataway campus.

The successful candidates will join a multidisciplinary and rapidly expanding group of investigators in the study of biomolecular structure, function, and design. We seek distinguished leaders with demonstrated ability and commitment to conduct vigorous research programs, to interface with molecular biology, to teach at the graduate level, and to assume other usual academic responsibilities. Because of the interface with molecular biology, two of the positions, protein engineering and protein chemistry, will be associated with a curatorship of two resource facilities, the fermentation laboratory, F1, and the biomolecular instrumentation laboratory, F2. F1 will be equipped with new microprocessor controlled vessels and state-of-the-art analytical equipment. F2 will run and maintain an oligonucleotide synthesizer, a peptide synthesizer, an amino acid analyzer, a high resolution protein sequenator, and HPLCs. Access to the supercomputer at the newly established John von Neumann Center for Scientific Computing, to several VAX computer systems, and to computer graphics equipment provides excellent computational facilities for this program. Salary and rank will be commensurate with experience, background and professional qualifications.

Applications must include an expression of interest in the position, and up-to-date publication list, a *curriculum vitae,* and confidential letters of recommendation from five professional references. Applications will be accepted until the position is filled.

Applications and nominations may be directed to:

Professors Joachim Messing and Wilma K. Olson
Chairpersons
Biomolecular Structure
Program Search Committee
Waksman Institute of Microbiology
Rutgers, The State University
PO Box 759, Piscataway, New Jersey 08854-0759

Rutgers University is an Equal Opportunity/Affirmative Action Employer
(NW1849)A

Nous sommes un important groupe pharmaceutique international et en expansion.
Pour faire face à l'extension de ses objectifs, notre R. & D., située à Braine l'Alleud près de Bruxelles, recherche 3 collaborateurs ou collaboratrices de haut niveau:

UN(E) BIOCHIMISTE (senior)
UN(E) PHARMACOLOGUE (généraliste)
UN(E) NEURO-PHARMACOLOGUE (physiologie et comportement)

Ils sont appelés à animer et diriger une ou plusieurs équipes de recherche et d'évaluation de produits nouveaux ainsi que des projets de recherche impliquant des contacts multidisciplinaires internes et externes.

Les candidats recherchés doivent être porteurs d'un diplôme de Doctorat en Sciences ou équivalent; une expérience confirmée de plusieurs années dans la spécialisation indiquée est requise.

La connaissance courante du français et de l'anglais est requise.

Les candidatures, accompagnées de C.V., sont à adresser à:
UCB s.A. - Direction du Personnel/Développement
326 Avenue Louise-Bte 7 - B-1050 BRUXELLES

(W1949)A

FACULTY POSITION IN IMMUNOPHARMACOLOGY

The School of Pharmacy, University of California at San Francisco seeks an outstanding scientist for a tenure track position at the Assistant or beginning Associate Professor level in the Department of Pharmacy. We are particularly interested in an individual whose research interests will complement the Departmental strengths in drug delivery and pharmacodynamics. Specific areas of interest might include immunomodulation for therapeutic purposes, drug targeting by antibodies, the development of immunologic therapeutic agents, the development of immunologic indicies to monitor drug efficacy and toxicity of the formulation of mathematical models of the immune system.

Applicants should have a Ph.D., M.D. or M.D./Ph.D. and be committed to develop or continue a first-rate active research program. Teaching responsibilities will be in the professional and graduate curriculum.

Applicants should submit curriculum vitae, three letters of reference, and a statement on present and future research interests by August 15, 1985 to: Dr. Francis C. Szoka, Jr., School of Pharmacy, 926-8 University of California, San Francisco, CA 94143. ***UCSF is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply***

(NW1829)A

UNIVERSITY OF GUELPH

COLLEGE OF BIOLOGICAL SCIENCE

Pre- and post-doctoral positions available for training in the Molecular Biology of post transcriptional control with special emphasis on structure and function of mRNP complexes and biogenesis of ribosome particles in procaryotic and eucaryotic systems Studies of this laboratory are directed to examining molecular events which occur during changes in growth rate and differentiation. Teaching opportunities available. Stipends for post-doctoral fellows are $17,500 to $21,000 and according to departmental guidelines for pre-doctoral fellows.

Please send résumé and names of three referees to:

Bruce H. Sells
College of Biological Science
University of Guelph
Guelph, Ontario
Canada N1G 2W1

(NW1848)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

SENIOR RESEARCH ASSISTANT

Applications are invited for the above postdoctoral position to work on the molecular biology of the expression of mammalian proteinases. The project is specifically concerned with the regulation of genes coding for kidney brush border endopeptidases. This post is funded by an SERC grant awarded to Dr R J Beynon and Dr P C Turner and is tenable for up to three years. Further information on the project may be obtained from Dr Beynon or Dr Turner on 051-709 6022, ext 2757 or 3183.

Initial salary within range £7,520 — £8,450 per annum (under review). The starting date is nominally 1 October, but may be open to negotiation for an outstanding applicant. Applicants should preferably be experienced either in protein chemistry/enzymology or gene manipulation

Applications, together with the names of three referees, should be received not later than 27 June, 1985 by the Registrar, The University PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/298/N.

(6701)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

DEPARTMENT OF SURGERY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post, supported by the Wellcome Trust, for three years The project is to study proteins of the endoplasmic reticulum membrane involved in intracellular transport and recognition, and will involve photoaffinity labelling, isolation of membrane receptor proteins, in vitro transcription/translation and preparation of DNA probes. Experience with membrane protein isolation or molecular biology would be an advantage, and the applicant should possess or expect to obtain shortly a PhD in biochemistry or related discipline.

Salary is on the IA scale for Research and Analogous Staff, with London Weighting, according to age and experience.

Applications including a curriculum vitae and the names and addresses of two referees should be sent to Dr B M Austen, Department of Surgery, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE (Tel 672 1255 Ext 5150) Closing date 4 July 1985.(6809)A

MIDLAND CENTRE FOR NEUROSURGERY AND NEUROLOGY

DEPARTMENT OF PATHOLOGY

BIOCHEMIST IN CHARGE OF MUSCLE LABORATORY

(Senior/Principal Grade)

required on a locum basis (available grading up to Principal as defined by Whitley Council PTA regulations). The muscle laboratory is part of the Clinical Biochemistry Department but has close links with other sections (Electron Microscopy, Histopathology etc) as part of a comprehensive diagnostic service in muscle, central and peripheral nervous system pathlogy, to Hospital and Region. Clinical Biochemistry is a well-housed department, with nine staff at present, including five graduate scientists, one of whom is mainly engaged on muscle chemistry. Equipment includes two Ultra centrifuges, isotope counting facilities, fluorimeter and a spark camera for autoradiography. The successful candidate will be requried to continue and develop a diagnostic biochemistry service for the investigation of human metabolic muscle disease. Collaboration with current research projects will be expected, and there is an excellent opportunity to develop a personal research programme with the readily available material

Intending applicants are welcome to visit by appointment with Mr R A Westhead, (Principal Biochemist) 021-558 3232, ext 257.

Job description and application forms from: The Administrator, The Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick, Warley, West Midlands B67 7JX.

Tel: 021-558 3232, ext 251.

(6826)A

UNIVERSITY OF NOTTINGHAM
Medical School

DEPARTMENT OF COMMUNITY HEALTH

TEMPORARY LECTURER OR RESEARCH FELLOW IN BEHAVIOURAL SCIENCE/HEALTH EDUCATION

A two year position of Temporary Lecturer or Research Fellow, with possible extension, is offered to undertake research on the development and implementation of an interactive computer based system of health risk appraisal for use in health assessment and education. Candidates should have expertise in the conduct of evaluative studies, and interests in health education, epidemiology, and disease prevention. Salary on the appropriate scale.

Potential applicants wishing to discuss the position or to receive further information and application forms should write to, or telephone, Professor J M Elwood, Department of Community Health, Queen's Medical Centre, Nottingham NG7 2UH; telephone 0602 700111, ext 3561. Closing date for applications 12th July 1985. (6778)A

UNIVERSITY OF SURREY

DEPARTMENT OF BIOCHEMISTRY

RESEARCH OFFICER

Applications are invited for two pos of Research Officer which a available imnmediately in th Department of Biochemistry. Fo both posts, applicants must have good degree in biochemistry or related subject.

Post 1 The Research Officer wi work with Mr B A Morris, Dr M Clifford and Dr B J Gould on a foo analysis project funded by th Ministry of Agriculture, Fisheri and Food. The objective is to develo an immunological method of analys capable of supporting legislation th controls which enzyme preparatio may be used as food additives. Th project should provide a challenge relently qualified young scientis interested in applying their skills this relatively new field. Previo experience in protein separatio immunoassay, especially ELISA, a knowledge of food manufacturi processes would be an advantag Further details of the post may b obtained from Mr Morris on 048 571281 ext 823 or Dr Clifford on e 435. Salary up to £7,520 per annu depending on age, qualifications an experience.

Post 2 A Research Officer required to work on a project funde by the Water Research Centre. Th project involves the production antibodies to pesticides and oth pollutants and the development non-isotopic immunoassay tec niques appropriate to the Wat Industry. Further details of the po may be obtained from Dr W Aher on 0483 571281 ext 497. Salary up £7,980 per annum depending on ag qualifications and experience.

Both posts are available for period of two years.

Applications in the form of curriculum vitae (3 copies) an including the names and addresses two referees, should be sent to th Personnel Office (JLG), Universi of Surrey, Guildford, Surrey, GU 5XH by 24th June 1985 quotin reference 398/N. Previous applican need not reapply. (6793)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSIOLOGY

Applications are invited for the post of

LECTURER IN PHYSIOLOGY

The Department is particular seeking a physiologist who is veterinary graduate. It woul however, also be interested receiving applications fro candidates with a medical degree o research interest in cardiac musc The salary will be within the ran £7,520 - £14,925 per annum togeth with superannuation.

Applications including the nam and addresses of two referees, shou be sent by 28th June 1985, to t Registrar and Secretary, Universi of Bristol, Senate House, Bristol B 1TH, from whom further particula may be obtained. Please quote ref ence TLJ (6772)A

THE UNIVERSITY OF THE SOUTH PACIFIC

LECTURER I/SENIOR LECTURER IN ANIMAL SCIENCE — Post 85/33

Applications are invited for the above post in the School of Agriculture located at the Alafua Campus in Western Samoa. The appointee will take charge and direct the work of the Animal Science section of the School and will be responsible for the co-ordination of the teaching and research of two other members of the lecturing staff who jointly offer courses in animal science to students reading for the Diploma in Tropical Agriculture, the Bachelor of Agriculture and the Master of Agriculture by research. He will in addition be responsible for operation and further development of the School's livestock enterprises for demonstration, research and commercial purposes. Applicants should possess a first degree in Agriculture, Animal Science or Veterinary Science with postgraduate qualifications, preferably at doctoral level, in a relevant field of animal science. Research interests in the field of reproductive physiology would complement those of the present staff and meet the needs of the region for improvements in this area.

Salary will be in accordance with qualifications and experience in the Lecturer I scale: F$13,921-18,919 or Senior Lecturer scale: F$19,525-22,815 (£1 = F$1.48 on 28/5/5). In addition the University provides gratuity amounting to 15% of basic salary, appointment allowance and, subject to the University's current housing policy partly furnished accomodation at a rental of 12½% of salary. The University will contribute a sum equivalent to 10% of the appointee's salary towards his superannuation obligations. Appointment will be for a contract period of three years and may be renewable by mutual agreement. Candidates should send three copies of their curriculum vitae with full personal particulars, names and addresses of three referees and date of availability, to the Secretary, The University of the South Pacific School of Agriculture, Alafua Campus, PO Box 890, Apia, Western Samoa, to reach him no later than 19 July 1985. Candidates in UK should also send an additional copy to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1935)A

RESEARCH POSITIONS

1) Postdoctoral Associate and 2) Research Assistant (BS or MS) positions available in a research group working with RIBOSOMAL PROTEIN GENES AND cDNA EXPRESSION LIBRARY from higher plants. DNA cloning and/or sequencing experience required. Write to: Dr Alap Subramanian, Group Leader, Max-Planck-Institut fur Molekulare Genetik, Abt Wittmann, Ihnestr 63, Berlin-Dahlem, Fed Rep Germany. (W1933)A

THE NATURE CONSERVANCY COUNCIL

HIGHER SCIENTIFIC OFFICERS

3 contract posts with a duration of up to 2 years in the Geological Conservation Review Unit.

Applications are invited from specialists in the following subjects:

1 Vertebrate Palaeontologist (fish)

2 Palaeobotanist/Westphalian Stratigrapher

3 Pleistocene River Terrace Stratigrapher

The successful candidates will be required to undertake the assessment, selection and documentation of key earth science sites.

Qualifications and experience. Applicants should have a first or second class honours degree in an earth science, with at least three years PhD experience in the specified discipline. Candidates should also have undertaken relevant post-doctoral research and have experience in conservation. A current driving licence is essential.

Salary, depending on qualifications and experience. £7,435 rising to £10,039 per annum.

The posts will initially be based in Newbury but will be moved to Peterborough in 1986.

For an application form and further particulars please contact Miss B A Meredith, Personnel I, Nature Conservancy Council, Northminster House, Peterborough, PE1 1UA. Closing date for receipt of completed forms is 10 July 1985.

The Nature Conservancy Council is an equal opportunity employer. (6801)A

UNIVERSITY OF KEELE

DEPARTMENT OF BIOLOGICAL SCIENCES

DEMONSTRATORSHIPS IN ZOOLOGY AND BIOCHEMISTRY

Applications are invited from individuals with some research and, ideally, teaching experience for the posts of Demonstrator, initially for one year from October 1985, at a salary of £6,600 per annum, together with membership of the USS. For the Demonstratorship in Zoology, candidates should have a good degree in zoology or biological science. Applicants with interests in helminthology (especially the biology of metacestodes) or behavioural endocrinology will be given preference, but other areas are not excluded. For the Demonstratorship in Biochemistry, candidates should have a good degree in biochemistry or cell biology and should be prepared to participate in existing biochemistry research projects in the department.

Further particulars and application forms are available from the Registrar, University of Keele, Keele, Staff, ST5 5BG to whom completed applications should be returned before Friday 5 July 1985. (6827)A

CSIRO

AUSTRALIA

RESEARCH FELLOW

BIOTECHNOLOGIST

$A26,001 - $A38,243

DIVISION OF TEXTILE INDUSTRY GEELONG VIC

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Textile Industry is a member of the Institute of Industrial Technology.

FIELD: Microbiology, biotechnology, surface chemistry.

GENERAL: The Division of Textile Industry conducts research aimed at improving the utilization of Australian wool in the world textile industry. It undertakes physical and chemical investigations related to the conversion of raw wool for mill processing, improving manufacturing techniques and equipment, reducing the environmental impact of textile-processing, and improving end product performance.

DUTIES: The appointee will conduct research to establish the potential of biotechnology systems in wool-scouring effluent treatment processes and for modfying fibre characteristics. The appointee will be part of a multidisciplinary team which has extensive knowledge of wool processing and access to processing facilities.

QUALIFICATIONS: A PhD degree or equivalent qualifications in a relevant field of science plus demonstrable research ability. Applicants should possess a sound knowledge of microbiology and/or biotechnology and surface chemistry, in particular techniques related to the identification and optimisation of active species in broad enzyme systems.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No C8603, should be directed to:

The Chief
CSIRO Division of Textile Industry
PO Box 21
BELMONT VIC 3216
AUSTRALIA

by 4 weeks from date of publication

CISIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W1946)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited for this post to work with Dr J Kay (in collaboration with Dr B M Dunn, University of Florida Medical School) on structure/activity relationships in aspartic proteinases and the selectivity of synthetic and naturally-occurring inhibitors. Duties to commence as soon as possible.

The appointment is for one year in the first instance, with a possible renewal for a second year. Salary range Grade 1B £6,600 - £7,055 per annum (under review). Research expertise with fractionation techniques including HPLC and experience of kinetic procedures would be an added advantage.

Applications (2 copies), together with the names and addresses of two referees should be forwarded to the Establishment Officer, University College, PO Box 78, Cardiff CF1 1XL. Closing date 4th July, 1985. Ref: 2956. (6775)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GENETICS

POSTDOCTORAL RESEARCH FELLOW

Applications are invited from persons with relevant research experience in genetics physiology or metabolism biochemistry to develop a programme on the quantitative and physiology of growth and metabolism using selected lines of mice The project is supported by the AFRC in collaboration with the AFRC's Animal Breeding Research Organisation and Poultry Research Centre and is supervised by professor W G Hill.

The salary will be on the Research Associate 1A scale, minimum £7,520 pa. The appointment will be for three years in the first instance.

Further information can be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH8 9YL to whom applications, together with CV and names and addresses of three referees should be sent, quoting ref 5257. (6818)A

Applications are invited for a position in neurophysiology at the Assistant Professor level in the Department of Physiology at the University of Manitoba Faculty of Medicine. Candidates should have a PhD or equivalent, with two or more years of post-doctoral training. Preference will be given to individuals with experience and a continuing interest in electrophysiological analysis of neural networks and/or neurotransmitter actions using *in vitro* or *in vivo* systems. Send curriculum vitae, an outline of future research plans, and arrange for three letters of reference to be forwarded to: **Dr. Henry G. Friesen, Professor and Head, Department of Physiology, The University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0WE Canada.** Deadline for receipt of applications is 1 September 1985. In accordance with Canadian immigration requirements this advertisement is directed to Canadian citizens and permanent residents. (NW1857)A

TRUDEAU INSTITUTE

VISITING INVESTIGATORS

The Trudeau Institute has openings for Visiting Investigators interested in spending up to two years working in the field of cancer immunology. The salary will range from $20,000 to $25,000, depending on experience University researchers interested in spending a sabbatical, or medical doctors wishing to obtain experience in a research laboratory are encouraged to apply

The Trudeau Institute is a not-for-profit pure research institute situated on the shore of Saranac Lake, 8 miles from Lake Placid, in the Adirondack Mountains of New York.

Send curriculum vitae and names of 3 references to: Dr. Robert J. North, Director, Trudeau Institute, Inc, PO Box 59, Saranac Lake, NY 12983. (NW1823)A

An Equal Opportunity Employer

Re-Advertisement

HOUGHTON POULTRY RESEARCH STATION

Grant-Aided by the Agricultural & Food Research Council

IMMUNOLOGIST

Applications are invited from immunologists to join a group studying cellular interactions in the immune system of chickens with the long term objective of providing a rational basis for the development and presentation of vaccines, particularly novel vaccines produced by genetic engineering techniques. The initial objective is the production and application of monoclonal antibodies specific for lymphocyte populations and subpopulations and the development of in vitro assays for soluble mediators such as interleukins.

Applicants should have a first or upper second class honours degree in a biological subject and preferably a PhD, with at least two years relevant post-graduate experience.

The appointments will be made in the Higher Scientific Officer grade, salary: £7,435 × 6 to £10.039.

The position is available for a period of three years, commencing on a date to be arranged prior to October 2nd, 1985.

For further details and application forms please contact the Secretary, John Andrews at Houghton Poultry Research Station, Houghton, Huntingdon, Cambs, PE17 2DA, tel St Ives (0480) 64101. Closing date for applications is 12th July 1985.

The Houghton Poultry Research Station is an Equal Opportunities Employer. (6802)A

UNIVERSITY OF NEWCASTLE UPON TYNE

READERSHIP IN CANCER PATHOLOGY

Applications are invited for an established Readership in Cancer Pathology in the Department of Pathology, jointly funded by the North of England Cancer Research Campaign and the University. Candidates should have substantial experience and interests in research in Cancer Pathology and must also have a good working knowledge of experimental techniques in molecular biology as applied to cancer research. The possession of a medical qualification and of the membership of the Royal College of Pathologists would be preferred, but other candidates with appropriate qualifications and experience will be considered. The successful candidate will be expected to take part in general departmental and teaching duties, under the direction of the Head of the Department.

Salary will be at a point on either the clinical Reader's scale or the non-clinical Reader's scale, with membership of the appropriate superannuation scheme. An appropriately qualified clinical appointee contributing at least two sessions to the clinical service would be offered honorary consultant status by the Newcastle Health Authority.

Applications (15 copies), giving the names of three referees must be lodged with the Senior Assistant Registrar, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, not later than 12 July 1985, from whom further particulars can be obtained. (Applicants from outside the British Isles may submit one copy only). (6779)A

THE UNIVERSITY OF NEW SOUTH WALES

Sydney, Australia

LECTURER IN SCHOOL OF ANATOMY

Applicants should have a degree in medicine or relevant science and appropriate postgraduate qualifications Clinical experience desirable. Duties will include teaching gross anatomy and at least one other subject, and undertaking research relevant to the School's interests

In addition to salary, a paraclinical loading of $A6,558 per annum will be paid to a medically qualified appointee.

Appointment will be on the basis of a contract with provision for conversion to tenure

Further information from Professor F W D Rost, Head of School (02) 697-2481 or c/o University of New South Wales, PO Box 1 Kensington, NSW 2033, Australia.

Applications close 8 July 1985

Salary: $A26,236 range $A34,467. Commencing salary according to qualifications and experience.

For further information about conditions of appointment and method of application write to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Equality of employment opportunity is University policy. (W1942)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF IMMUNOLOGY

SENIOR TECHNICIAN

required for large scale production of monoclonal antibodies for in vivo therapy by large scale screening, and investigation of reagent combinations on selected leukaemias and lymphomas.

Salary range £7,521 — £9,935 (under review) plus £1,042 London Allowance. 5-year appointment.

Further particulars and application form available from the School Office, RFHSM, Rowland Hill Street, London, NW3 2PF (010-794 0500 Extn 4262). Closing date 5th July 1985. Please quote reference J/ST. (6824)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

RESEARCH ASSISTANT & RESEARCH TECHNICIAN (2 POSTS)

Applications are invited for a Research Assistant, Grade RA 1A, and a Grade 3 Research Technician to work on a project investigating neuronal 5-hydroxytryptamine receptors using in vitro electrophysiological methods. The posts are funded by the Wellcome Trust and both are for 3 years. Commencement of duties to be arranged.

Applicants for the Research Assistant post should have a good Honours degree in a relevant biological science, but Post-doctoral applicants will be considered. Experience of electrophysiological techniques will be an advantage. Salary Grade 1A £7,520 per annum.

Applicants for the Grade 3 Technician post should have as minimum qualifications ONC, TEC or equivalent or a degree in biological science. Starting salary will be in the range £5,399 - £5,701 per annum. Some experience in the field of electrophysiology and/or pharmacology will be an advantage.

For further details, write or telephone Professor D I Wallis, (0222) 44211 Ext 2259.

Applications (2 copies) giving details of age, qualifications and experience, together with the names and addresses of two referees, should be forwarded to the Establishment Officer, University College, PO Box 78, Cardiff, CF1 1XL. Closing date 4th July, 1985. Ref: 2954. (6774)A

UNIVERSITY OF OXFORD

DEPARTMENT OF AGRICULTURAL SCIENCE

SOIL CHEMIST

A post-doctoral research assistant with experience of soil chemistry and the simulation of complex dynamic systems is required as soon as possible to carry out research under the supervision of Mr P H Nye on the development and testing of a model of nitrogen losses from soil by ammonia volatilization. The post is for two years on salary scale 1A, and candidates should preferably be aged 28 or under. Applications, including a curriculum vitae and the names of two referees, should be sent by 28 June to the Administrator, Department of Agricultural Science, Parks Road, Oxford, OX1 3PF. (6764)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

LECTURESHIP IN BIOCHEMISTRY

Applications from candidates with appropriate qualifications and research interest in Molecular Cell Biology are invited for a Lectureship which is available from 1 October 1985 in the above Department, in which Professor G B Warren has recently been appointed to the Chair of Biochemistry.

Salary scale currently £7,520 - £14,925 but due for review. Initial placing dependent on qualifications and experience of the appointee. Grant towards removal expenses to Dundee.

Applications, containing full career details and the names of three referees should be lodged as soon as possible with the Personnel Officer, The University, Dundee, DD1 4HN. Please quote reference EST/18/85J. (6768)A

THE UNIVERSITY OF SYDNEY

CHALLIS CHAIR OF ANATOMY

Reference No 19/02

Applications are invited from suitably qualified candidates for the Challis Chair of Anatomy. The Chair has become vacant following the retirement of Professor M J Blunt.

Applicants should have a wide experience in the teaching of Anatomy and should be familiar with the requirements of teaching Anatomy to medical and dental students. Candidates will be expected to have an established reputation in research and to be actively engaged in research in anatomy or related field

Possession of a medical degree is preferred. Non-medical graduates are also invited to apply.

Salary will be at the rate of $AS54,948 per annum plus a pre-clinical loading of $A4,919 per annum where appropriate.

Applications in duplicate, quoting reference no, including curriculum vitae, list of publications and the names and addresses of three referees, by 12 July 1985, to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information is available.

The University reserves the right not to proceed with any appointment for financial or other reasons.

Further information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

(W1936)A

THE SCHOOL OF PHARMACY

University of London

CANCER RESEARCH CAMPAIGN PROTEIN SEQUENCING FACILITY

RESEARCH ASSISTANT

Applications are invited from persons suitably qualified in protein chemistry and/or biochemistry to manage the Applied Biosystems gas phase automatic protein sequencer facility funded by the CRC This new centre for microsequencing proteins and peptides related to the study of cancer has the latest design of on-line automatic HPLC equipment and computerised data storage. It is located in the Department of Pharmaceutical Chemistry, which has excellent facilities to pursue multi-disciplinary research in modern chemistry, biochemistry and molecular biology.

Applicants should be graduates (or equivalent), preferably with PhD, and have experience in protein chemistry and in running similar equipment. The successful candidate will be encouraged to pursue a research programme towards a higher degree or at postdoctoral level.

Appointment will be in the Research Staff 1A range (£7520 — £12150, plus London Allowance of £1233), at a point commensurate with qualifications and experience

Further details may be obtained from Dr Alastair Aitken, on 01-837 7651, be sent to: Personnel Officer, School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, to arrive by 5 July 1985.

(6786)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF IMMUNOLOGY

SENIOR RESEARCH FELLOW

(Senior Lecturer grade) IN CLINICAL IMMUNOLOGY

required for co-ordination of immunotherapy with monoclonal antibodies and cyclosporin-A in patients undergoing kidney, bone marrow (BMT), heart and liver transplantation This is an opportunity for a physician to specialize in Clinical Immunology including aspects of immunotherapy.

Salary range £16,240 — £22,620 (under review) plus £839 London Allowance 5-year appointment.

Further particulars available from the School Office, RFHSM, Rowland Hill Street, London, NW3 2PF (01-794 0500 Extn 4262) to which applications (full curriculum vitae and names and addresses of two referees) should be sent by 5th July 1985 Please quote reference IMM/SRF.

(6822)A

TECHNICIAN or EMBRYOLOGIST with experience in mammalian embryology, required to work for IVF clinic in Athens. Salary negotiable. Interested persons should write in confidence to Dr H G Massauras, 3 Marasli Street, Athens 106-76, Greece. (W1920)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSICS

Applications are invited for the post of

EXPERIMENTAL OFFICER

to work with a research group on the physics of semiconducting Low Dimensional Structures (LDS) using samples prepared by Molecular Beam Epitaxy (MBE).

The person appointed, who will be qualified to graduate level, will be expected to assist with the growth, processing and subsequent characterisation of layers grown on a Varian GEN II MBE machine.

Familiarity with some or all of the following techniques would be an advantage: ultra high vacuum systems, Auger and RHEED spectroscopy, mask aligning and bonding and working under "clean room" conditions.

This is a permanent University appointment in an area of Physics which is currently receiving major funding from the Science and Engineering Research Council and the University.

Salary will be within the range of £6,600 — £10,330 per annum (under review).

Further particulars and forms of application, returnable not later than 30 June 1985, may be obtained from the Staff Appointments Officer, Univeristy of Nottingham, University Park, Nottingham, NG7 2RD. Ref No. 1000. (6791)A

U M I S T

POST-DOCTORAL RESEARCH ASSISTANT

Protein Engineering

A Post-doctoral Research Assistant is required to work on an SERC-funded project which will analyse and alter the active site of eukaryotic Superoxide Dismutase both to elucidate the enzyme's catalytic mechanism and to confer novel activities upon it. The project will exploit site-directed mutagenesis to introduce defined changes in the enzyme's active site and will analyse their effect on protein structure and function using X-ray absorption spectroscopy (XAS) The mutagenesis and related biological experiements will be performed in the laboratory of Dr S G Oliver in the Department of Biochemistry and Applied Molecular Biology. This superbly equipped, modern Department has a dedicated facility for both oligonucleotide and peptide synthesis. The XAS and structural analyses will be carried out in the laboratory of Dr N J Blackburn (Dept. of Chemistry) and at the SERC Synchrotron Radiation Facility in nearby Daresbury

The post, which is funded for three years, would suit a PhD molecular biologist with experience in recombinant DNA technology. An interest in, and knowledge of, biophysics or physical chemistry would be advantageous but not essential. Commencing salary will be within the scale £7520 - £12150 per annum (under review).

For further details contact Dr S G Oliver (061-236 3311 extn. 2172) or Dr N J Blackburn (extn. 2109). Applications, quoting reference BIO/87, including a curriculum vitae and the names of two referees should be sent to Dr S G Oliver, Department of Biochemistry and Applied Molecular Biology, UMIST, P O Box 88, Manchester M60 1QD.

RESEARCH ASSISTANTS

Molecular Biology Of Lignin Biodegradation

Vacancies exist in the Department of Biochemistry and Applied Molecular Biology for two Research Assistants in a Group which is funded jointly by the AFRC and BP's Venture Research Unit The interests of the Group are in lignin solubilisation by white rot fungi and actinomycete bacteria. A wide range of molecular genetical, microbiological and analytical methods are currently being used in work to identify and characterise genes coding for the enzymes involved, and their controlling elements. These include HPLC analysis, protein purification, cDNA and genomic cloning, use of synthetic oligonucleotide probes, DNA sequence analysis and site-directed mutagenesis There is also extensive collaboration with other groups in this excellently equipped and interactive department

Applicants must have relevant experience at the graduate or post-doctoral level and must be available for interview.

The appointments will be for 18 months in the first instance, with the prospect of renewal and the commencing salaries will be within the range £6600 - £12150 per annum (under review)

Applications, quoting reference BIO/88, including a curriculum vitae and the names of two referees should be sent to Professor P M A Broda, Department of Biochemistry and Applied Molecular Biology, UMIST, P O Box 88, Manchester H60 1QD, from whom further information can be obtained (Tel): 061-236 3311 ext. 2119).

The closing date for ALL the above posts is 28 June, 1985.

(6795)A

ENDOCRINOLOGY BIOCHEMISTRY/ PHYSIOLOGY

Postdoctoral Opportunities

Broad range of Endocrine research projects: (i) isolation and identification of pituitary and lung peptides; (ii) physiologic and pharmacologic action; (iii) growth and control of function of fetal adrenals; (iv) role of growth factors in fetal development

Send curriculum vitae, bibliography, three letters of recommendation, and short statement regarding career goals, interests and experience to: Dr Samuel Solomon, Endocrine Laboratory, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec H3A 1A1. (NW1772)A

POSTDOCTORAL/ ASSISTANT PROFESSOR

position available immediately in the Surgical Immunology Laboratory, V A. Medical Center, Miami, Florida, affiliated with the Departments of Surgery and Urology at the University of Miami, School of Medicine. The research revolves around new applications of multiparameter flow cytometry in the characterization of solid tumor cell kinetics, antigens and metalolic states. The candidate should be familiar with the principles of flow cytometry and monoclonal antibody staining/production techniques. Some computer programming experience is desirable. Send curriculum vitae to: **Norman L. Block, M.D., Department of Urology, University of Miami, School of Medicine, P.O. Box 06217, Miami, Fla. 33101; telephone: 305-547-6630.** *An Equal Opportunity/Affirmative Action Employer.* (NW1860)A

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THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH ASSISTANT

Department of Applied Mathematics and Theoretical Physics

This post, funded by SERC, involves work on maintaining an Atomic Data Bank for basic atomic data which will be used in a number of branches of theoretical physics including atomic physics, fusion physics and astrophysics. Extensive use of computers will be involved; the Data Bank is set up on a local VAX 11/750, and its main use is to capture data from a CRAY-1S at the University of London Computer Centre, via data links.

Applicants must have a good honours degree *and* postgraduate research experience in a relevant field. A knowledge of atomic physics, astrophysics, or information retrieval would be an advantage.

Salary up to £9390 per annum. The post is tenable for one year, starting as soon as possible.

Curriculum vitae, giving the names and addresses of two referees, should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland Closing date: 1 July 1985 (Please quote Ref. 85/D). (6810)A

IMMUNOLOGY PhD

wanted to run transplant immunology laboratory. Must have experience in cellular immunology — in vitro immune responses, cell separation and culture techniques, safe use of radioactive isotopes — and be willing to direct and assist research technicians in performance of simple and complex procedures. Some experience with UV irradiation and epidermal cell reactions preferred; 2 to 3 years of postdoctoral experience required. Applicants should submit curriculum vitae to: **Jay C. Fish, MD, Department of Surgery, University of Texas Medical Branch (UTMB), Galveston, Texas 77550.**

UTMB is an Affirmative Action/Equal Opportunity Employer. (NW1839)A

MOLECULAR BIOLOGIST

To engage in research regarding causes of and cures for autoimmune diseases; studies growth, structure, development and general characteristics of micro-organisms believed to cause arthritis and other autoimmune diseases.

Specifically, successful applicant will study interactions between La protein, virus RNA molecules, and patient anti-La antibodies. Req.'s PhD in Molecular Biology, Virology, Biochemistry or Medical Biophysics; 2 yrs. exp in job being offered or as a Postdoctoral Fellow/Molecular Biologist; and thorough knowledge of an substantial exp. in adenovirus VA RNAs and La protein antigens. La Jolla, California, job site. $35,000 per year. If offered employment, must show you have the legal right to work.

Send this ad and your résumé to job no 1760 PO Box 865, Sacramento, California 95804, not later than June 28 1985. (NW1840)A

UNIVERSITY OF WISCONSIN-MADISON

MYCOLOGY/MICROBIAL ECOLOGY

RESEARCH ASSOCIATE (postdoctoral) position available 1 June 1985 on the BIOTECHNOLOGY and microbial ecology of fungal pathogens (biocontrols) to watermilfoil Research aspects include mass culture and strain improvement techniques, interaction with other control measures, and tracking marked populations in water and sediment Funding for two years. Salary competitive and commensurate with qualifications. **Send résumé with names of 3 references to: Dr. John H. Andrews, Department of Plant Pathology, 1630 Linden Drive, Madison, WI 53706.** *jAn affirmative action/equal opportunity employer.* (NW1835)A

HLA LABORATORY DIRECTOR:

Ph.D. immunologist with previous experience and research interest in human histocompatibility testing needed to develop the histocompatibility laboratory at The University of Texas Health Science Center at San Antonio. Please send inquiries or curriculum vitae, current laboratory procedure manual, and UCLA and/or SEOPF quality control data for the past year to: **Callann T. Lum, M.D., Director, Organ Transplantation Programs, Department of Surgery, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284.** (NW1861)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL

Food Research Institute

PROTEIN BIOCHEMIST

A vacancy has arisen for a protein biochemist to investigate what structural features in the collagen molecule determine the mechanical strength of gelatin gels. The appointed scientist will work closely with both the Collagen Section and Myofibrillar Structure Section. The project will require the characterisation of a variety of gelatin preparations, collagen chains and peptide fragments, together with mechanical studies on gelatin gels and electron microscopy. The post is financed by industry and is for three years.

Qualifications: First of upper second class honours degree in biochemistry or related subject with at least 2 years relevant post-graduate research experience for appointment at HSO, 4 years for SSO. A PhD and experience of electorn microscopy or mechanical measurements would be an advantage or equivalent experience.

Salary: £7,435 — £12,020 according to qualifications and experience.

Please apply to: Personnel Officer AFRC Food Research Institute Langford Bristol BS18 7DY (Ref 85/11)

The AFRC and the Grant-Aided Institutes of the AFRS are Equal Opportunities Employers. (6788)A

CAMBRIDGESHIRE COLLEGE OF ARTS AND TECHNOLOGY

DEPARTMENT OF SCIENCE

LECTURER I in Geology

Required from 1st September 1985 to teach either sedimentary and organic geochemistry or geotechnical engineering to final honours degree level. A strong interest in research and field work is necessary. Other teaching will include supervision of student projects. Development of links with industry is encouraged.

Salary scale LI £5910 — £10512 (award pending), starting point according to qualifications and experience.

Details and form, to be returned by 28th June, from Administrative Assistant, Department of Science, CCAT, East Road, Cambridge CB1 1PT, Tel (0223) 352992. (6782)A

UNIVERSITY OF EDINBURGH

DEPARTMENTAL SUPERINTENDENT

Applications are invited for the above post in the Department of Zoology, West Mains Road. The successful applicant will be responsible for the day-to-day running of the Department including general supervision, selection and training of technical staff, purchasing and maintenance of equipment, safety and security. Applicants should have several years of supervisory or middle management experience preferably in University laboratories. Salary on scale £10,504 — £11,396 (under review). Ref NT546

Further particulars, where available, and application forms may be obtained from the Personnel Office, University of Edinburgh. 63 South Bridge, Edinburgh, EH1 1LS. Tel 031-556 2930. (24 hour answering service) (6815)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF ANIMAL PHYSIOLOGY AND NUTRITION

LECTURER

Applications are invited for the above post available from 1 August 1985. Candidates should have a PhD in animal science, preferably involving some study of pigs, and should have some postdoctoral research experience.

Salary on a scale for Lecturers (£7,520 - £14,925) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no 61/19.

Closing date for applications 4 July 1985. (6797)A

UNIVERSITY OF LONDON

Institute of Neurology

Applications are invited for a

RESEARCH ASSISTANT

post available until September 1986 to work on a project to determine the in vitro chemosensitivity measurement of human glioma within the Neuro-oncology section of the Department of Neurological Surgery. Aplicants should have experience in tissue culture work. Salary within the range £7,980-£8,920 plus £1,233 London Allowance.

Further particulars may be obtained from Mr D G T Thomas, FRCSE, Department of Neurological Surgery, Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG, to whom applications should be forwarded within two weeks of the appearance of this advertisement. (6803)A

ROYAL HOLLOWAY AND BEDFORD COLLEGES

Egham Hill, Egham, Surrey TW20 0EX

SCIENTIFIC OFFICER

Monoclonal Antibody Production

Well qualified graduates in a Biological Science required for three years for the posts (UGC funded) of Scientific Officer to develop a central service unit for the production of monoclonal antibodies. At least three years experience in mammalian cell culture and preferable hydridoma production and screening, is essential.

Salary scale £7,833 — £10,153 inclusive of London allowance

Please send self addressed envelope for further details to Mrs D J Odds, Personnel Officer. (6785)A

BLAND-SUTTON INSTITUTE OF PATHOLOGY

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL, London W1P 7PN

RESEARCH ASSISTANT

required to raise monoclonal antibodies with a group investigating the expression of antigens in breast cancer. For 1 to 2 years, (starting salary up to £7,833 per annum), to start as soon as possible Experience in monoclonal antibody production/ tissue culture would be advantageous.

Applications in the form of cv plus names and addresses of 2 referees to Dr A Leathem at the above address. (6828)A

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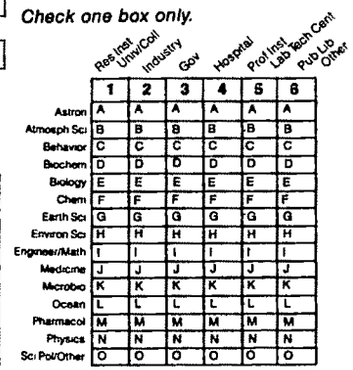
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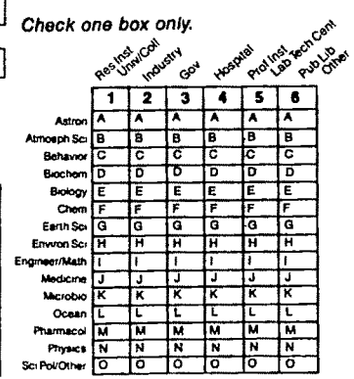
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ASSISTANTSHIPS

UNIVERSITY OF CAMBRIDGE
UNIVERSITY CHEMICAL LABORATORY

POSTDOCTORAL ASSISTANTSHIP

Applications are invited from molecular biologists for work on the cloning and expression of the genes for the key enzymes on the biosynthetic pathways to haem, vitamin B_{12} and related macrocycles. This will be in collaboration with Dr C Abell and Professor A R Battersby.

The appointment from a mutually agreed date in 1985 is for up to 2 years at a starting salary within the Scale £7520 (for 24 years of age) to £8920 (for 27 years of age) with an increment after one year. Applications with curriculum vitae and 2 referees' names should be sent as soon as possible to: Professor A R Battersby, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW (6812)P

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY

MICROBIAL ECOLOGY

Applications are invited for an NERC POSTDOCTORAL RESEARCH ASSISTANTSHIP

tenable for three years from 1 October 1985. The project is to study the recycling of chitin in estuaries with Professor G W Gooday and Dr J I Prosser.

Salary within range £7,520 - £12,150 on the 1A scale for research and analogous staff (under review).

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 1st July 1986. (6767)P

DURHAM UNIVERSITY
DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP

tenable for up to 3 years, to work with Dr D Parker on the synthesis of new anti-cancer drugs. Applicants should either have a strong background in synthetic organic chemistry (particularly bio-organic chemistry) The project is in collaboration with Celltech Ltd, and the starting salary will be £7520 or £7980 per annum on Range 1A plus superannuation (under review).

Applications (3 copies) naming 3 referees should be sent by 1 July 1985 to the Registrar, Science Laboratories, South Road, Durham, DH1 3LE (6790)P

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF AGRICULTURAL BIOLOGY

MAFF RESEARCH STUDENTSHIP

"Investigation of earliness and frost resistance in winter rye"

The aims of the project are to investigate the physiological basis of the earliness and frost resistance of winter rye, to assess intercultivar variation in these properties and to evaluate both tetraploid and diploid winter rye cultivars in comparison with perennial ryegrass and triticale as sources of early forage in both lowland and upland pastures.

While all the aims will be included in the programme of research, the emphasis of physiological, agronomic or genetic aspects will depend on the interests of the graduate appointed.

Applicants should have, or expect to obtain this summer, a first or upper second class honours degree, or equivalent, in Agricultural Botany, Botany or a related subject.

Applications in the form of a curriculum vitae should include the names and addresses of two academic referees and should be sent to Dr W S Stewart, Department of Agricultural Biology, The University, Newcastle upon Tyne, NE1 7RU, from whom further details can be obtained. Closing date 5 July 1985 (6821)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

SERC RESEARCH STUDENTSHIP

Applications are invited for an SERC Research Studentship, commencing October 1985, leading to the degree of PhD The successful candidate will join an active research group investigating the molecular basis of glucose transport and its regulation in the membranes of mammalian cells.

Applicants should have, or expect to obtain, a first or upper second class honours degree in Biochemistry or a related discipline, and should have strong interest in cell and/or molecular biology. Applications, containing a CV and the names, addresses and telephone numbers of two professional referees, should be sent as soon as possible to Dr S A Baldwin, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, from whom further details may be obtained. (6776)F

STUDENTSHIPS

UNIVERSITY OF LEICESTER/CRANFIELD INSTITUTE OF TECHNOLOGY

POSTGRADUATE STUDENTSHIP IN MOLECULAR GENETICS

Applications are invited for a postgraduate studentship in the Department of Genetics, University of Leicester, for three years commencing October 1st 1985, leading to the degree of PhD The project will involve the application of recombinant DNA techniques to the study of secretion of a bacterial toxin by E coli. The project involves collaboration between the Institute of Biotechnology at Cranfield, the Department of Genetics, Leicester and ICI, Agricultural Division, Billingham. The work will be carried out at Leicester as part of a small team investigating the molecular biology of haemolysin secretion. Applicants who hold or expect to obtain at least a 2i degree in a relevant Biological Science should apply to: Professor I B Holland, Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH enclosing a brief curriculum vitae and the names and addresses of two Referees Closing date for applications July 1st 1985. (6817)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF NEUROLOGICAL SCIENCE

SERC-CASE PhD STUDENTSHIP
(Jointly with Eli Lilly Research Ltd)

Immunology/cell biology graduate required to study the functions of germinal centre cells in mice and man, in an MRC-funded group of clinical and basic scientists working on the immunology of myasthenia gravis. The 'industrial' component will be at Lilly Research, Windlesham, and will probably require about six months.

For further information please contact Dr N Willcox, Department of Neurological Science, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF (01-794 0500 Extn 4386) to whom applications giving the names of three referees should be sent by 28 June 1985. (6799)F

UNIVERSITY OF EDINBURGH MEDICAL SCHOOL
DEPARTMENT OF SURGERY

RESEARCH STUDENTSHIP IN ENDOCRINOLOGY AND METABOLISM

Applicants are invited for a postgraduate studentship tenable for 3 years starting in October 1985 and leading to a PhD Degree The research topic includes an investigation on the mechanism of action of prolactin and other growth hormones in the human prostate.

Applicants should have or expect to have at least, Upper Second Class Honours in Biochemistry, Pharmacology or other biological sciences. Applications, which should include a curriculum vitae and the names of two referees, should be sent to Dr F K Habib, Department of Surgery, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG. (6816)F

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF PHYSICS

RESEARCH STUDENTSHIP IN SOLAR PHYSICS

A studentship is available for a suitably qualified and motivated student to undertake reearch with the solar physics groups to study solar oscillations leading to a PhD degree. The work involves the development of new resonant scattering spectrometres, the collection of data at remote sites (Australia, Hawaii and Tenerife) and the subsequent analysis and interpretation of the data.

For application form please contact Mrs Muriel Mason, Department of Physics, University of Birmingham, PO Box 363, Birmingham B15 2TT. (021 472 1301 ext 3265) (6825)F

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF BIOCHEMISTRY

RESEARCH STUDENTSHIPS

Applications are invited from suitably qualified graduates or those expected to graduate shortly for Research Studentships tenable in the Department of Biochemistry for the Session 1985-86. Studentships are the same value as those awarded by the various Research Councils or the DES plus tuition fees The following research fields may be available to suitable candidates: (1) Membrane bound enzymes. (2) Glycosaminoglycans of blood vessel surfaces. (3) Alginate modifying enzymes. (4) Hepatic anti-tumour agents.

Further particulars can be obtained from Professor K S Dodgson, Department of Biochemistry, University College, PO Box 78, Cardiff CF1 1XL. Ref: 2961. (6773)F

FROST EXHIBITION RESEARCH STUDENTSHIP in Freshwater Biology

The Freshwater Biological Association is offering one three-year postgraduate Studentship to start in summer 1985 at one of its Laboratories. Research projects in several fields of freshwater biology are available for interested students. Alternatively students may propose their own research project in any field of freshwater biology. A good honours degree in science or its equivalent is essential for registration at Lancaster, Reading or another appropriate university. The studentship will be awarded in open competition to the person showing most promise of making a successful career in creative research in freshwater biology. Basic maintenance grant of 10% above the normal Research Council grant and allowances pa plus fees etc.

Full details from The Assistant Director, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP (Tel 096-62 2468). Closing date 26th June 1985. Selected candidates will be interviewed on 12th July 1985. (6796)F

Continued on pages 18, 20

Special Training Fellowships in Recombinant DNA Technology

Applications are invited for fellowships for any period from 6 months to three years commencing on or after 1 May 1986 from scientists requiring training in recombinant DNA techniques. Applicants may be young postdoctoral scientists (or those just completing PhD's) or more mature scientists with established reputations in other areas wishing to enter this field. Permanent academic staff with unpaid leave of absence may apply. Applicants will be expected to propose their own training programme prepared in collaboration with the proposed supervisor.

These fellowships are tenable in any UK institution offering training appropriate to the applicant's needs. UK residents temporarily working abroad may apply.

MRC
Medical Research Council

Application forms, and further details may be obtained from Training Awards Group, Medical Research Council, 20 Park Crescent, London W1N 4AL. (Tel: 01-636 5422 Ext 448)

Closing date: 15 October 1985

(6766)E

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

POST DOCTORAL FELLOWSHIP

Applications are invited for a Research Fellowship funded by the Medical Research Council to study recognition signals in adenovirus replication. The applicant should prefereably have some experience in either cloning or hybridoma technology and will become part of the Molecular Virology Group in the Department. The project will be under the supervision of Professor W C Russell from whom further information may be obtained (Telephone 0334 76161 ext 295).

The appointment will be for 3 years, starting as soon as practicable. Starting salary at appropriate point within range £7,520 to £10,330 per annum on the 1A Research scale, plus superannuation

Applications (two copies preferably in typescript) with the names of two referees should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ to arrive not later than 2nd July 1985. (6813)E

Wellington Medical Research Foundation Inc.
MALAGHAN RESEARCH FELLOWSHIP

The Wellington Medical Research Foundation Inc. invites applications for the position of Malaghan Research Fellow to be held at the Wellington Cancer and Medical Research Institute, Wellington Clinical School of Medicine, Wellington, New Zealand. The applicant should be a research worker with evidence of capacity to undertake original research. The applicant must possess a higher qualification and the field of research will be in the general field of cancer with preference for haematological malignancies. The annual salary will depend on qualifications and experience but will be on the University Lectureship scale and within the range of NZ$23,622 - NZ$27,928. A particularly well qualified person could be appointed at a higher level on the salary scale. Adjustments will be made to cover increases in cost of living in line with the wage adjustment of University staff. The appointment is for four years. Assistance towards transport expenses is available. **Conditions of appointment, method of application and other information may be obtained from the Director of the Wellington Cancer and Medical Research Institute, Wellington Clinical School, Wellington Hospital, Wellington 2, New Zealand, or from the office of the High Commission, New Zealand House, London. Applications close with the Secretary of the Wellington Cancer and Medical Research Institute on 31st August, 1985.** (W1940)E

UNIVERSITY OF SURREY

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Fourier-Transform Infra-red Spectroscopy

Applications are invited for a Post-doctoral Research Fellowship to develop applications of Fourier-Transform Infra-red Spectroscopy in conjunction with Dr G J Buist. The Department has recently purchased a Perkin-Elmer model 1750 FTIR spectrometer, and wishes to develop applications to polymer chemistry, food science, biochemistry and microbiology. Applicants should have had research experience in infra-red spectroscopy. The appointment is available from Sept/Oct 1985 for one year at a salary of £7,520 per annum. Superannuation is under USS conditions.

Applications in the form of a curriculum vitae (3 copies) including the names and addresses of two referees should be sent to the Personnel Office (JLG), University of Surrey, Guildford, Surrey, GU2 5XH by 12 July 1985 quoting reference 397/N. (6794)E

EMBO
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
Autumn 1985 — Awards
Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany.

(W1893)E

COLUMBIA UNIVERSITY
Department of Biochemistry and Molecular Biophysics

A postdoctoral research fellowship is available immediately for studies on the structure of a Na/K-ATPase The applicant should have a background in either synthetic organic chemistry or the biochemistry of integral membrane proteins. The work will involve both the preparation of heavy atom labeled ligands, and the reassembly and reconstitution of the ATPase subunits Applications, including a curriculum vitae and the names of two referees should be sent to.

Dr. I.S. Edelman
Department of Biochemistry & Molecular Biophysics
Columbia University
630 West 168 Street
New York, NY 10032

COLUMBIA UNIVERSITY IS AN EQUAL OPPORTUNITY/ AFFIRMATIVE ACTION EMPLOYER

(NW1862)E

The John Hopkins University
Department of Anesthesiology and Critical Care Medicine

POST DOCTORAL FELLOWSHIP IN RESEARCH

Applications are invited for this position available immediately to study the molecular biology of human cytochrome P450. The applicant must be creative and highly motivated to carry out independent experimentation utilizing human cytochrome P450 under study.

M D. or Ph.D. required with 1–2 years experience in molecular biology.

Contact: Roy C. Levitt, M.D., The John Hopkins University, Department of Anesthesiology and Critical Care Medicine, Blalock 1410, Baltimore, Maryland 21205. Telephone (301) 955-7609.

(NW1822)E

SANDOZ RESEARCH INSTITUTE
Vienna, Austria

is one of the three research centres SWISS based pharmaceutical company SANDOZ

A post doctoral fellowship in a research group working on the CLONING OF LYMPHOKINE GENES is available for a

CELL/MOLECULAR BIOLOGIST

with interest in the expression and function of lymphokines. Previous experience in molecular biology, immunology and/or gene cloning techniques in bacteria or yeasts will be of advantage.

The successful candidate will find excellent working conditions in well equipped laboratories. The appointment will be for an initial 2-year period. Moving expenses will be paid.

Please send cv, description of scientific experience and names of two referees to

SANDOZ

SANDOZ Forschungsistitut
Personnel Department
Brunner Strasse 59
A-1235 Wien, Austria (W1951)E

THE OPEN UNIVERSITY
Faculty of Science
BIOLOGY DEPARTMENT
POSTDOCTORAL FELLOWSHIP IN MOLECULAR GENETICS

Applications are invited for an immediately available MRC funded postdoctoral fellowship to work on the molecular genetics of human lactase using recombinant DNA techniques. This is a collaborative project between the Open University and the MRC Human Biochemical Genetics Unit, University College, London. The aim is to understand the control of lactase expression and the evolution of lactase persistence. The appointment will be for 3 years on the 1A Scale for Research and Analogous Staff (£7520 — £12150 pa under review). Maximum initial salary will be £8920.

Application forms and further particulars are available from Dr J W R Smith (4817/1), Administrative Assistant (Science), Faculty of Science, The Open University, Walton Hall, Milton Keynes, MK7 6AA or telephone Milton Keynes (0908) 653993: there is a 24 hour answering service on Milton Keynes 653868.

Closing date for appllications: 5th July 1985. (6808)E

POST-DOCTORAL FELLOWSHIP POSITION

Applications are invited from chemists, pharmacologists and biochemists to work on the application of mass spectrometry to nutrition based research. Applicants should have experience in modern methods of analysis together with an interest in mass spectrometry. The position will involve collaboration with a multi-disciplinary group of scientists working in the Clinical Nutrition Research Unit. It is envisaged that two major areas of research will be involved. First, determination of glucose production rates in children with glycogen storage disease and second the analysis of urinary prostaglandin metabolites. Salary for this position will be commensurate with training and experience.

The mass spectrometry laboratory is equipped with 3 magnetic sector instruments and 5 quadrupoles. Capabilities of the laboratory include FAB/MS, Thermospray LC/MS, Accurate mass determinations and capillary column GC NICI/MS. **Applications including full personal details togehter with the names and addresses of at least two referees should be sent to Ian A. Blair, Ph.D., Department of Pharmacology, Vanderbilt University, Nashville, TN 37232 not later than June 21, 1985.** (NW1855)E

ANNOUNCEMENTS

American Teknos, Inc.

American Teknos, an international technology development and transfer company is seeking health-care technologies in the early development or invention stage.

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Mr. Joseph Fanelli, Chairman
American Teknos, Inc.
14710 Greenleaf St, Sherman Oaks, CA 91403

(NW1854)G

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SEMINARS and SYMPOSIA

NOVO RESEARCH SEMINAR

"The Biogenesis of Regulatory Peptides"

22nd-23rd AUGUST 1985
Novo Industri A/S, Bagsvaerd, Copenhagen, Denmark

Organising committee: J.F. Rehfeld, T.W. Schwartz, L. Olsson, U.V. Lassen.

There will be five sessions:

I. Post-Transcriptional Processing:
chairman Niels Ful, Novo's Genetic Laboratory
Lecturers:
S. Nakanishi, Kyoto University
J.F. Habener, Massachusetts General Hospital, Boston
D. Richter, Hamburg University

II. Intra-Cellular Targeting of Precursors:
chairman Ove Noren, University of Copenhagen
Lecturers:
D.I. Meyer, EMBL, Heidelberg
R.B. Kelly, University of California, S.F.

III. Proteolytic Processing:
chairman Howard S. Tager, The University of Chicago
Lecturers:
J. Thorner, University of California, Berkeley
T.W. Schwartz, MRC, University Hospital, Copenhagen
K. Docherty, University of Birmingham
G. Kreil, Austrian Academy of Science, Salzburg

IV. Amino Acid Derivatization:
chairman G. Kreil, ibid.
Lecturers:
D.G. Smyth, MRC, London
B.A. Eipper, The Johns Hopkins University, Baltimore
C.C. Glembotski, University of Pennsylvania
G.S. Baldwin, Royal Melbourne Hospital
W.B. Huttner, Max-Planck Institute, Munchen

V. Specific Peptide Systems:
chairman Jens Vuust, Arhus University
Lecturers:
H.S. Tager, the University of Chicago
T. Yamada, the University of Michigan
J.F. Rehfeld, University Hospital, Copenhagen

Participation in the seminar itself is free.
Travel and accommodation expenses will have to be met by participants.
Closing date for registration is 1st August 1985.
For details and registration, please contact:
Mrs. Karen Rimmer, Novo Industri A/S,
Novo Alle, DK-2880 Bagsvaerd, Denmark
Tel: (02) 982333, extension 3531.

NOVO

(W1928)M

GRANTS and SCHOLARSHIPS

Research Award in Immunoregulation

Centocor, Inc., will award a $10,000 unrestricted research grant for the most outstanding human Interferon-Gamma scientific paper published (or accepted for publication) between 1/1/85 and 6/30/86.

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(NW1726)H

STUDENTSHIPS

THE SCHOOL OF PHARMACY
University of London
DEPARTMENT OF PHARMACOLOGY

MRC RESEARCH STUDENTSHIP

Applications are invited from graduates holding, or expecting to obtain, an upper second or first-class degree in Pharmacology or a related subject, for a three year MRC studentship. The successful candidate will study the pharmacology of excitatory and inhibitory synaptic transmission in identified spinal neurones evoked by stimulation of specific brain stem areas. This project will utilise, in particular, the microiontophoritic technique.

Letters of application, together with a full CV and the names, addresses and telephone numbers of 2 referees, should be sent to: Dr J Davies, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, to arrive by 1 July 1985. (6804)F

Continued on page 20

UNIVERSITY OF WALES COLLEGE OF MEDICINE
DEPARTMENT OF CHILD HEALTH

(re-advertisement)

Applications are invited for a postgraduate scholarship to investigate androgen action and metabolism in disorders of sexual differentiation in humans, using tissue culture and cell fractionation techniques.

Applicants should have, or expect to gain, a first or upper second class honours degree in Biochemistry or a related science discipline.

The scholarship will commence in September 1985 and is tenable for a 3 year period (subject to a probationary year). The results are expected to be submitted for a PhD degree.

Further particulars and application forms (quoting ref No C10/61) may be obtained from the Registrar and Secretary, University of Wales College of Meducine, Heath Park, Cardiff, CF4 4XN (Tel No 0222/755944 Ext 2296) as soon as possible. Closing date for application: 12th July, 1985.

(6830)H

CONFERENCES and COURSES

INSTITUTES OF PSYCHIATRY, NEUROLOGY AND OPTHALMOLOGY
(British Postgraduate Medical Federation, University of London)

MSc in NEUROCHEMISTRY

This is a one-year Course leading to the degree of MSc in Neurochemistry. It offers graduates in science and medicine training in basic and applied neurochemistry. The Course consists of lectures, seminars and laboratory work and, in the second and third terms, research on a neurochemical topic.

Applicants should have a First or Second Class Honours degree in Biochemistry or a cognate subject, or be medically qualified.

Applicants for the Academic Year October 1985 - July 1986 should be addressed to the Secretary, Course in Neurochemistry, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, England.

(6805)C

EUCHEM Conference on Stereochemistry

The 22nd EUCHEM Conference on Stereochemistry, covering a wide range of topics in chemistry with emphasis on its inderdisciplinary character in natural sciences, will be held on the Burgenstock near Lucerne, Switzerland, from April 27 - May 3, 1986. The number of participants will be limited. Inquiries and applications (no special forms are required) should be addressed before January 10, 1986, to the President:

Prof. E. Winterfeldt
Institut für Organische Chemie
Universität Hannover
Schneiderberg 1 B
3000 Hannover 1
Germany

(W1931)C

The New York Academy of Sciences Conference on

Metabolic Regulation: APPLICATION OF RECOMBINANT DNA TECHNIQUES

6 November to 8 November 1985

The Barbizon-Plaza Hotel New York City

This symposium is intended to facilitate exchanges of ideas and information between scientists who study (1) regulation of the expression of genes of metabolic interest, (2) regulation of the catalytic activity of enzymes, and (3) development and use of the techniques of gene transfer, site-specific mutation and expression of cloned genes in heterologous cells.

Invited speakers include: P.P. Cohen, P. Felig, R. Fletterick, R.E.K. Fournier, S. Gluecksohn-Waelsch, J.L. Goldstein, A.G. Goodridge, M. Gottesman, D.K. Granner, R.W. Hanson, R.A. Jungmann, R.J. MacDonald, R.C. Mulligan, S.J. Pilkis, G.M. Ringold, J.M. Rosen, M. Rosenberg, C.S. Rubin, G. Schutz, S.S. Taylor, H.C. Towle, I.M. Verma, S.J. Wakil.

There will be two contributed poster sessions. The deadline for submission of abstracts is 15 July 1985. The abstracts should include the name of the author(s), institution, and a 200-word summary of the work to be presented. Send abstracts to: Dr. Alan G. Goodridge, Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio 22106.

Conference Co-chairmen: Alan G. Goodridge, Ph.D. and Richard W Hanson, Ph.D., Case Western Reserve University.

Inquiries should be addressed to: Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021. (NW1864)C

ADVANCED COURSE IN FLOW CYTOMETRY: ANALYSIS AND FLUORESCENCE ACTIVATED CELL SORTING

Paris, September 9–14, 1985

Sponsored by: The Cancer Research Association (ARC) – Jacques Crozemarie, President, and the "Centre Technique pour le soutien de la Recherche sur le Cancer des Laboratoires et Formations du Centre National pour la Recherche Scientifique" (C.N.R.S.)

A Course in Flow Cytometry will be offered with special emphasis on cell biology. Themes will include Multiparameter cell sorting, cell lineage studies, tumor cells characterization, gene amplification selection, cloning of genes by transfection, somatic cell genetics, hybridoma variant selection, liposome endocytosis, cell cycle analysis, Brd U and cytokinetic studies, mathematical modeling of the cell cycle, computer system for multiparameter data analysis, cytochemistry and new fluorochromes, karyotype analysis, pumped dye lasers, emission anisotropy.

The Course will consist of lectures, laboratory demonstrations and problem solving sessions.

Course Directors: Harry Crissman (Los Alamos National Laboratories), Claude Rosenfeld (INSERM, Villejuif, France)

Organizers: L.A Herzenberg (Standford University, USA), P. Le Bouteiller (Centre d'immunologie de Marseille-Luminy, France), M. Monsigny (Centre de biophysique moléculaire, Orleans, France), J.L. Roti-Roti (Washington University of St. Louis, USA)

Lecturers will include the above, plus: B. Bagwell, S. Cram, Z. Darzienkiewicz, P. Dean, M. Goldberg, J. Gray, P. Horan, C. Julien, M. Loken, H.S. Micklem, K. Muirhead, D.R. Parks, A. Truneh.

Course tuition: $500 (5 000 FF) includes laboratory fees, manual, coffee breaks, lunches and an optional Flow Cytometry Certificate.

Send C.V. and tuition deposit $50 (500 FF) to the local Course Director (max. 50 persons).

Dr. Claude Rosenfeld,
INSERM Unite 253,
Groupe Hospitalier Paul Brousse,
16 bis avenue Paul Vaillant Couturier,
94804 Villejuif, France

Application deadline June 30 1985, priority given to early applicants. (W1919)C

Microbe 86 IUMS

XIV INTERNATIONAL CONGRESS OF MICROBIOLOGY

7th-13th September 1986, Manchester, England.

To be held under the auspices of the International Union of Microbiological Societies.

Sponsored by the Royal Society of London, the Society for General Microbiology, the Society for Applied Bacteriology, the Society of Chemical Industry and the British Mycological Society.

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STUDENTSHIPS continued

London School of Hygiene and Tropical Medicine
(University of London)
Keppel Street, London, WC1E 7HT

MRC RESEARCH STUDENTSHIP

Applications are invited for a three year MRC studentship, tenable from 1 October 1985. The project will involve the use of biochemistry, histochemistry, and molecular biology in an investigation into oocyte formation in mosquitoes.

Applicants should have, or expect to have, a first or upper second class honours degree in a relevant biological subject. Applications should be sent with a CV and the names of two academic referees, not later than 30 June 1985, to Professor A.N. Clements, Department of Entomology, from whom further details can be obtained. (6789)F

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF PATHOLOGY
RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship in the Department of Pathology to study the carotid bodies in heart disease. The Studentship is financed by the Mersey Regional Health Authority and is tenable, year by year, to a maximum of three years. Candidates should hold or expect to obtain a First or upper Second Class Honours degree in Zoology, Life Sciences or Cell Biology. The successful applicant will be expected to register for a PhD.

Applications, together with the names of two referees, should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool, L69 3BX. Quote Ref RV/105/N. (6769)F

The University Of Oxford
MRC CELLULAR IMMUNOLOGY UNIT

A research studentship, leading to a D Phil degree will be available starting in October 1985. Basic research in immunoregulation especially with respect to the role of T cell subsets in autoimmune disease will form the topic of the thesis

Applicants should submit a CV and the name of two referees to: The Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. (6771)F

UNIVERSITY OF LEICESTER
Department of Genetics

Applications are invited for a SERC research studentship in Genetics tenable for 3 years from October 1985. The award is 'earmarked' by SERC for research in the genetic analysis of Drosophila behaviour. Applicants should have or expect to have a degree (1st class or 2/1) in a Biological subject and have an interest in molecular, genetic or evolutionary aspects of behaviour.

The Department is well equipped for research in genetics and has excellent facilities for molecular biology.

Enquiries and applications may be made to Dr C P Kyriacou, Department of Genetics, Adrian Building, University of Leicester, Leicester LE1 7RH. (6811)F

UNIVERSITY OF DURHAM
DEPARTMENT OF GEOLOGICAL SCIENCES
BP PhD STUDENTSHIP IN GEOPHYSICS

Applications are invited for a 3 year research studentship funded by BP to work on borehole-to-borehole seismic surveying techniques and applications. The studentship will be associated with a SERC grant to Dr N R Goulty and Dr R K Taylor (Dept of Engineering) for instrument development and fieldwork. Candidates should possess or expect to receive a good honours degree in Physics, Engineering or a related subject. Applications, together with the names of two academic referees should be sent as soon as possible to Dr N R Goulty, Department of Geological Sciences, South Road, Durham DH1 3LE. (6820)F

Trent Polytechnic
DEPARTMENT OF LIFE SCIENCES
SERC STUDENTSHIP

Applications are invited for the above positions from candidates who hold or expect to obtain at least a 2(i) honours degree in an appropriate discipline
Topic areas available for study are:
Elimination of drugs through liver and kidney (Ref LS/1)
Neurotransmitter pharmacology (Ref LS/2)
Calcitonin on gastric function (Ref LS/3)
Acid rain on amphibia (Ref LS/4)
Locomotion in stick insects (Ref LS/5)
Metal toxicity to fish (Ref LS/6)
Please quote the reference number of projects which are of interest in any correspondence.
Further details and forms of application are available from the Staff Officer, Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Closing date 5 July 1985. (6765)F

THE UNIVERSITY OF SHEFFIELD
AMORPHOUS AND CRYSTALLINE QUANTUM WELL SEMICONDUCTORS
SERC PhD STUDENTSHIP IN THE DEPARTMENT OF PHYSICS

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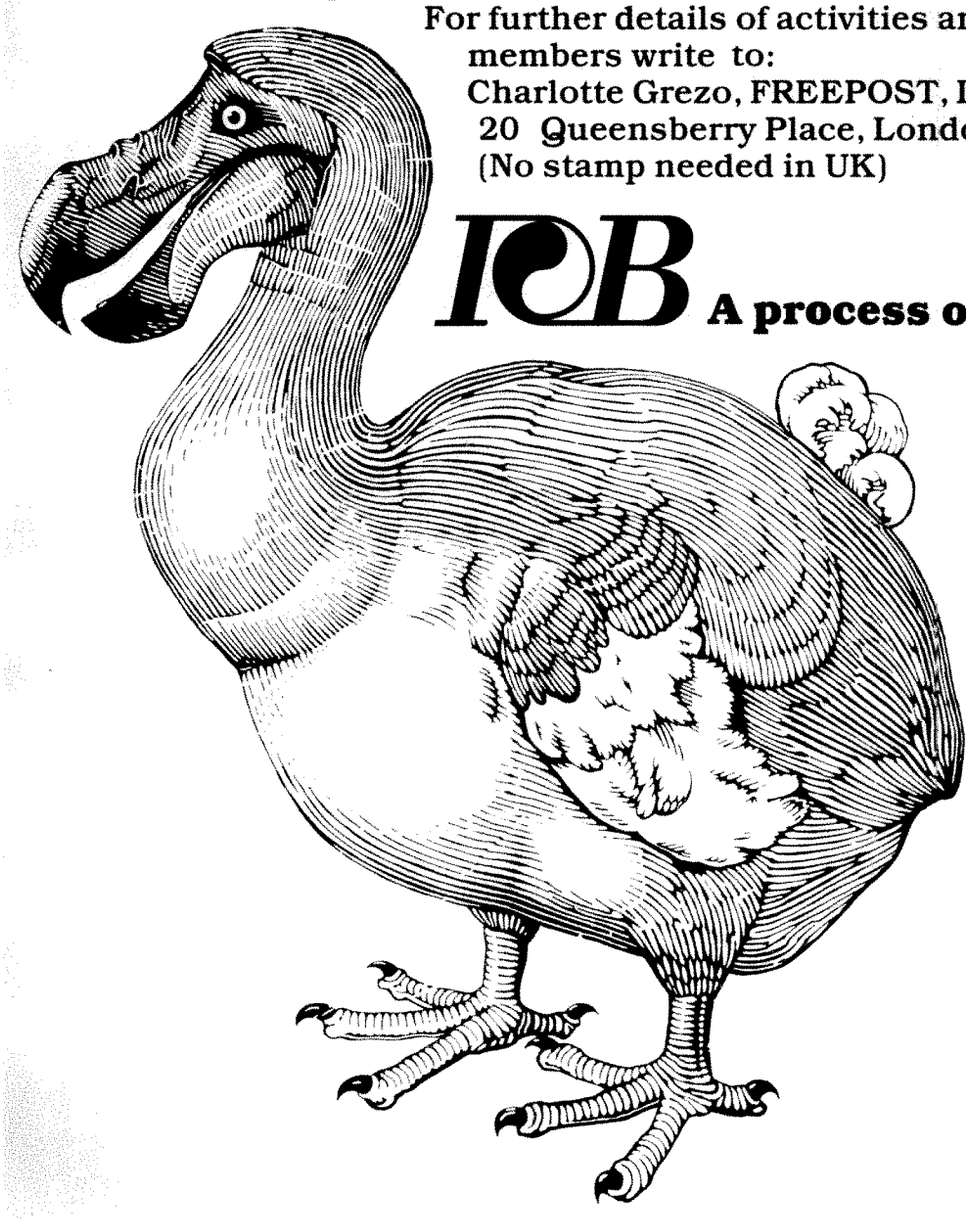
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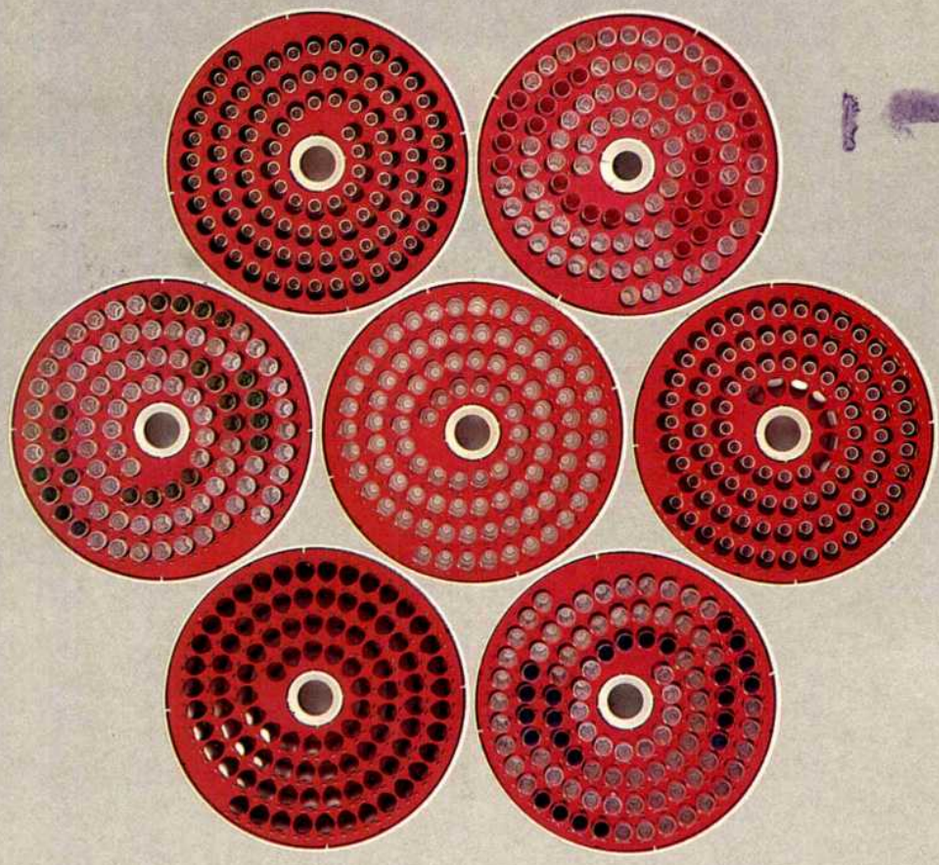
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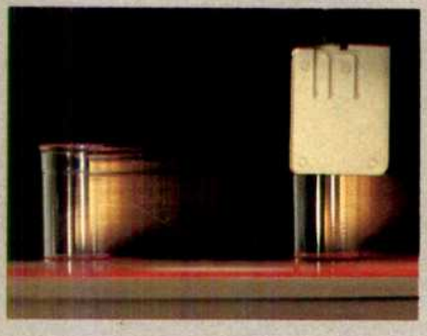
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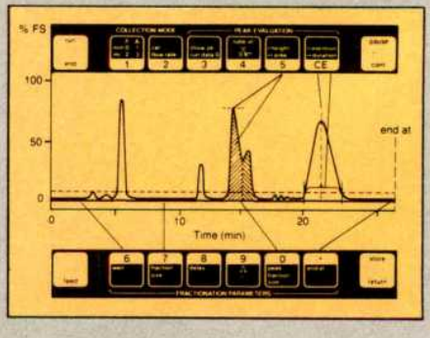
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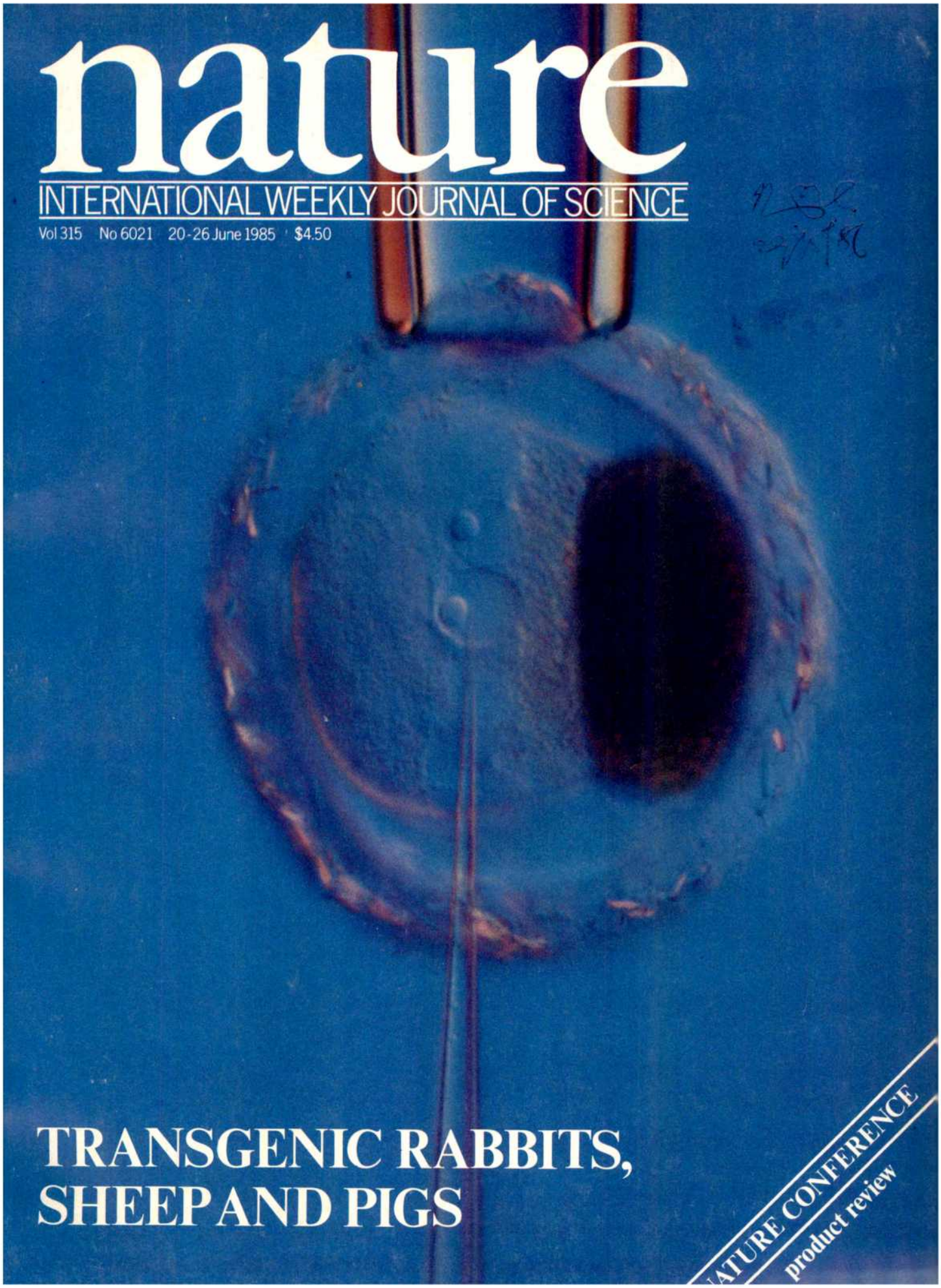
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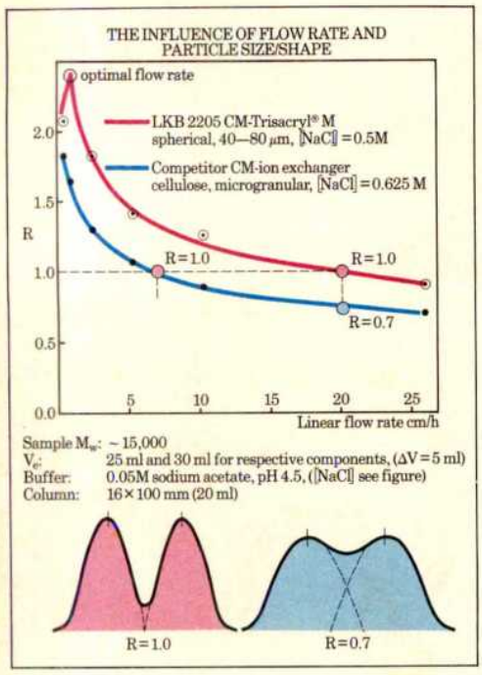
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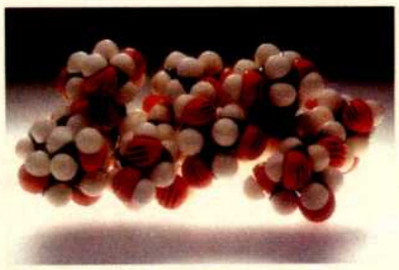
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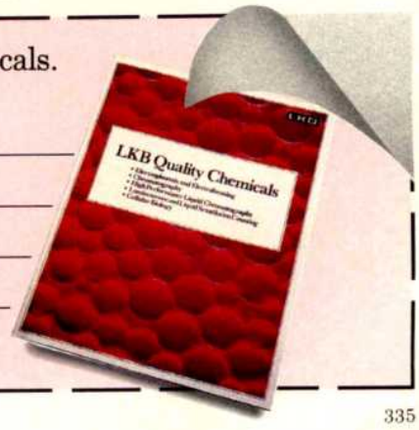
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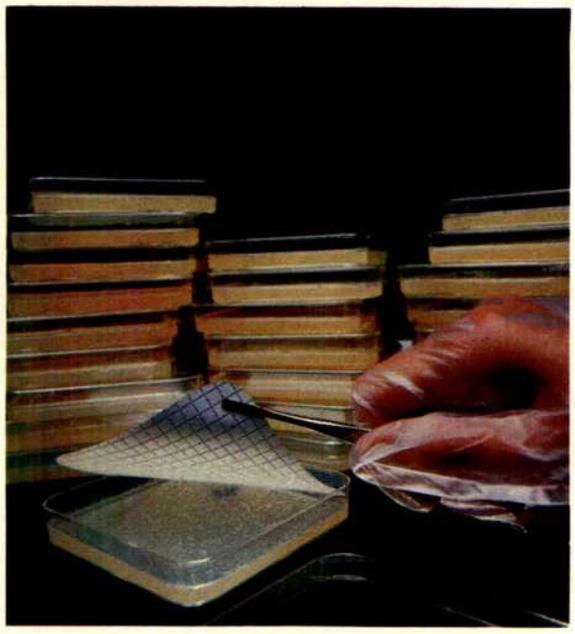
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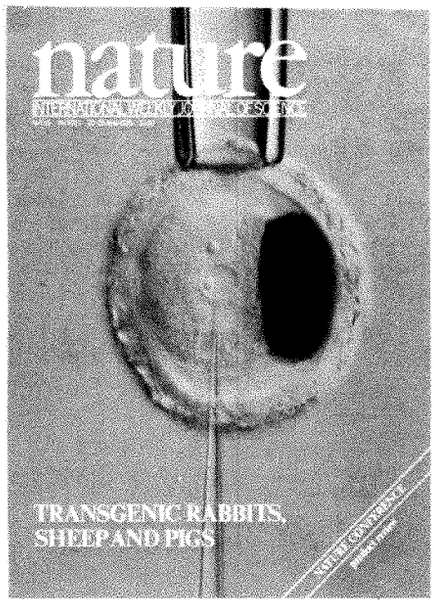
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Interference-contrast photomicrograph of a pig ovum. The cell has been centrifuged to reveal the normally obscure pronuclei. Visualization of the nuclei enables foreign genes to be microinjected into the cell, to become stably integrated into the germ line of even large animals such as pigs, sheep and rabbits. (See p.680, and *News and Views* p.628.)

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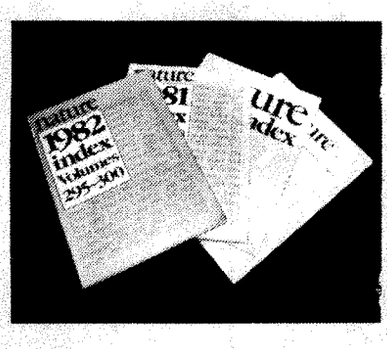
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References should appear sequentially indicated by superscripts, in the text and should be abbreviated according to the *World List of Scientific Periodicals*, fourth edition (Butterworth 1963-65). The first and last page numbers of each reference should be cited. Reference to books should clearly indicate the publisher and the date and place of publication. Unpublished articles should not be formally referred to unless accepted or submitted for publication, but may be mentioned in the text.

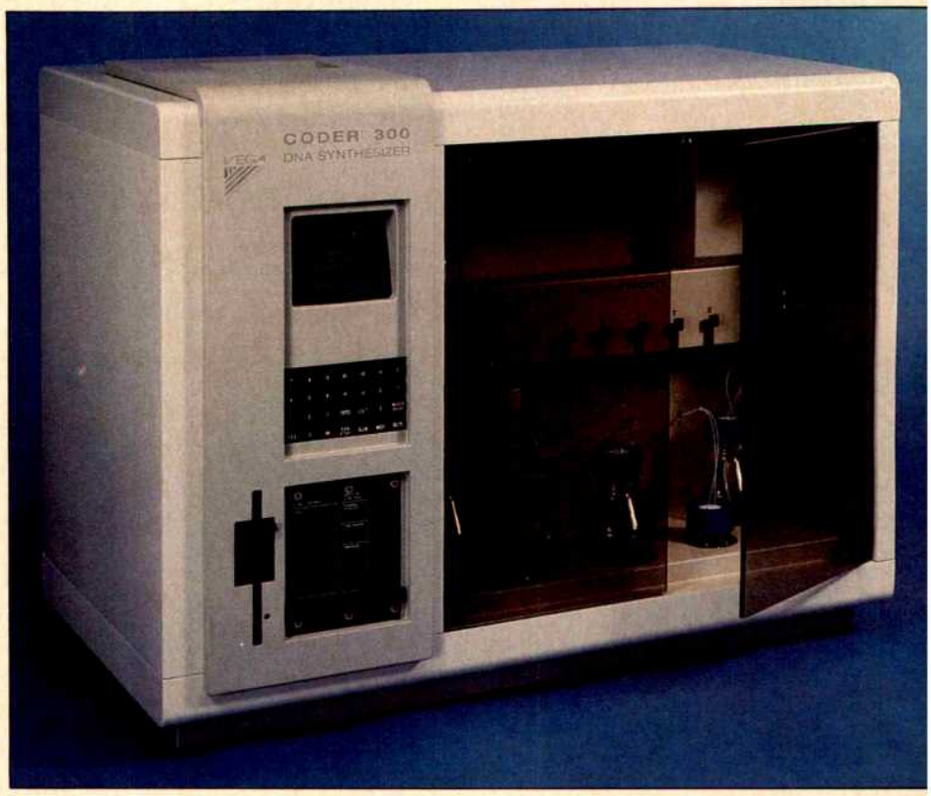
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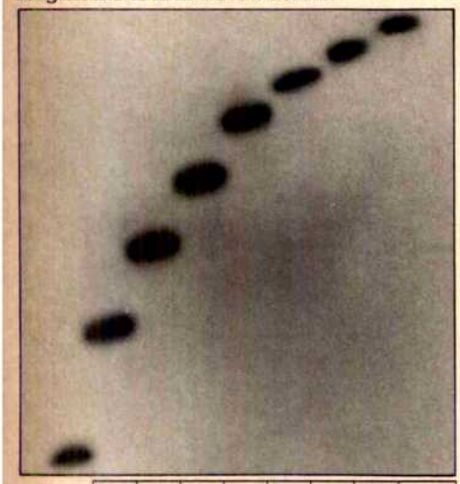
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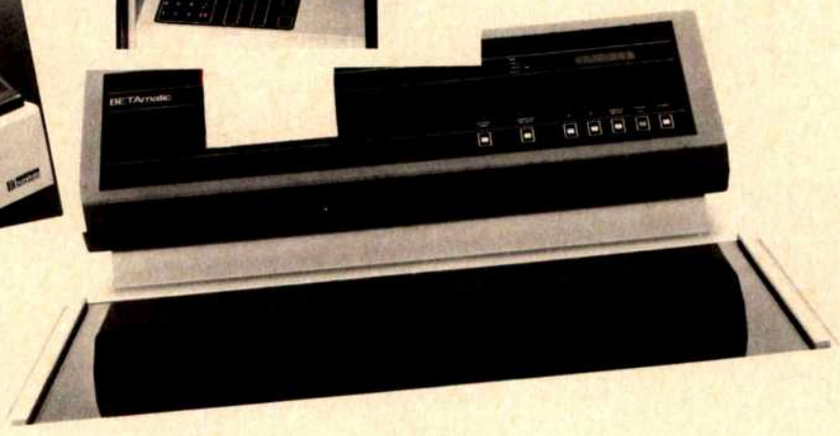
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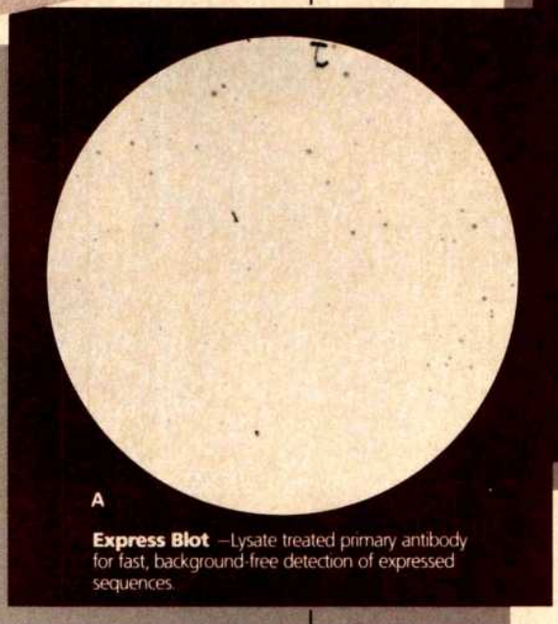
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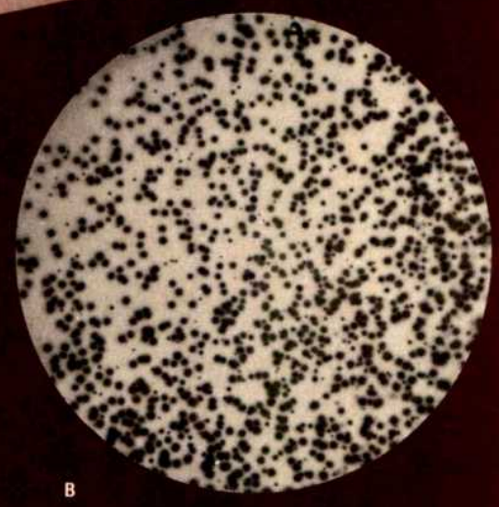
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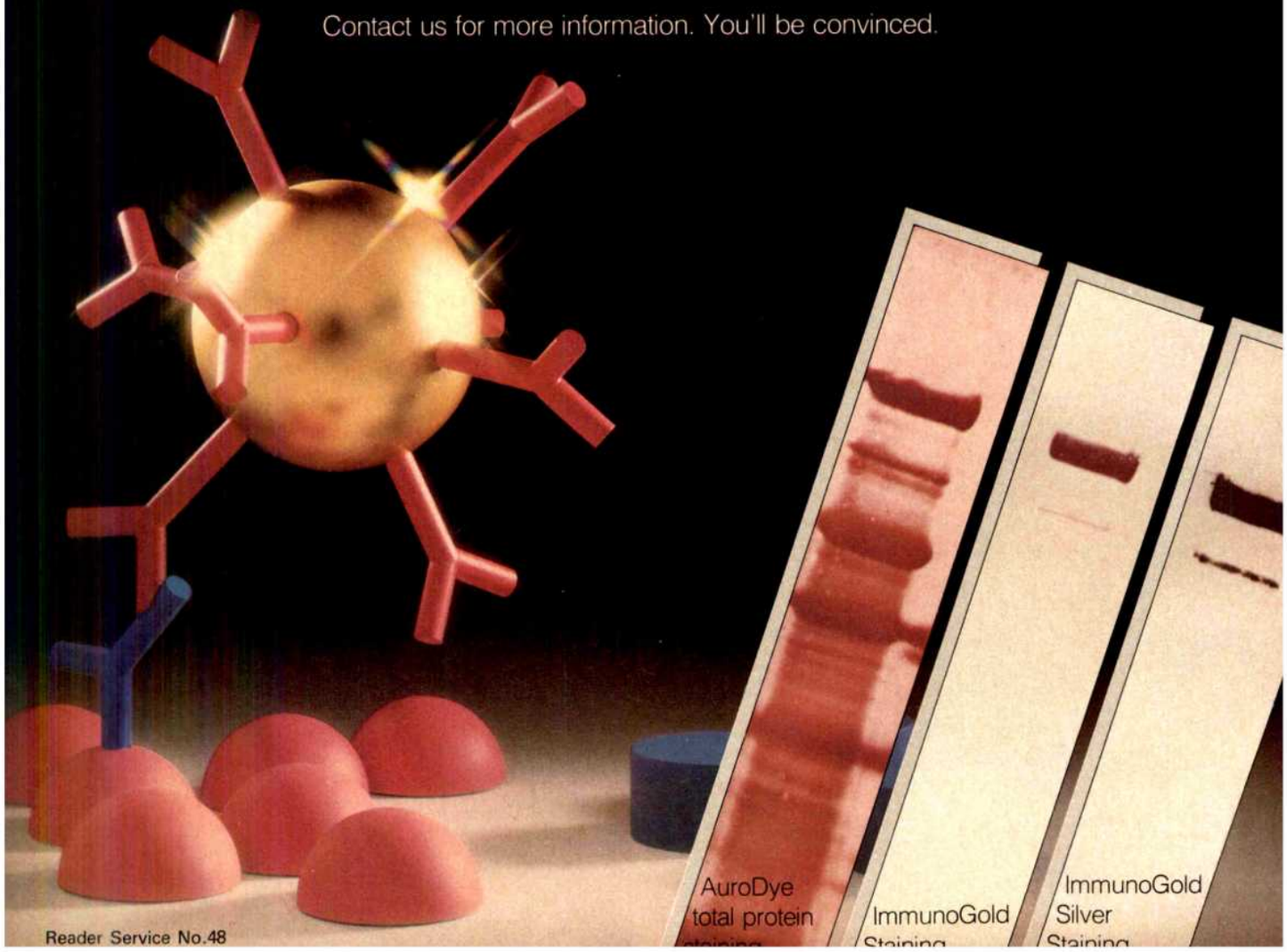
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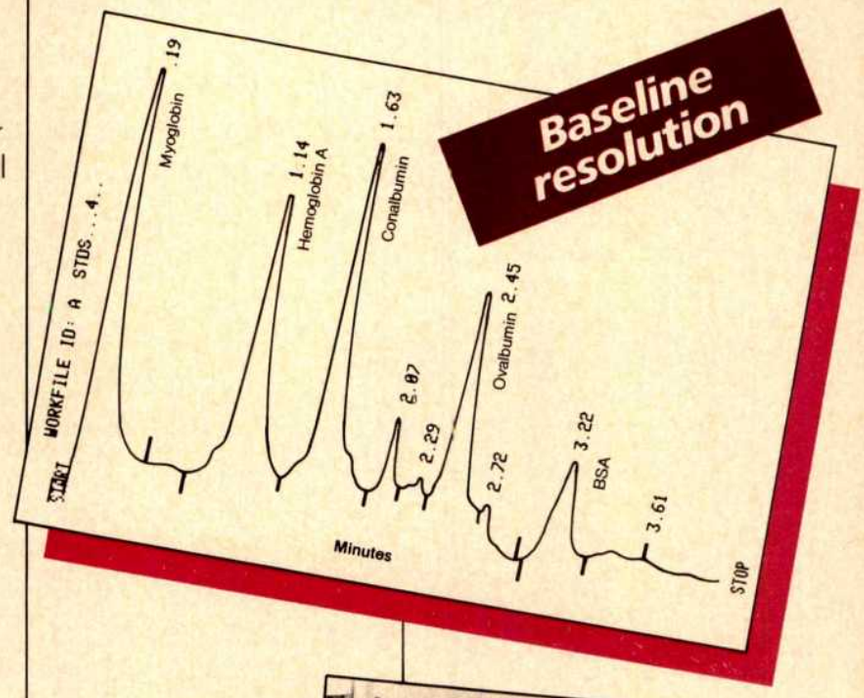
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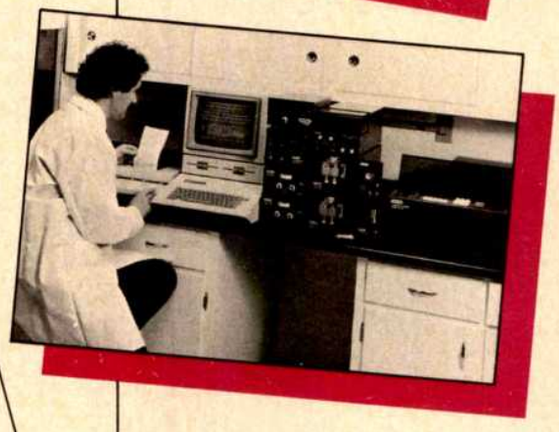
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  RT          AREA TYPE    AR/HT
0.19        55[illegible]500   BB      0.130          23.326
1.14         5861300   BV      0.159                  21.122
1.63         5274500   VV      0.132                  22.812
2.07         1140100   VV      0.092                   4.758
2.29         1865500   VV      0.169                   4.447
2.45         3495800   VB      0.129                  14.589
2.72          435380   PV      0.080                   1.817
3.22         1683800   VB      0.143                   7.027
3.61          216130   VB      0.260                   0.902

TOTAL AREA= 2.3962E+07
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Arms control.

λgt 11 Arms

Expression Cloning. The λgt 11 Cloning Vector has been digested with EcoRI and dephosphorylated. It accepts 0 to 7 Kilobase EcoRI inserts.

Kits contain strains Y1088, Y1089, Y1090 and a test insert. Efficiencies are greater than 10^7 recombinants per microgram of λgt 11 Arms using Gigapack™ packaging extract.



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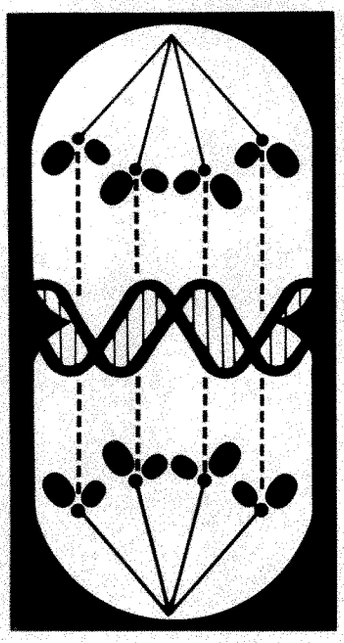
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A major exhibition of scientific products and services will be held in conjunction with the Conference.

PROGRAM SCHEDULE*

DAY 1: MONDAY, OCTOBER 7

SYSTEMS

Speakers	*Topics*
F. Bloom (Research Institute of Scripps Clinic)	Vertebrate neural systems
J. Gerhart (U. of California at Berkeley)	Early development—Xenopus
C. Nüsslein-Volhard (Tübingen)	Early development—*Drosophila*
C. Goodman (Stanford)	Development of invertebrate neural systems
H.R. Horvitz (MIT)	Caenorhabditis

DAY 2: TUESDAY, OCTOBER 8

CELLS

Speakers	*Topics*
M. Davis (Stanford)	T-cell receptors
U. Storb (U. of Washington at Seattle)	B cells
J.M. Bishop (U. of California at San Francisco)	Oncogenes
M. Ptashne (Harvard)	Regulatory proteins
I. Herskowitz (U. of California at San Francisco)	Yeast development
E. Jaworski Monsanto	Plant transformation

DAY 3: WEDNESDAY, OCTOBER 9

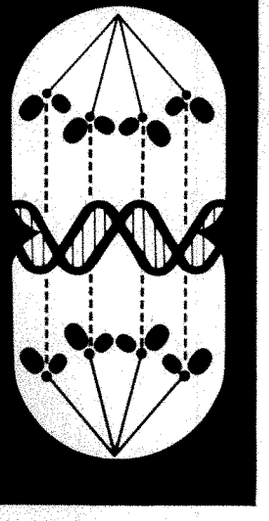
GENES

Speakers	*Topics*
R. Palmiter (U. of Washington at Seattle)	Transgenic mice
D. Hogness (Stanford)	*Drosophila* homoeotic genes
W. McGinnis (Yale)	Mammalian homoeotic genes
T. Friedmann (U. of California at San Diego	Human therapy

*Additional speakers will also appear on the program.

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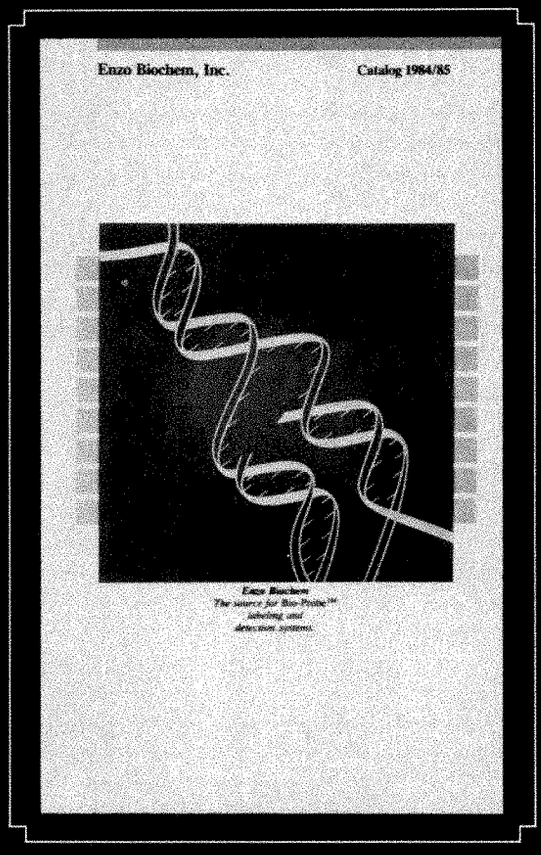
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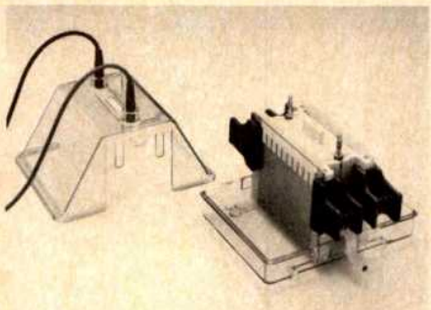
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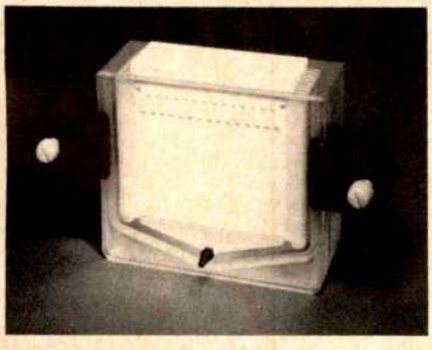
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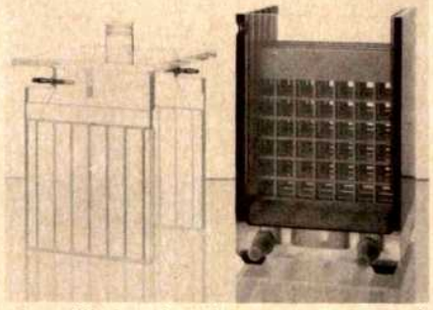


double-sided pod. The pod consists of two discretely separate upper buffer chambers and a serpentine coolant chamber between. *Cooling is the key feature of the Mighty Small design.* The back plate of each gel sandwich is in contact with the upper buffer and is made of sturdy alumina. Alumina is an excellent heat conductor, 40 times faster than a comparable thickness of glass. It rapidly conducts joule heat away from the gel sandwich into the buffer. A serpentine coolant chamber, permanently sealed between the two buffer chambers, assures further heat dissipation. This excellent cooling system means you can get flat, side by side sample lanes with no smile in 45 minutes.
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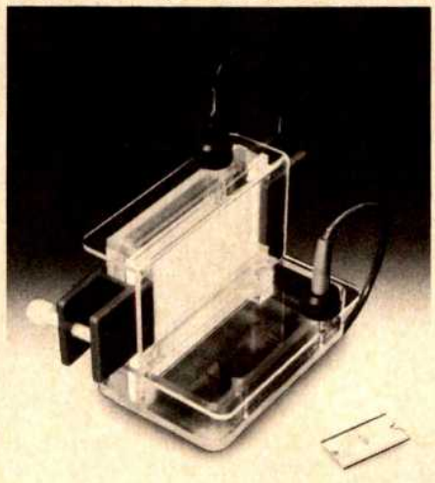


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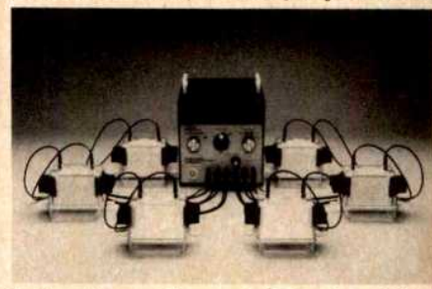
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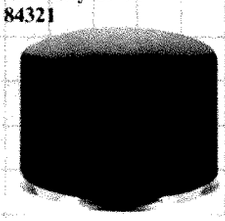
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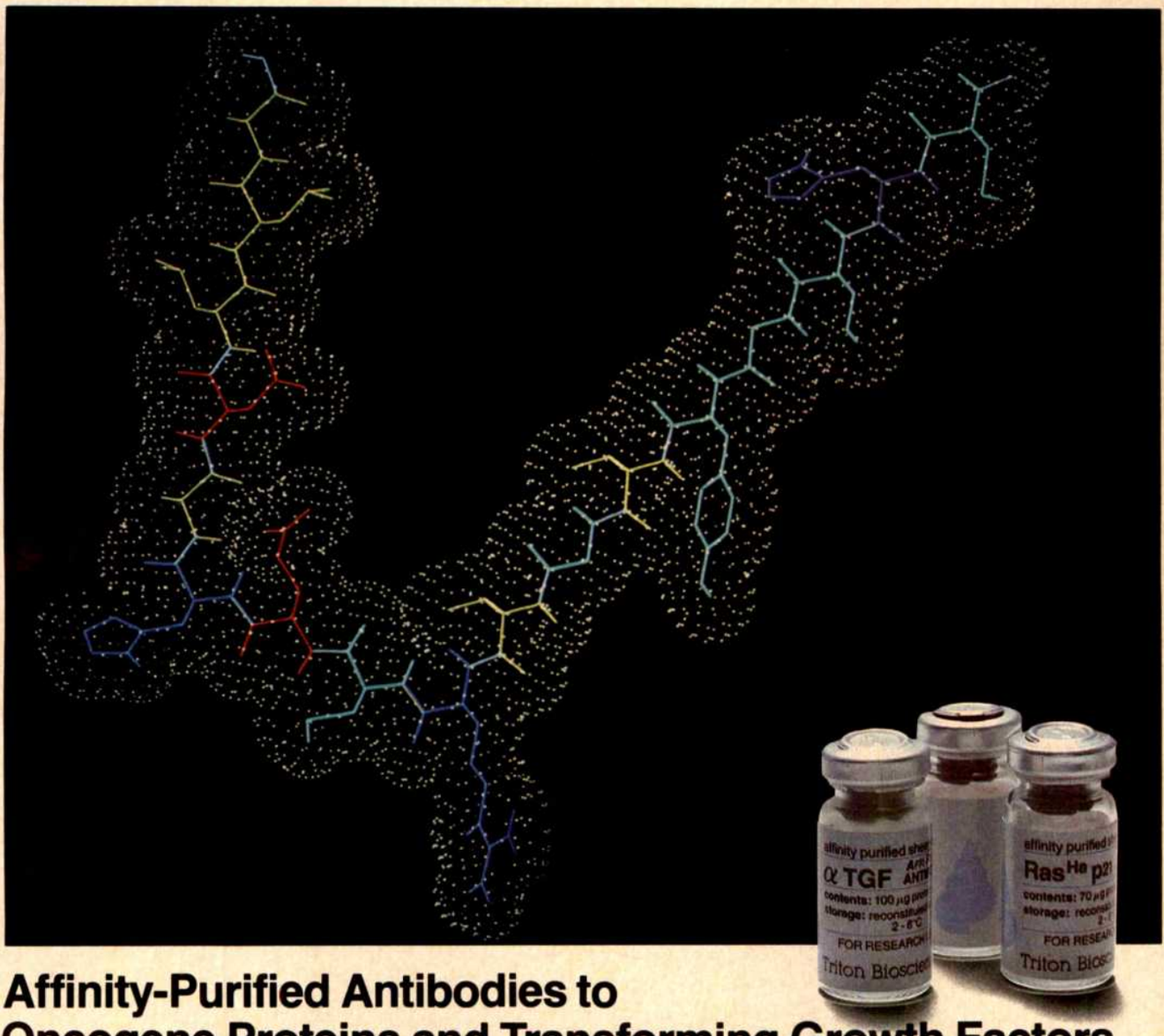
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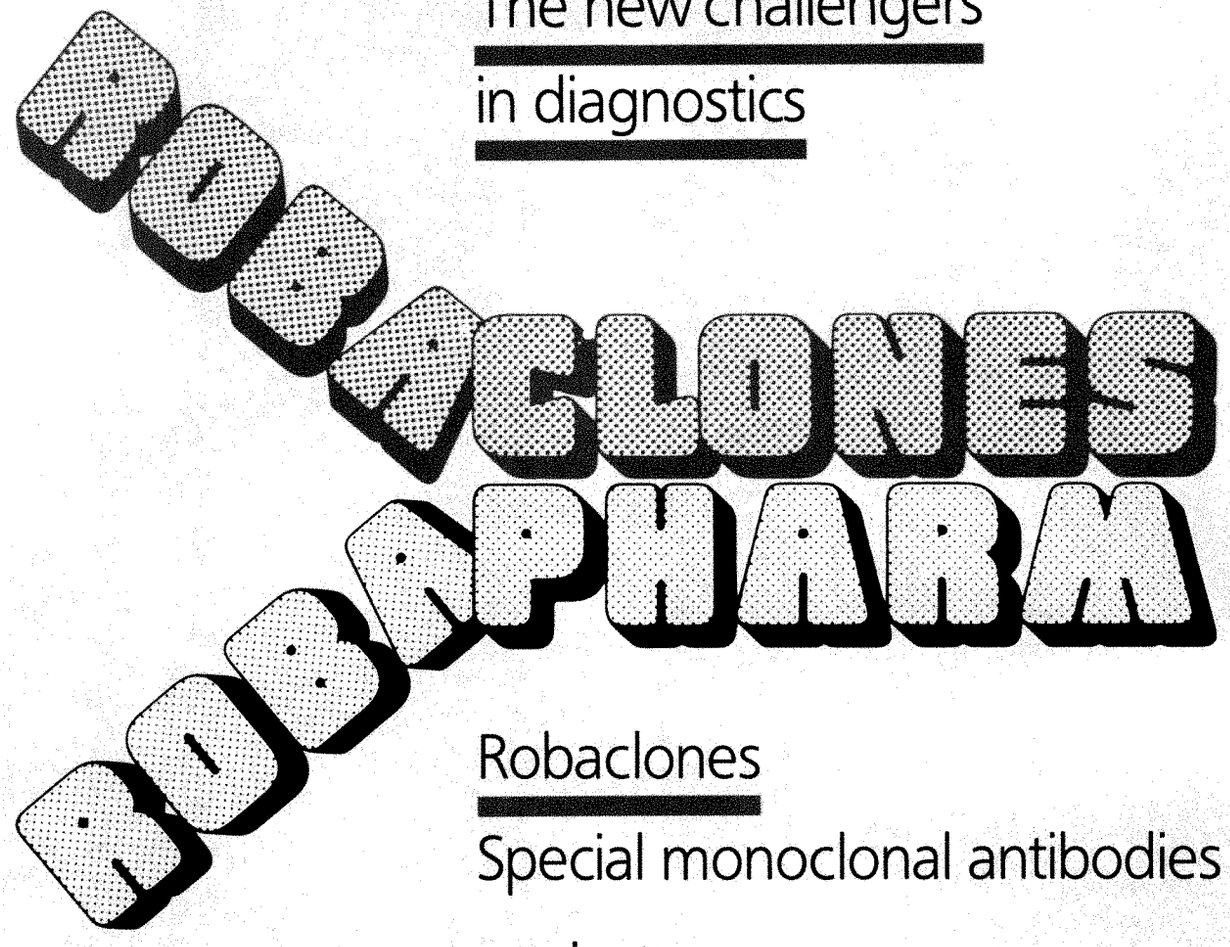
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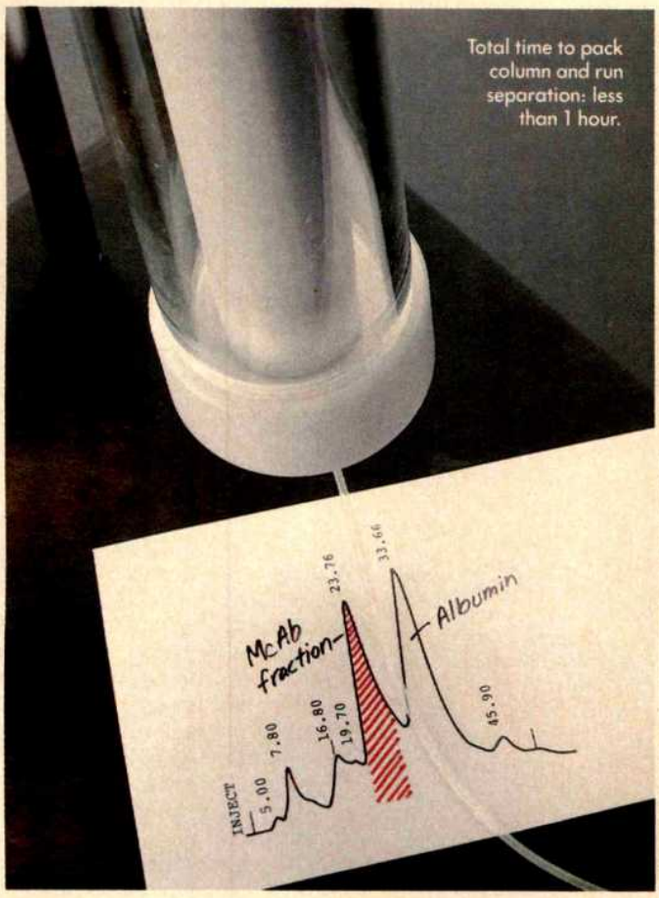
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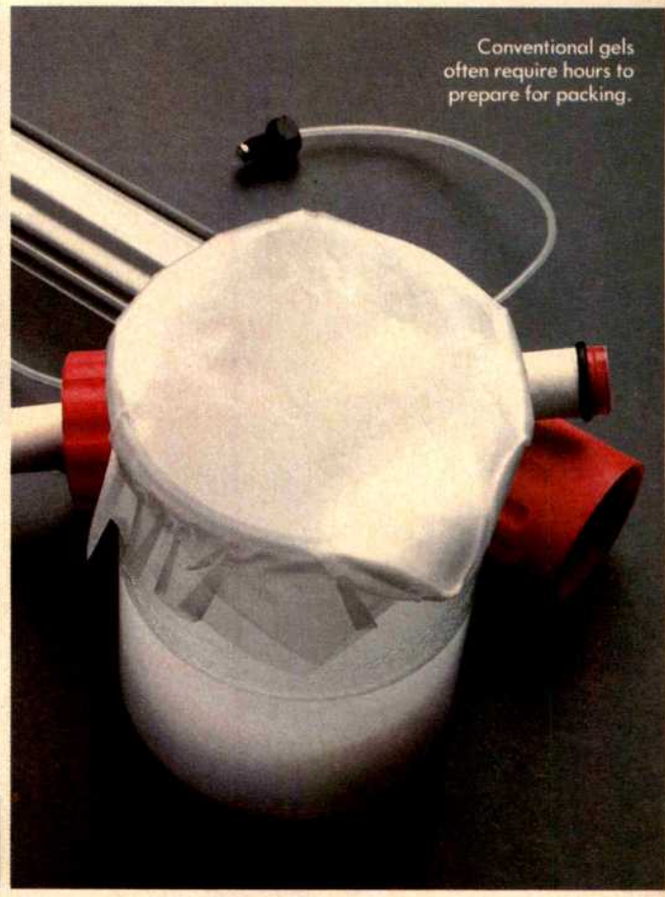
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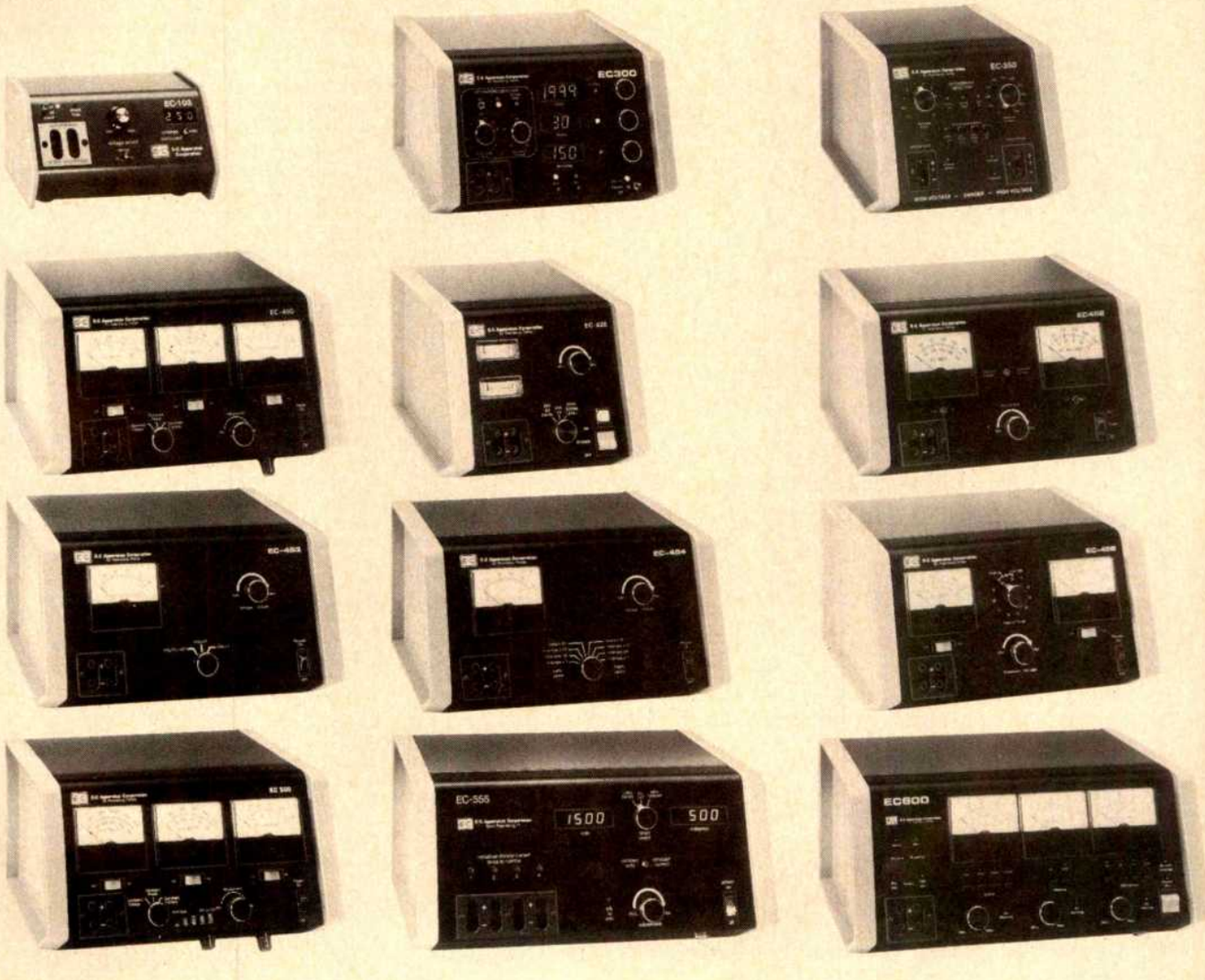
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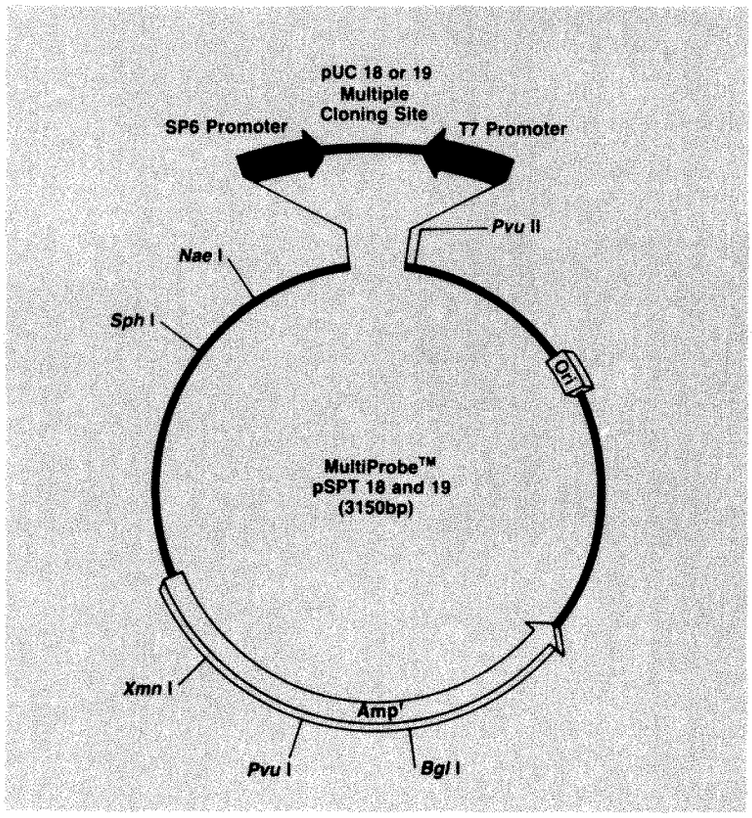
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NATURE VOL 315 20 JUNE 1985

Retreat from particle physics?

The Kendrew committee has given the expected opinion on whether Britain should pull out of high-energy physics. But was it asked the right question?

THE Kendrew committee has had a thankless task in deciding whether Britain could continue to afford high-energy physics. Its report now published (see p 619) faithfully reflects the difficulty of reaching a clear decision on such a complicated issue. The presenting symptom of the trouble that has arisen is that British spending on high-energy physics, taken entirely from the budget of the Science and Engineering Research Council (SERC), although much reduced in the past decade, is still (at £55 million) an uncomfortably large fraction of the total (£288 million for SERC in the present financial year). The consequences for the general support of science in Britain have been all too clear in the past few years. Good projects even in basic physics have languished for lack of funds and even, latterly, because those best qualified to execute them have been persuaded by the general climate that they had best try their luck elsewhere. Yet, curiously, it remains a fact that British spending on high-energy physics is not entirely out of line with what is spent by the other members of the European Organisation for Nuclear Research (CERN). As a fraction of gross national product, British spending on high-energy physics is less than that of France, Italy and West Germany but greater than that of Austria, the Netherlands, Norway and Greece. So why is it that Britain, for the time being at least, is the only member squealing about the cost?

There are some extenuating circumstances, of which the chief is the curious way in which the British government stolidly refuses to guarantee the costs of international collaborations against the effects of currency fluctuations. Occasionally, there may be special concessions from the Treasury, but more frequently there are not. The result is that research councils locked into international agreements for collaboration that have the force of international treaties must raid their provisions for domestic spending whenever sterling moves against them. The Kendrew committee has rightly asked that the British government should follow a more generous policy towards the research councils' difficulties in this regard. Indeed, it could well have made the point less temperately. For how can the government expect the research councils to plan carefully for the future, while following the rule that funds cannot be held over from one year to the next, when they can never know when it will be necessary to scrape the barrel for an unbudgeted few million pounds or so?

Structure

A more serious problem is structural, and particularly concerns SERC, which differs from other research councils in that its function is exclusively to support research in universities and polytechnics, and to provide the necessary central facilities. This is the spirit in which, in more fulsome days, SERC provided not only the annual contribution to CERN but domestic high-energy physics laboratories (long since converted to other purposes) as well. The trouble with high-energy physics is that even the reduced costs of the present operations are too large a proportion of the budget to be easily digested, but this is not the only hostage to fortune that SERC has given. Who can say whether the cost of maintaining SERC's spanking new observatories, on Hawaii and at La Palma in the middle of the Atlantic, will not become some years from now the kind of embarrassment that CERN seems now?

Commitment

The truth is that SERC's total budget of £288 million (this financial year) is not freely disposable income in the true sense. Large parts of it are committed in advance, not merely for the payment of annual instalments of research grants awarded in previous years and for maintaining central facilities and their staffs, but also because SERC has been persuaded, in what seems to have been the national interest, that it should support with its own funds substantial government initiatives, for example, information technology, and at the same time increase support for engineering research at universities. The CERN subscription, however onerous, is only one of the several commitments that explain why SERC has had so little to spend on the core of scientific research. So the Kendrew committee might reasonably have argued that it might have been spared the trouble of existing if SERC, over the years, had more zealously managed its affairs for flexibility. Somewhat delphically, all that Kendrew says is that "the organization of science in Britain should be rationalized so that funds available are used more effectively". That, presumably, is what people have been attempting for decades past.

The course of action now suggested is an awkward compromise. Nothing much will be saved in the next three years or so, although there will no doubt be a flight by graduate students from high-energy physics into other fields. Since there is very little chance of the government relenting, and allowing a substantial increase of the funds available for civil science, SERC will continue to be unable to support the projects now sent away begging for at least that long. And even if the negotiations with CERN are successful (which is improbable), there will not be much extra in the kitty — perhaps £15 million a year seven years from now, a mere 5 per cent of the annual budget. In the circumstances, it would have been better that the Kendrew committee should have bitten hard on the bullet put between its teeth, should have counted the real British moral obligation to CERN as a small consideration compared with the survival of British science, and should have plumped for withdrawal at the end of 1986 (the earliest date possible). It could have added that it would have preferred to have been asked a different question, and might even have formulated some of the more intelligent questions that need answering. But that way, at least, there would have been an early prospect of relief from the present crisis.

Ructions

But would not such a course have been a recipe for ructions? Of course, Llewellyn-Smith (see p.619) is entirely right in most of what he has to say about the consequences for CERN, and for

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the British reputation in European collaboration, of even the compromise course of action now recommended. Outright withdrawal would have caused a bigger row, but one not qualitatively very different. Moreover, such a decision would have laid the trouble squarely where it belongs, with the government whose niggardliness has brought about the crisis of the past several years. Indeed, it is entirely possible that if the British government as distinct from SERC were faced (as it may be still) with the task of engineering withdrawal from CERN, it would find the task politically unpalatable.

Whatever happens now, the Kendrew committee has demonstrated vividly that something must be done about the condition of academic research in Britain. One of its most compelling passages is the account which it has gleaned from the public grant-making bodies of the projects the research councils are unable to support for lack of funds. This is also the theme of last week's report from the Advisory Board for the Research Councils, published early so as to inform last Friday's debate in the House of Commons (see p.623). (By all accounts, Kendrew would have been similarly dealt with if only the Department of Science and Education had had an extra word-processor.) Quite why that occasion took place is still far from clear. The initiative was that of the Secretary of State for Education and Science, Sir Keith Joseph, but on this occasion the apostle of management by cash limits spent much of his time reciting the difficulties attending British academic research, the flight of people, for example. Was the debate Sir Keith's own way of acknowledging that his critics have won the day, and of recruiting their help in talking down his colleagues in the government?

However that may be, it seems not to be fully appreciated that an urgent remedy is required. Some good ideas have always been wasted because there are no funds to back them. What matters now, in Britain, is the sheer volume of talent going to waste. Even the good ideas that, with luck, find backers are often supported with such economy that their success is jeopardized. Last week's advisory board report gives several examples of projects with industrial importance which are less successful than they might be for lack of adequate support. Is it any wonder that, in these circumstances, people's spirits have evaporated? Or that those not yet entirely demoralized think first of taking their skills elsewhere?

Yearning

The simple solution is that the government should find some extra money, on a strictly short-term basis, to see that projects of outstanding quality (and their authors) should not be lost during the painful but necessarily cumbersome reorganization of the research enterprise now in prospect. An extra £100 million spread over the next three years would do an immense amount to help. If the government is afraid of distributing such a bonus among the research councils in the usual way for fear of giving them ideas above their station, it might think of distributing the money by some different mechanism. It would be by no means unreasonable or inappropriate that there should be a national competition for shares in the central fund. If the government's yearning for industrial collaboration is seriously intended, grants from such a special fund might be made contingent on matching contributions from outside. Is that modest request too much to ask?

Otherwise or perhaps in any case, the research community should take what steps it can to hasten the reorganization of resources on which it has embarked, but which will work its way through the system only after several years. The University Grants Committee and the research councils have the common objective of concentrating resources for research on the institutions best qualified to make use of them, but the process will not begin until next year. Why wait that long? And why, in the interests of fairness, contemplate a long period of transition from the present indiscriminate distribution of resources to a less egalitarian system? Why not move faster, recognizing that the pursuit of fairness may be a luxury when the danger is that the quality of British research will be irreparably damaged? □

The logic of Europe

The plan to turn Europe into a true customs union by 1992 deserves a welcome.

At long last, there is a chance that Europe will become the integrated economic entity of which the founding fathers dreamed thirty-five years ago. Or, at least, the member governments (now twelve, with the accession of Spain and Portugal) will be compelled at next month's annual summit meeting at Milan to come off the fence and to make plain what kind of Europe they wish to see emerge in the remainder of this century. Ordinarily, governments struggle to avoid answering questions of such a general character. The need now to face them arises because the European Commission, the executive branch of what passes for the government of the European Communities, has produced an unexpectedly radical reply to a request last year from member governments for an outline of the steps that would have to be taken if internal customs posts were to be abolished. What the Commission says is simple. There is no reason why customs posts should not be swept away, but it will first be necessary for European governments to make more similar the domestic taxes they impose on the sale of goods.

There is the rub. Over the years, European governments have allowed the accumulation of a bewildering variety of indirect taxes on goods as different as alcoholic drinks, tobacco products and commodities considered to be luxuries, motor cars included. Then, membership of the European Communities has compelled the development of a novel system of indirect taxation, called value-added tax (VAT), which is levied at different rates on different classes of goods by the several members of the Communities. The continued existence of customs barriers within Europe is partly a consequence of this patchwork system of indirect taxation. The Commission now proposes that member governments should be constrained in their freedom to levy indirect taxes. During a transitional period, they will be expected not to allow the rates of tax to range outside prescribed limits. The Commission says that it should be possible to arrange that everything could be ready for the abolition of internal customs posts by 1992, which is asking a great deal. The immediate objection, of which much will be heard at Milan, is that the member states are not ready to relinquish such a visible sign of national sovereignty as their freedom to fix domestic taxes.

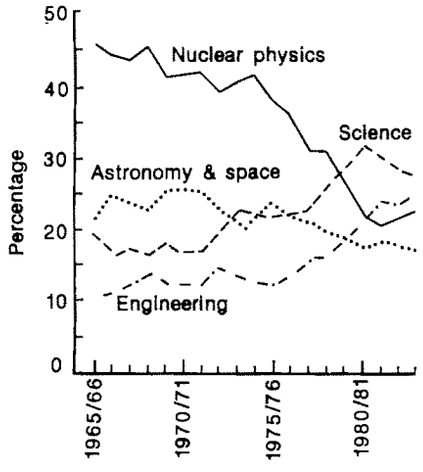
Pragmatically (which is in itself unusual but welcome), the Commission does what it can to defuse this argument by pointing out that the differences between the taxes levied on goods at the point of sale are not so great that their approximation during the next seven years will be impossible. And, in any case, the argument continues, the experience of federal systems such as the United States has shown that even substantial differences between sales taxes levied in neighbouring states do not send consumers scurrying across state lines in search of bargains in the shops. But there is an important issue that the Commission overlooks — the possibility that member states may wish to engineer substantial changes in the balance between direct and indirect taxes in their national accounts. For entirely good reasons, the government of New Zealand has already shifted the burden of taxation from income to consumption taxes (and lost the Timaru by-election at the weekend chiefly as a result). The government of Australia is planning to follow suit, while even the British government has, in previous years, talked of similar proposals. So the European Commission's proposals are more radical than they may seem. Prudent member governments will accept them — and they should — only if there is a mechanism by which general questions of taxation policy can be raised and decided on a European basis in the decades ahead. But that will imply a substantial step along the road to a federal Europe, as the objectors at Milan will be quick to say. The logic of the circumstances that have now arisen, however, is that Europe can not hope to be an integrated market of nearly 400 million people without becoming at the very least what many outsiders think it is already, an effective customs union. □

CERN

Kendrew takes knife to high-energy physics

THE technical consultant to the Kendrew committee on British involvement in high-energy physics, Dr Christopher Llewellyn-Smith, had repudiated the committee's chief conclusion (see *Nature* 29 May, p.266) within minutes of its formal publication earlier this week (*High Energy Particle Physics in the United Kingdom*). Of the recommendation that Britain should seek a reduction in its contribution to CERN, the Geneva high-energy physics laboratory, of "at least 25 per cent by 1991", Llewellyn-Smith says that the "unsubstantiated assertion" that such a reduction could be attained without detriment to CERN's position is, simply, "false".

That the Kendrew report would be contentious has always seemed probable. In the event, however, the report is a model of clarity, which should comfort both its supporters and its critics. Briefly, the conclusions are that Britain should remain a member of CERN "on the present basis" until 1989, but that it should continue beyond that time only if that can be done "at significantly lower cost". The timing is determined by the construction programme for the new electron collider LEP.



The committee notes that British scientists continue to make important contributions to high-energy physics and that the subject is at present at an exciting stage, but it also says that British spending on high-energy physics is too great a proportion of the present or "any realistic" budget of the Science and Engineering Research Council (SERC). It recommends that modest economies should be made as soon as possible; principally in the budgets of groups working on experiments at CERN, does not expect these to be substantial before the financial year 1988–89, but would expect them to increase to "at least 25 per cent"by 1991.

The committee says that the proposed reduction of the CERN budget would be an "attainable adjustment" that would "still allow the laboratory to maintain itself at a world class standard albeit at a reduced pace of development". The proposed reductions of domestic expenditure would entail "a significant decrease in the size of the experimental community" (now estimated to consist of 400 senior people and 240 graduate students), but the committee says that it should be possible to arrange for an orderly contraction while maintaining an "appropriate" British contribution to the field

The report argues that the financial circumstances of the United Kingdom which have led the committee to its conclusions "may not be unique" among CERN members, that "the overall level of expenditure on the subject is too high" and that "it would not be counter to the long-term interests of the field" if the pace of development were reduced "worldwide and not merely at CERN".

The committee acknowledges that the total budget of CERN has decreased by 20 per cent in real terms over the past decade, and that British spending on high-energy physics has declined during the same period "from what was an extraordinarily high level". It also says that there is insufficient support for first-class research proposals both in high-energy physics and in other areas

The report suggests that there is at present little room for manoeuvre within the British budget, pointing out that there would be no point in remaining a member of CERN if it were not a first-class laboratory and if domestic funds were insufficient British physicists to make full use of it. The report also says that there is a "strong moral obligation" on the United Kingdom to remain a member of CERN until LEP is finished as well as to continue development of the three particle detectors being built for use with LEP.

The Kendrew committee therefore argues that the British delegation to CERN should press for an immediate review of the financial structure of the CERN laboratory, with the objective of reducing costs after 1989. Allowing for both domestic savings and reduced expenditure at CERN, the committee calculates that it should be possible to save a total of $55 million over the next seven years, or an annual $13.8 million (at present prices) in 1992–93 and succeeding years. The Kendrew committee asks that the British government should help to avoid future difficulties by arranging that the budgets of the research councils will not be further eroded by inflation, and that the research councils should be insulated from fluctuations of exchange rates.

The committee goes on to say that even the saving likely under its proposals will not "in itself solve the financial problems of other areas of science", and that extra funds will be needed if the British research base is to meet "the challenges of the next decade".

Formally, the report is a recommendation to SERC and the Advisory Board for the Research Councils, on whom will fall responsibility for deciding what should be done. The most likely next step is that negotiations will be opened with CERN on the proposed reduction of the budget. Most probably, there will be no biting of bullets unless and until those negotiations seem about to fail. □

Informed dissent

THE dissenting opinion from Dr Christopher Llewellyn-Smith, reader in theoretical physics at the University of Oxford, is strong meat. It begins by saying that the real issue facing the whole of British science is "the erosion of our superb scientific tradition" by the inadequacy of funds. Llewellyn-Smith says that he informed the Kendrew committee of his dissenting opinion before making it public.

Among Llewellyn-Smith's objections are the following.

- **The demand that the British contribution to CERN should be reduced by 25 per cent implies a similar reduction of the total budget.**
- **Given that salary costs at CERN are roughly half of the total budget, reducing the budget by a quarter would require either a disproportionately larger cut of scientific activity or a reduction of the staff employed (which would not yield immediate savings because of the high cost of redundancy).**
- **It is inconceivable that the necessary two-thirds majority for a 25 per cent cut in the CERN budget could be found among the present membership, for which reason the committee's main conclusion "implies withdrawal . . . the committee fails to face up to this consequence."**
- **The suggestion that the proposed domestic reduction of 25 per cent could be orderly "is ludicrous"; because of the long timescale of high-energy physics experiments, the cuts would fall on those whose projects are about to be completed.**
- **As recently as 1981, SERC offered the opinion that the budget for nuclear physics should remain constant, since when high-energy physicists have made long-term commitments.**
- **The recommendations in the report would seriously damage particle physics, would damage the credibility of the United Kingdom as a collaborator in science projects and yet would make only a small contribution to the "solution of the crisis in science funding".** □

Sandia Labs

Looking to the future

Albuquerque, New Mexico

A CARTOON circulated recently at Sandia National Laboratory shows a wizard complete with pointed hat gazing intently into a crystal ball; a sign on the door in the background reveals the location to be the head office of the Future Options Group (FOG) an 8-man team of defence scientists which spends its time trying to do just that. Glenn Kuswa, manager of the FOGs (as they are known) for the past 10 months, admits that the mysterious-sounding group of futurists that he leads is a fair target for cartoonists, but he insists that the group is taken perfectly seriously by Sandia's senior management. In a national laboratory with primary responsibility for defence, future-gazing is a serious business.

FOG was formed to advise on long-term planning at Sandia only three years ago, but has already had a significant impact on both the weapons development and the basic science at Sandia. Its concern about the importance of high-speed computing, for example, led to a new laboratory division devoted to computer architectures. Sandia, in addition to carrying out contract research (mainly for the Department of Energy), is charged with anticipating future defence needs, and several of its major research efforts, such as sub-micrometre semiconductor technology, were initiated by the laboratory management

While the laboratory's primary mission is still research into nuclear weapons, the scope of this work has broadened considerably in recent years, so much so that the laboratory recently had to be reminded by Washington that biological research, at any rate, could not be considered part of its mandate. More and more projects are carried out in collaboration with commercial companies, and the number of staff has grown to match Sandia now employs more than 8,000 people, a figure that is not expected to increase further.

FOG is the creation of an informal group of laboratory managers seeking a mechanism for looking beyond the normal course of events, pondering how Sandia should anticipate the future

The group started by concentrating on strategic defence, and gave advice on the subject to the White House long before President Reagan's famous star wars speech of March 1983. Missile defences continue to take up a large part of the FOGs' time. Electrothermal guns might, according to Kuswa, be capable of accelerating projectiles to even greater velocities than the rail guns now being intensively investigated for the Strategic Defence Initiative: the physics of impacts at extremely high velocities and their implications for weapons design are one focus of the FOGs' attention.

So far, most of FOG's divinings have been delivered as briefings to Sandia's senior management, with some informal contacts with outside bodies such as the Congress's Office of Technology Assessment Kuswa thinks FOG has played an important, if little-known, role in fostering communications between different factions at Sandia, and wants to see it move towards producing more formal written reports in future.

Not surprisingly, the successful maintenance of nuclear defence is counted the group's primary objective. The issues tackled range from the particular — for example, hypervelocity guns — to the general — brainstorming and arranging training courses in global security issues such as the world's energy supply.

Kuswa has thought about how the "nuclear winter" hypothesis might affect deterrence, but FOG is not yet committed to studying the topic in earnest. By and large FOGs just think, rather than do experiments, and Kuswa thinks that the group should wait until there are more data on nuclear winter before addressing it.

FOG can see further into the future of photonics and fibre optics, which might lead to revolutionary advances in computing speeds and which Kuswa says will certainly have important implications for the way high-speed data are recorded. Such matters are crucial for the strategic balance of power, and Kuswa believes computing is important enough to warrant at least one FOG member permanently tracking it Such is the speed of progress that Sandia boasts that it has recently produced an experimental computer nicknamed the "VAX in a lunch box"

Software engineering is another topic FOG is studying; Kuswa is alert to the possibility that, despite what many see as a disappointing track record so far, artificial intelligence research could yet rock the strategic boat. In one attempt to make the future come a little sooner, Kuswa's group recently sought to apply expert systems, a spin-off of artificial intelligence research, to some of the laboratory's work. A retired employee had his expertise on the electrical wiring and connectors of nuclear warheads programmed into an expert system for the laboratory's future use. Kuswa hopes to persuade Sandia to make further use of expert systems for similar tasks in future.

A related subject is the likely availability of custom integrated circuits. Whereas circuit design is today still the province of the expert, the increasing sophistication of computer-aided equipment suggests that before too long the design and manufacture of a high-performance integrated circuit engineered for a specific task will be within the reach of most engineers. Contrary to FOG's usual approach, the group recently staged a demonstration of the production of specific integrated circuit.

Kuswa would like ultimately to establish a database of all military hardware, as a resource for studies of what the Department of Defense's shopping list is likely to look like in future (future bombing "platforms", including unmanned ones, are the subject of a current study). This, however, has proved difficult and is unlikely to be accomplished soon. In the meantime, there are plenty of other subjects to think about, and Kuswa can apparently see far enough into the future to know that FOGs are going to be in continuing demand. "We have," he says, "earned our keep."

Tim Beardsley

Cell lines jump US customs

Washington

THE US Department of Agriculture (USDA) has agreed to simplify and streamline its quarantine rules for the import of cell lines. Biotechnology companies have been complaining of delays of as much as a year while tissue cultures and tissue-culture products await testing at USDA's Plum Island quarantine facility. Industry sources say they hope the new procedures will cut that time to two months.

USDA's quarantine rules apply to imports of any materials that could contain foot-and-mouth disease virus and other livestock diseases not already present in the United States. Thus cell lines prepared with bovine serum or the enzyme trypsin (which is derived from cows and pigs) come under the quarantine. The safety test involves inoculating a steer, waiting 4–6 weeks and analysing a blood sample.

During negotiations between USDA and representatives of the biotechnology industry on speeding up the process, it became clear that most of the delays were bureaucratic. Companies would, for example, be sent a bill for a test that had to be paid before testing would begin. Under the new procedures, companies will be able to establish deposit accounts with USDA so that the tests can be run as soon as products arrive at Plum Island.

The new rules will not, however, provide exemptions from the strict testing requirements that the industry had been seeking in respect of materials to be used only in clinical applications or *in vitro* diagnostics. The industry argues that so long as such materials are disposed of properly, there is virtually no chance of livestock being exposed. USDA officials say that discussions with the industry on this issue will continue.

Stephen Budiansky

Polish universities

Reforms threaten independence

POLAND'S Main Council for Science and Higher Education, the representative body of the university community which serves as an intermediary between the universities and the Minister of Science and Higher Education, stands to lose most of its powers in the planned reform of the 1982 Higher Education Act. Since the discussions on reform began last autumn, the Main Council has resolutely opposed the proposals which would effectively annul the university autonomy won during the Solidarity era. A vote taken on the proposed changes early in March showed only one member of the council in favour of the changes, with a handful of abstentions Now, a new draft of the proposed reforms, which reached us last week, threatens the status of the Main Council itself.

The 1982 Act established the Main Council as the supreme elected organ of the university community. It has seventy members, fifty from the universities and colleges under the ministry and the rest from the medical, physical training, art, drama and maritime colleges. The council has power to decide on the direction of research and the organization of studies, to set the conditions for the awarding of academic degrees and has the right of consultation with the minister on a wide range of financial and administrative matters. The original version of the reform strengthened ministerial control and disciplinary powers over the universities, bringing the "students' self-government committees" within the framework of the Party youth organizations, but the role of the council was left virtually unaltered.

With the Main Council resolutely opposed to the proposals, however, the government clearly decided to think again. Of late, the issue has no longer been in the hands of the minister of science, Dr Benon Miskiewicz, but had been handed over to the government's Committee for Social and Political Affairs, under Deputy Prime Minister Mieczyslaw Rakowski. The new draft which has emerged would impose considerable restrictions on the Main Council. First and foremost, the members of the Main Council would no longer be elected, but instead would be "appointed by the chairman of the Council of Ministers at the request of the Minister of Science and Higher Education" (Art. 18.1). "Directions of study" will now be decided not by the Main Council but by the minister (Art. 8 2) And, under the greatly revised Art. 2, the Main Council will no longer "decide at the request of the Minister" but only "give an opinion" on "possible and definite plans for the development of higher education". The same article does, indeed, leave decision-making on research within the universities and in the competence of the Main Council, but with the proviso that its decisions must be within the framework of the central economic plans. On matters affecting the terms of employment of academic staff, the minister will decide "after hearing the opinion of the Main Council" instead of, as at present, "in agreement with the Main Council".

The latest version of the reform will therefore reduce the Main Council to, at best, a powerless talking shop for the universities and, at worst, a rubber stamp for the decisions of the minister and the economic planners. During a special plenary session of the Central Committee of the Polish United Workers' Party last month, General Jaruzelski reiterated the official viewpoint, that the universities cannot any longer be allowed to be the breeding ground of political opposition, but must be more closely incorporated into the socialist structure This triggered nationwide student protests, as a result of which Rakowski and Miskiewicz found it necessary to assure university rectors that the reforms would not be made an excuse for a political purge.

Vera Rich

Electricity supply

Cross-Channel power games

FRANCE will begin pumping 1,000 MW of its cheap nuclear electric power across the English Channel into the British grid later this year, when the first stage of a new 2,000-MW undersea link is completed.

The new cables — four of them — run from Les Mandarins, south of Calais, to Folkestone. They will increase the French-British power linkage more than tenfold, from the present 160 MW transferred along a line between Dungeness and Boulogne mainly to even-out peak demand on each side of the Channel.

But the new link will open in two 1,000-MW stages And while the English utility, the Central Electricity Generating Board (CEGB) and its French partner, Electricité de France (EDF), have reached agreement on the first stage, for France to supply Britain with cheap power, the use of the second 1,000 MW is still in dispute

On its side, EDF would like CEGB to buy another 1,000 MW of "baseload" continuous power at French marginal supply prices, which EDF is quoting at attractive rates. This is not surprising, as within a few years the French electricity supply will be more than 70 per cent nuclear. Nuclear reactors are hard to turn on and off, so the responsiveness of the French network to demand fluctuations has been decreasing, despite efforts to make French pressurized water reactors more variable.

CEGB, on the other hand, in considering a second 1,000 MW from France, is said to be concerned about its agreements with the British National Coal Board (NCB), now recovering from its year-long coal strike. CEGB is by far the coal industry's biggest customer, and is contractually committed to buying large amounts of NCB's coal. CEGB cannot buy too much French power without endangering those agreements, and the British coal industry, it is claimed

Or, to put it differently, it seems that while 1,000 MW of French electricity is convenient enough to make a sharp point to the government and NCB about the value of nuclear power — and a warning that coal prices had better be kept down — 2,000 MW from France might be contractually uncomfortable.

Robert Walgate

CSIRO on the right rails?

Canberra

THE Australian Minister for Science, Mr Barry Jones announced last week the appointment of Dr N.K. (Keith) Boardman as the next chairman of the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Dr Boardman, for the past eight years a member of the three-man CSIRO full-time executive and formerly chief research scientist of the CSIRO Division of Plant Industry, will take the reins for nine months in the first instance, beginning in September when the present chairman Dr J.P. (Paul) Wild retires. Mr Jones also announced the appointment of two new part-time members of the executive, Dr Adrienne Clark, a Melbourne University botanist, and Dr Kevin Foley, chairman of the Australian Industrial Research and Development Board. Like that of Dr Boardman, their appointments are for nine months pending the outcome of a series of reviews into government-sponsored science in Australia being undertaken by the Australian Science and Technology Council, in the course of which CSIRO is only the first research establishment to come under scrutiny.

The conclusion of the CSIRO review is meant to coincide with Dr Wild's retirement. A former chief research scientist of the CSIRO Division of Radiophysics and 1980 Royal Medallist of the Royal Society, Dr Wild has personally lifted the public profile of CSIRO through highly-visible and commercially-orientated work on the new aircraft landing system Interscan and, more recently, his plan to link Australia's two largest cities with the national capital Canberra using trains operating at speeds of up to 350 kilometres per hour.

Jeffrey Sellar

Arianespace

Europe's launcher cashing in

ARIANESPACE, the French-based company that sells the successful European space launcher Ariane, has tripled its turnover compared with projections made when the company was set up five years ago, according to company president Frédéric d'Allest last week. Bursting with enthusiasm, and apparently happy to challenge competitors in the United States and anywhere else, d'Allest said Arianespace has 35 satellites and FF7,000 million (£600 million) on its order books.

"There has been a very dramatic increase of activity" and "we at Arianespace are the only launch agency that can balance its accounts", d'Allest said. Arianespace was planning three launches a year and now is planning a firm six, with up to eight a year possible.

A further new launch pad, ELA 2, will be inaugurated at the Kourou launch site in French Guyana, north of Brazil, in October. At only five degrees of latitude from the Equator, compared with Cape Canaveral's 28 degrees, the site has the benefit of greater natural launch velocity (from the Earth's rotation).

At the same time, d'Allest dismisses all competition in launch vehicle manufacture. In the United States, attempts are being made to commercialize the "obsolete" Atlas Centaur and Thor Delta rockets. But there will be no significant improvement in the technology of expendable launchers in the United States, he claimed. For, d'Allest argues, no commercial company can bear the costs of launcher development while the US government is unlikely to make a substantial contribution to the development of expendables for fear of undercutting the economics of the space shuttle.

Meanwhile Ariane development goes on apace, he said The penultimate Ariane-1 should launch the Halley probe, Giotto, Ariane's first interplanetary mission, on 2 July, while the slightly bigger Ariane-3 has become the main Arianespace workhorse. Ariane-4, with strap-on liquid and solid-fuel boosters, designed for a new generation of telecommunications satellites, will be launched in mid-1986 and enter commercial service the following year.

After Ariane-4, a completely new design of Ariane will take over, a design using HM-60 cryogenic engines now under development by the French national space agency CNES. This "Ariane-5", capable of launching the bigger communications satellites being developed, will carry Arianespace from 1995 to the end of the century.

Arianespace could face some competition from Japan's H2 launcher, but H2 is too small, more comparable with Ariane-4 than Ariane-5, said d'Allest. Moreover, Japan is constrained like the United States by the northern location of its launch site.

The problem with the Soviet Union, a potential competitor, is that its launch sites are under military control and very tight security, so very little information emerges on launch technologies, communications facilities and so on, too little to make possible fruitful cooperation with a commercial client. In China, the Long March 3 rocket, at the testing stage, is similar to Ariane-1 Chinese technology will develop, but like Japan, China is hampered by a northern launch site. And the painful West German experience in the private OTRAG venture, which attempted, unsuccessfully, to use ultra-cheap technology and a Zaire launch site, showed that there was no easy route to space. d'Allest acknowledges that fibre optics may become competitive with communications satellites on certain trunk routes, but considers that there will be enough launcher business left to keep Arianespace healthy.

That leaves Arianespace, which has taken 48 per cent of the world commercial satellite market since 1981, "in the clear", given that the US space shuttle has proved expensive. The company's objective, according to marketing manager Jean-Claude Bichet, is to take one third of the market, which is to be found 40–50 per cent in the United States, 18–20 per cent in Europe, 10 per cent in the international bodies Inmarsat and Intelsat, and 20 per cent in the rest of the world. Arianespace hopes soon to be launching 8 to 12 commercial satellites a year.

Robert Walgate

Rocketing insurance

ARIANESPACE may have to wait a year before it can announce reductions in the insurance premiums for satellites launched on Ariane, even though these premiums have reached punishing levels after satellite losses on the space shuttle (see *Nature* 6 June, p.447). So said M. Charles Bigot, director-general of Arianespace, who was in London last week with his president, Frédéric d'Allest, to discuss, among other things, new insurance terms with City of London brokers.

"We had hoped to announce new terms at the Paris air show a few days ago", said Bigot, but the problem has proved more difficult than expected. "The insurance companies are treating all launchers as if they were the same kind of car", Bigot complained. But Arianespace is not going to fight dirty and use French government pressure, as it could now that most French sources of finance are nationalized. "This is a commercial question and we shouldn't mix it with politics", he said.

Robert Walgate

Exhaust emissions

No agreement in Europe

Brussels

VEHICLE emission limits announced two weeks ago by European environment commissioner Stanley Clinton-Davis are expected to receive a very cool reception from the ten environment ministers when they meet in Luxembourg on 25 June, which could yet again delay a decision to control car pollution.

Last March, environment ministers of the European Community had agreed to stagger deadlines for the introduction of new emission standards according to engine capacity, and had asked the European Commission to provide exact figures for emission limits for carbon monoxide (CO), hydrocarbons and nitrogen oxides (HC and NOX) and for nitrogen oxides on their own (see *Nature* **314**, 304; 1985). These figures, it was agreed, would achieve an environmental effect equivalent to that reached in the United States, and would allow the use of lean-burn technology in the case of the medium category of car (1.4 – 2.0 litre).

But the limits suggested last week for medium cars (30 grams per test for CO, 8 g/t for HC plus NOX and 4 g/t for NOX) seem to be unobtainable with lean-burn technology. When differences between the European and US test cycles are taken into account, the suggested limits are stricter than the standards applied in the United States and could remove 20 per cent of present large cars from the roads.

Several countries consider these new figures unrealistic and feel they do not correspond with what was agreed at the March council The United Kingdom had suggested 35 g/t for CO, 12 g/t for HC plus NOX and 6 g/t for NOX, while Italy and France, which have carried out their own tests in the meantime, maintain that 4 g/t for nitrogen oxides is not achievable except by using the catalytic converter.

Mr Clinton-Davis is determined to take a firm stand. His aim is to see NOX emissions in the Community cut by half from 3 million tonnes to 1.5 million tonnes.

But the Commission's proposals are unlikely to please those countries seeking a more flexible approach Furthermore, they will once again put West German interior minister Friedrich Zimmerman in a difficult position. At the last council, he obtained Community approval to go ahead with plans to introduce national tax incentives for clean cars, but these incentives have to be tied to clear-cut emission standards. If Community ministers cannot agree, Zimmerman will either have to rely on US standards when the incentives come into effect on 1 July or continue to hold up the project, which could further adversely affect an already depressed car market.

Anna Lubinska

British science

Commons debate cuts little ice

LITTLE blood was drawn, and no concessions won from the British government, during last Friday's debate on science in the House of Commons. But the government had agreed to the demand of Dr Jeremy Bray, the Labour Party spokesman on science, that this year's advice from the Advisory Board for the Research Councils (ABRC) should be published for the debate, it asks that the government should give the research councils £85 million extra over the next three years and wrings its hands over the rate at which younger scientists are leaving Britain.

Sir Keith Joseph, Secretary of State for Education and Science, admitted at the outset that a "significant" part of the science budget has had to be spent on activities other than research, but insisted that the government has protected the budget in gross terms. This assertion was disputed by many members of parliament (MPs) during the debate (see also *Nature* 23 May, p 271), for example by Dr John Marck, who pointed out that the Agricultural and Food Research Council (AFRC) is facing cuts of £10 million this year and £20 million next.

Sir Keith also acknowledged that the number of scientists leaving Britain to work abroad where the potential rewards are greater is a matter for concern On the other hand, he was encouraged by the increased collaboration of the research councils (which fund much of Britain's civil research) with industry in a selective and efficient way. He applauded the reduction of the research council's long-term commitments and their consequent increased flexibility and concluded that, for British science, "the way forward must be to scrutinize carefully the priorities implicit in the present balance of funding between subject areas and to consider whether some adjustments may be needed better to serve the national interest". He promised to assess ABRC's recommendations in this year's public expenditure survey, but demanded more investment in basic science from industry, particularly in engineering.

Opposition and Conservative MPs alike demanded action from the government to keep Britain in the front line of international science research. Dr Bray said that the least the government could do is to guarantee ABRC's recommended increases. His worries about the enforced drift of scientists elsewhere were epitomized by the £25 million laboratory set up by a US pharmaceutical company for Dr Leslie Iversen, formerly head of the Medical Research Council Neuropharmacology Unit at Cambridge. Dr Bray said that the work should be available to science generally and to British companies for commercial development, rather than being "pre-empted" by a US company He argued for the creation of a Minister of Science, and for an annual survey of industrial research and development

Mr Ian Lloyd (Conservative) endorsed Dr Bray's proposals but had one of his own to make as well, the setting up of an Office of Technology Assessment similar to that in the United States This independent body would serve both houses of Parliament, would be non-political and would address fundamental issues of current interest such as Mr Enoch Powell's embryo protection bill, acid rain, nuclear power and the commercial implications of biotechnology

ABRC has set up its own working party, chaired by Professor Mathias, to examine funding of research by the private sector and to consider how it is to be extended. Its findings will be published in September of this year.

Among many other suggestions as to how the government should support science was that of Mr Robert Jackson MP to find money from the defence research and development budget. In 1981, Britain spent $3,000 million on civil and $3,256 million on defence research and development. Equivalent figures for France and West Germany in the same year were $4,370 and $2,591, and $6,698 and $646, respectively. Britain could, therefore, adjust its balance of spending accordingly

● Mr Enoch Powell used the debate to discuss once more his bill to protect human embryos. His argument was twofold. First, he failed to find any evidence, by correspondence with this journal, scientists and doctors, that any research of the kind that would be forbidden by his bill is actually being performed, arguing that his bill should therefore be passed. Second, he suggests that those who opposed his bill were the unwitting dupes of corporate giants that stand to gain by developing contraceptive and other drugs using human embryos as experimental material. Both these arguments, as well as the appropriateness of the issue in this debate, cut little ice **Maxine Clarke**

US agencies

NSF's hypothetical spree

Washington

THE idea of a federal Department of Science and Technology, which briefly resurfaced last winter for approximately the 100th time in the past 20 years, according to a tally by the Congressional Research Service, has appeared yet again, this time in a slightly veiled form. This week, the National Science Board, the governing body of the National Science Foundation (NSF), is to receive a study of what the agency would do if its budget were to increase by a factor of three over the next five fiscal years. The study, commissioned by NSF's director, Erich Bloch, included the assumption that as well as the extra money, NSF would take over the extramural research programmes of the Departments of Agriculture, Energy and Defense.

Although last winter's proposal rapidly fizzled out and while there is little chance that a budget-deficit-minded Congress would consider such a proposal even if it were submitted, the NSF study makes clear that those in high places would like a major reorganization of US science agencies.

One often-discussed idea, less drastic than consolidating all the science agencies into a single cabinet-level department, is a merger of NSF and the National Bureau of Standards, as proposed by the Reagan administration in 1983 as part of a plan to replace the Department of Commerce with a Department of International Trade and Industry. The proposal of last winter (in a study by the Presidential Commision on Industrial Competitiveness), took that as a starting point; the commission also considered adding in the research portions of the Department of Energy, the National Aeronautics and Space Administration and the National Oceanic and Atmospheric Administration.

Among the supporters of a cabinet-level department at that time was George Keyworth, the president's science adviser. And Roland Schmitt, the president of the National Science Board (and vice-president for corporate research and development at General Electric) said in a talk last winter at George Washington University that while he was unsure whether a Department of Science and Technology was the answer, the present organization of science agencies needed to be reexamined in the light of a failure to provide adequate support for the "science base" in the country. Schmitt said that NSF had traditionally supplied this base, which he said included the training of new scientists and engineers as well as research on new concepts too risky for industry to invest in, with help from the "mission agencies".

But now, he said, the mission agencies had become too involved with their missions to keep up their end "Maybe what we need is not a Department of Science and Technology but an NSF that is three times the size of the present one", said Schmitt.

Last week, however, NSF officials denied that the study ordered by Bloch was anything more than an "intellectual exercise" to identify research priorities and said that NSF had no wish to take over the research responsibilities of other agencies. **Stephen Budiansky**

Deep-sea diving

Japan finds clams and trouble

Tokyo

ON its second dive to the Tenryu Canyon off the Pacific coast of Japan, the French deep-sea submersible *Nautile* struck gold with the discovery of colonies of giant clams at a depth of 3,830 m, the deepest record so far of "vent communities" of organisms. But no sooner did news of the find break than *Nautile* sustained serious damage when surfacing in rough seas. Repairs to the submersible will probably take at least a month and may require the postponement of the Franco-Japanese project Kaiko until next year.

The colonies of clams were found on 6 June by Professor Claude Rangin of the University of Paris during a dive to the deep-sea fan at the mouth of the Tenryu Canyon, a steep-sided submarine valley that cuts into the accretion wedge of sediments being stacked up along the edge of the Eurasian plate as the subducting Philippine plate plunges beneath Japan. The giant clams are up to 15 cm in length and have been provisionally identified by Dr Suguru Ohta of the Ocean Research Institute, University of Tokyo, as *Calyptogena* sp , a genus of bivalve typical of the "vent communities" of organisms reported from mid-ocean spreading ridges and the subduction zone off the coast of Oregon.

According to Asahiko Taira, professor of sedimentology at the Ocean Research Institute, who made a dive to the clam site on 10 June, the colonies of *Calyptogena* sp. are circular, up to 60 cm in diameter and contain phenomenal densities of clams stacked on top of each other. One box core 250 cm^2 in cross-sectional area is said to have contained about 40 living clams, and during sampling some were crushed in the jaws of the box core releasing clouds of bright red blood. (The blood of vent organisms typically contains large amounts of haemoglobin.) On opening the box core on deck, a putrid smell was given off that made everyone feel sick, but hydrogen sulphide, which provides the "life-blood" of vent communities at spreading ridges, has not been found in anomalous amounts within the core and overlying waters, according to Dr Taira.

The colonies are aligned along north-east/south-west trending reverse faults associated with subduction and to a lesser extent along north/south strike-slip faults. The Tenryu Canyon runs along a major north/south transcurrent fault system which cuts into the mainland of Japan along the valley of the Tenryu River. Water inside the colonies shows positive thermal anomalies of a few tenths of a degree centigrade which may arise from connate water upwelling along the fault planes as the accretion prism of sediments flanking Japan is "squeezed" by subduction. The clam colonies thus in many rein the subduction zone off the Oregon/Washington coast, where it has been suggested that the vent organisms are powered by oxidation of methane contained in upwelling "old seawater" derived from underlying sediments (see *Nature* **313**, 339; 1985).

Other organisms found in the vicinity of the clam colonies include squat lobsters, gastropods, several of sea anemone and polychaete worms, but no giant tube worms have yet been found. Dr Ohta hopes to present the preliminary biological findings at an international symposium on deep-sea biology to be held in Hamburg, West Germany, next week. The specimens have been divided equally between the French and Japanese.

Cameramen from the Japanese national broadcasting corporation, NHK, will now be allowed to make two dives on the *Nautile* to film the seafloor. Japan's biologists, who have not been included in the dive schedule despite predictions that "vent communities" might be found (see *Nature* **313**; 1985), hope that NHK will choose to film the Tenryu Canyon clam colonies and perhaps collect some more specimens.

All this, however, depends upon *Nautile* being repaired in time to resume operations this summer. The electrical circuit of the submersible has been damaged and technicians and equipment must be flown out to Japan from France. At worst the Kaiko project may have to be delayed until next year, which could be a blessing in disguise as it would open a way for biologists to participate in the project. A decision on the future of Kaiko is expected this week. **David Swinbanks**

Soviet science

Gorbachev calls for new reform

ACADEMIC institutes in the Soviet Union must be "turned around sharply" to concentrate on research which is "technological in its thrust", the Soviet Party leader, Mikhail Gorbachev, told the Central Committee last week. Among other things, he proposed the establishment, within the Soviet Academy of Sciences, of "integrated, inter-industry scientific and technical centres".

This, in effect, would reverse Khrushchev's reform of almost 25 years ago, when its academy was stripped of its applied science institutes. Since 1976, party plans and directives have increasingly called for the academy to play a greater role in "coordinating" the implementation of research. Industrial centres of the type proposed by Gorbachev have already been established; one example is the Paton Electric Welding Institute under the aegis of the Ukrainian Academy of Sciences. This experience, Gorbachev suggested, should now be applied at the all-union level.

This turnabout would not, however, mean a decline of interest in pure research. On the contrary, said Gorbachev, it must be given priority, with no quarter given to "weakness" or "sluggishness". The research potential of the universities, in particular, must be better deployed. According to "available assessments", Gorbachev said, higher education establishments could increase the volume of their research by between 100 and 150 per cent (presumably without additional resources). Gorbachev's emphasis on the academy and university sectors is due, it appears, to the poor performance of the research establishments of the various production ministries. The Soviet Union spends 5 per cent of the national income on science, almost 90 per cent of that in ministry institutes More than half of all Soviet scientists work in them, and, according to Gorbachev, many of them, notably the Ministry of the Chemical Industry, have become "overgrown", with too many scientific institutes and pilot plants. Yet these are precisely the industries where there have been major shortcomings in developing new materials.

In his speech last week, Gorbachev complained that too many administrators, instead of finding a proper solution to a problem, shelve the issue by explaining that they are "carrying out an experiment", Gorbachev's answer is to strengthen the State Planning Committee, and to put into practice Lenin's dictum that the committee must be transformed into the country's "economic science organ, gathering together major scientists and leading specialists".

On the practical side, he urged that since the main weakness of Soviet industrial research lies in its isolation from production, many of the so-called "industrial" institutes should be immediately amalgamated with production enterprises. An immediate review should be carried out of how far the applied research institutes correspond to the current needs of industry.

Gorbachev also urged that something should be done about the declining prestige of engineers and the falling recruitment of talented young people into the technical sciences, which seems to have alarmed the Academy of Sciences. Gorbachev offers no immediate solution, but suggested that it may be time for a "serious reorganization" of higher education, meanwhile urging "measures to raise the social recognition" of engineers and technologists, including new pay deals.

Vera Rich

Animal rights

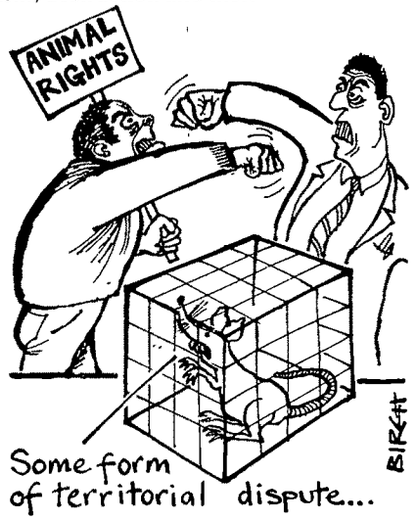
Battle joined on animal lib

Los Angeles

CALIFORNIAN investigators whose research animals have been stolen, whose laboratories have been vandalized, whose work has been disrupted and whose very lives have been threatened by "animal liberation" activists, are fighting back.

Earlier this month, several of them testified before a Californian Assembly committee on public safety which subsequently approved a bill that would stiffen the penalties for stealing research animals. An animal "liberator" currently faces misdemeanour charges, punishable by up to three months in county gaol and a fine, provided the animal is worth less than $400. The bill, if passed into law, would allow a judge to punish such a thief as a felon, with a 16-month to 3-year state prison term plus fines, no matter how inexpensive the animals.

"We are addressing the basic problem, which is interruption of research", says assemblyman William J. Filante, a northern Californian Republican who introduced the legislation. As the state legislature's only physician, Dr Filante said he feels an obligation to stiffen the penalties: "We are talking about vandalism, terrorism, destruction and theft".



Californian research facilities are threatened by ever bigger and bolder attacks, according to Sandra Bressler, executive director of the California Biomedical Research Foundation. The organization was set up last year, she says, to educate the public about how and why animals are used in biomedical research. California's leading universities, research clinics and other health organizations have joined the "fight back".

The problems began in 1982 when two monkeys used in brain research at the University of California (UC) at San Francisco were videotaped by interlopers. The tape was shown in Canada to raise money for animal rights groups. In 1983, two cats were stolen from a UC Berkeley campus psychology laboratory and twelve dogs used in cardiovascular research were taken from the Harbor-UCLA Medical Center in Los Angeles. The dogs, each valued at $5,000, wore pacemakers, research was delayed one year and the total loss was estimated to be £100,000.

In 1984, there were incidents of animal theft and vandalism at UC Sacramento and at the City of Hope Medical Center in Duarte. A $400,000 cancer study at Duarte was set back three years. There were also cases of threats and paint-spraying at UC Davis and San Diego.

In January this year, 11 rabbits were taken from a Napa county animal shelter and the chairman of the department of surgery at UC San Diego received a death threat. Two months later, the home and car of the Los Angeles county animal control director were vandalized. And in April 467 animals were stolen from laboratories at UC Riverside, ruining eleven projects and causing damage assessed at $683,000.

UC San Diego biology professor Mark Chappell lost 305 deer mice that he had painstakingly bred for five years to study variations in haemoglobin. Also in April, a student spirited away a cat being used to demonstrate premature infant resuscitation techniques at Stanford University.

None of the thieves, most of whom claim to belong to the Animal Liberation Front, has been caught, although there is one suspect from the Riverside incident and one person has been identified as passing on rabbits stolen from the City of Hope Medical Center.

The California legislature is under considerable pressure both from the biomedical community, which is powerful in California, and the animal rights activists, who are extremely vocal. Most legislators would like the problem to "go away", said an aide to Dr Filante. "But we expect it to stay a major issue for a long time. It'll be a big fight."

Sandra Blakeslee

Plant biotechnology

Young men go East to Georgia

Washington

THE University of Georgia, hoping to establish itself as a world-class centre for plant biotechnology, has acquired as a job lot a 20-strong research group from the University of Colorado. It has also committed $5 million over the next five years to establish 25 new faculty positions in molecular biology. The state of Georgia has demonstrated its support with a promise of $32 million for a new biotechnology building on the university's campus at Athens. The newly-hired research group is that of Peter Albersheim, who will be director of the new Center for Complex Carbohydrate Research. Albersheim let it be known last fall that he was planning to leave Colorado: Georgia was able to persuade him to Athens with an offer of almost 10,000 square feet of unused laboratory space in the Richard B. Russell Research Building, a facility of the US Department of Agriculture (USDA).

The new carbohydrate research group will work in collaboration with USDA scientists, and is expected to expand to a total staff of up to 40. Another reason Albersheim chose Georgia is the university's commitment to expand its biological research activities, which (including contract research) amounted to $75 million last year. About half of the promised 25 new faculty positions are likely to be in Albersheim's group, which brings with it from Colorado $1 million in research grants. Albersheim sees an impending golden age for the study of carbohydrates, which have tended to receive far less attention than other biopolymers. Now, he points out, it is becoming clear that carbohydrates, both individually and combined with proteins, play an important role in controlling metabolism and may dominate immunological reactions. Genetic engineering companies that have hitherto concentrated attention on proteins will in future have to pay more attention to carbohydrates, Albersheim believes. The University of Georgia has long nursed aspirations to be renowned for something. When president Fred Davison started looking at the available options, plant biotechnology seemed a likely choice.

It is planned that the faculty expansion over the next few years will be complemented by an international Information Center for Plant and Animal Biotechnology. This scheme, still rather vague, is intended to provide information to plant and animal breeders who may be aware of the potential of the new biotechnology but who do not know where to find researchers working in relevant areas.

According to Leon Dure, a professor of biochemistry at Georgia, there is a "distinct gap" in the knowledge of plant breeders about how recent developments might help their work. Dure expects that the member institutes of the World Bank's Consultative Group for International Agricultural Research, which includes crop and animal breeding centres all over the world, will be among the users of the new centre. In its initial phase the centre will concentrate on putting researchers and potential clients into contact with one another; later, it is hoped that more technical information, including gene and protein sequences and possibly complete scientific papers, will be included.

Tim Beardsley

Safety cabinets and AIDS

SIR — Many laboratory workers will be dismayed at the recent recommendation from the Advisory Committee on Dangerous Pathogens (ACDP) regarding the use of microbiological safety cabinets for the acquired immune deficiency syndrome (AIDS) agent human T-cell lymphotropic virus type III (HTLV-III). In this ACDP report[1] it is stated: "It is now clear that infection with HTLV-III may result in a fatal disease for which there is no effective prophylaxis or treatment. While there is still uncertainty about all the possible routes of transmission in the laboratory environment, care must be taken to avoid the dispersal of aerosols and for some work microbiological safety cabinets will be required. Because there are reservations about the level of operator protection which can be maintained by Class II cabinets under working conditions, for the time being, they must not be used for work with materials which contain the virus."

In many respects this conflicts with paragraph 5, Appendix A of the ACDP document *Categorisation of pathogens according to hazard and categories of containment* again published in 1984[2], only a few months before the AIDS recommendations. This appendix states that "In certain conditions some Class II cabinets will provide a protection factor of the same order as Class I cabinets (that is, a factor of 10^5 or better. BS 5726: 1979[3]).

If it can be shown by in-use tests that this level is achieved consistently (as demonstrated by tests carried out at regular intervals, the frequency of which will depend on use) and provided that the local safety arrangements will allow, a Class II cabinet may be used for some work with group 3 pathogens where protection of the work is essential. . . ."

In the six years since BS 5726 was published, many laboratories have invested heavily in both Class I and II cabinets that meet the operator protection requirements of the standard. Cabinet manufacturers have responded well to these requirements with the result that many open-fronted cabinets can consistently provide more than the minimum protection factor of 10^5. In addition, there has been a great improvement in the routine maintenance and certification of such cabinets. Many institutions now require certification to the filtration and containment requirements of the standard to be obtained twice per year for each cabinet.

The latest ACDP recommendations on the use of AIDS material in safety cabinets are questionable on two counts. In the first place they indict Class II cabinets in favour of Class I types and in so doing ignore the fact that Class I types are at least as easily disturbed by operator movements, by ventilation systems, by door movements and by people circulating within the laboratory. It is quite possible to reduce operator protection factors by one to two orders of magnitude by poor operator technique and lack of correct discipline.

Secondly, the recommendations totally disregard the current performance of many new Class II type cabinets which can produce consistent operator protection factors up to and beyond 10^6. It has to be remembered that BS 5726 makes no distinction between the operator protection of Class I and II cabinets – both must have factors of at least 10^5.

If there is so much uncertainty about the virulence of HTLV-III, then the very sensible procedures regarding the testing of operator protection factor initially outlined by ACDP for Class II cabinets[2] should be just as vigorously implemented for Class I types. As the weight of evidence does not favour transmission of HTLV by the airborne route, it is difficult to understand why Class I cabinets are allowed whilst similarly performing Class II cabinets are disallowed. Some of the work of this laboratory indicates that a number of the recently produced high performance Class II cabinets do, in fact, have higher nominal protection factors and are more resistant to disturbances than many Class I cabinets. Much of this cabinet evaluation work has been published in the scientific literature[5] and has formed the basis for containment policy in the Medical Research Council. It is naive to think that tissue culture work will always escape contamination in Class I cabinets or that they are intrinsically safer than Class II types. The banning of Class II cabinets by ACDP for AIDS work is inconsistent with their other recommendations and will cause grave difficulties for laboratory workers who require both operator and product protection without the difficulties associated with Class III cabinets. On this aspect of containment the ACDP committee should think again.

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1 Advisory Committee on Dangerous Pathogens *Acquired Immune Deficiency Syndrome (AIDS) — Interim Guidelines* (Health and Safety Executive, London 1984)
2 Advisory Committee on Dangerous Pathogens *Categorisation of Pathogens According to Hazard and Categories of Containment* (Health and Safety Executive, London 1984)
3 *BS 5726 Specification for Microbiological Safety Cabinets* (British Standards Institution, London, 1979)
4 Clark, R P *Occupational Hygiene Monograph No 9* (Science Reviews, Northwood, 1983). *Phil Trans R Soc Lond* B302, 593-604 (1983)
5 Clark, R P & Hill, J *J clin Path* **34**, 12, 1404 (1981)

Life and death

SIR — Rapid innovations in the biological sciences and technology are creating new and difficult ethical problems, as is evident to readers of *Nature* from numerous articles and letters. Most prominent are problems concerned with the early phase of the human life cycle. (See for example recent letters to *Nature* on the question "When life begins", *Nature* **314**, 492; 1985.) The "rights" of a human zygote, embryo and fetus, but for some reason not gametes, seem to evoke strong emotions.

New problems are also arising about when life ends. Problems of organ exchange necessitate a legal definition of the death of an individual.

At another level, what should be the status of human cells cultivated *in vitro*? These cells, in common with zygotes, have the potential, theoretical at the moment, to be regenerated if properly nurtured.

Further problems are now raised with the news of cloning of DNA extracted from an Egyptian mummy (*Nature* **314**, 576 and 644; 1985).

The unique genome of a human being apparently does not die when a person stops breathing or when electrical brain activity ceases.

The DNA molecules can be extracted and under proper conditions can be replicated. Thus by at least some definition of living matter the DNA is "alive". If the DNA is still alive, should we not change our accepted procedures of disposing of human corpses?

I suggest that the procedure of cremation, sometimes associated with the saving of the ashes as a memorial, is improper. Instead it would make much more "biological sense" to save at least a sample of DNA from the deceased.

I envision "mausoleums" where sealed test tubes containing these DNA samples will be kept as an appropriate memorial to individuals. With the rapid progress of biotechnology, the potential for "resurrection" of at least some on one's traits is not remote.

AHARON GIBOR
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Cows and goats

SIR — While Andrew Hill (*Nature* **315**, 222; 1985) shows deep understanding of the evolution of early African Hominoidea, his knowledge of Kalenjin nomenclature appears limited.

John Kimengich is surely John Kimngetich, which in Kalenjin means "born between 10 and 11 a.m. when the cows leave the cattle enclosure".

I am sure Dr Hill appreciates how in Kalenjin, as in English, omitting certain letters, for example the "o" from hominoid, can dramatically alter the sense.

MONIQUE BORGERHOFF MULDER
(born between 9 and 10 a.m when the goats go out, also known as Chemngeno)
Tabarit village,
PO Gorgor,
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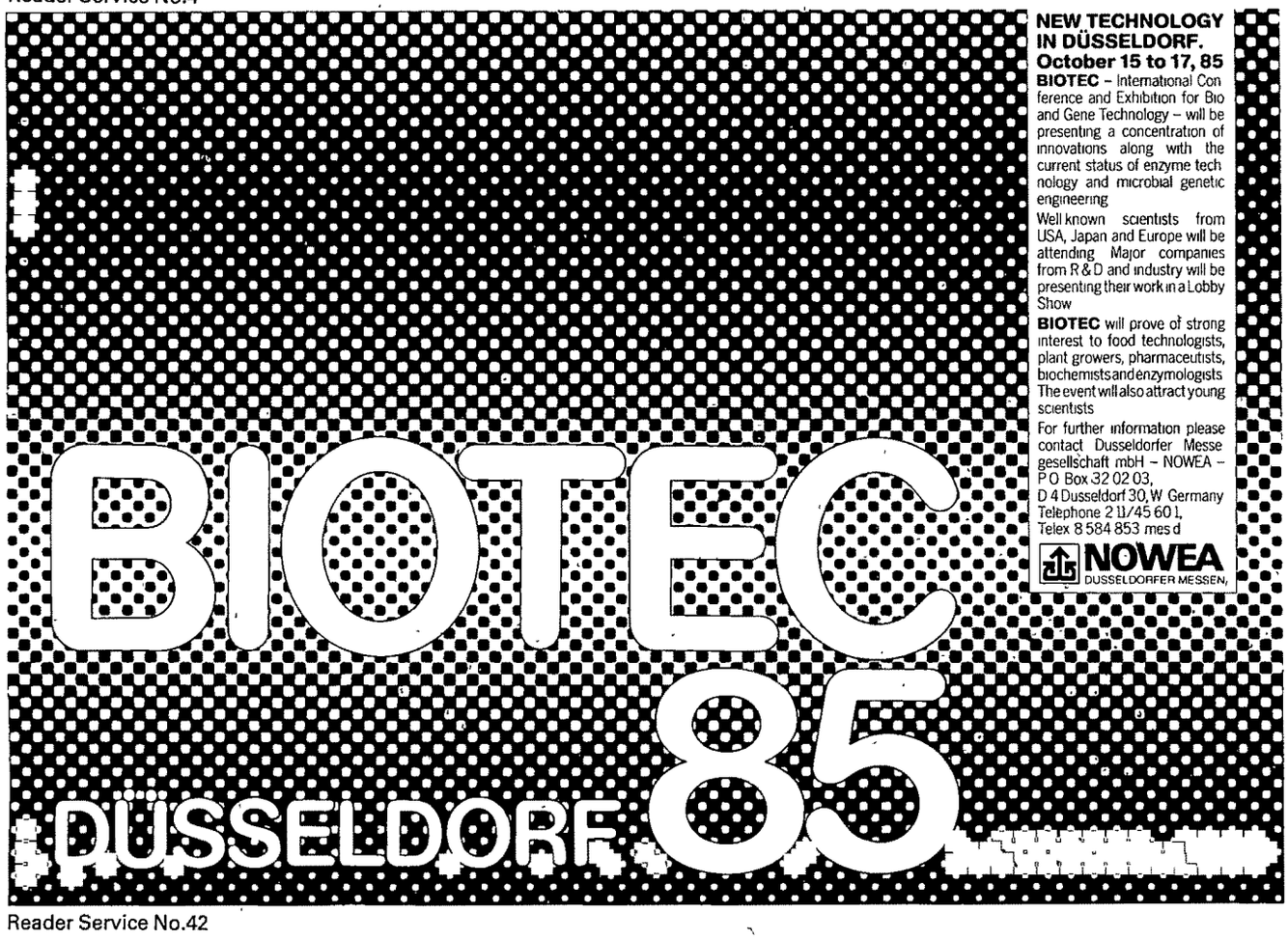
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Periodic extinctions undermined

Last year's fashion for explaining a supposed 16-million-year periodicity in mass extinctions of species has been made to seem a little spurious. Defining a mass extinction is a more urgent need.

THOSE who, last year, were enthusiastically speculating on the mechanism that might have been responsible for mass extinctions of living things at intervals of 26 million years will be given uncomfortable second thoughts by the article by Antoni Hoffman on p. 659 of this issue. For on the face of things, Hoffman has undermined the assumption on which all the excitement was based, the belief that there is a 26 million year periodicity to be explained But human nature being what it is, it seems unlikely that the enthusiasts for catastrophism will now abandon their quest

What Hoffman has done is to re-examine the palaeontological data on which rests the supposition that there is, or at least has been, a regularity in the timing of mass extinctions. The data consist of a catalogue of disappearances from the fossil record of families of marine animals compiled by J.J. Sepkoski and published, during 1982 and 1983, in the journal put out by the Milwaukee Public Museum. The first analysis of this material appeared in February 1984, in a paper by D.M. Raup and J J Sepkoski (*Proc. natn. Acad. Sci., U S A.* **81**, 801-5, 1984). It advanced the conclusion that mass extinctions of marine animals have taken place at times in the geological past separated by intervals of 26 million years or an integral multiple thereof

That Sepkoski's original data may not be robust enough to support this dramatic conclusion has always been a possibility, as Raup and Sepkoski themselves acknowledged The most obvious difficulty is the unavoidable uncertainty in assigning absolute ages to the successive geological stages recognizable only by the fossil assemblages that define them. For the past 100 million years, including the Cretaceous/Tertiary transition at 65 million years, the dating of the stages is reasonably good, with the result that most geological chronologies are consistent during that period, but disagreement is rife for earlier times. Raup and Sepkoski gave reasons, in their paper, for adopting the chronology due to W.B. Harland, the first column (labelled a) in Hoffman's Fig. 1 shows how the times of mass extinctions would have been changed if they had followed another equally plausible chronology

The nub of Hoffman's argument is, however, more subtle. Because palaeontological stages are defined by their fossil assemblages, each must differ from its predecessor and its successor by the appearance or the extinction of at least one family. So, Hoffman argues, distinguishing between a normal stage and one that qualifies as one of mass extinction requires the consistent application of some objective criterion He would prefer a criterion based on the rate or probability of extinction per unit time (say a million years) rather than one based on counting the number of family extinctions in a stage, which he says is biased towards periodicity.

So much is evident in the work of Raup and Sepkoski, where it is a necessary condition for associating a particular stage with a mass extinction that the number of families extinguished should be greater than in the preceding and succeeding stages. The effect of that is to exclude the possibility that consecutive stages could be associated with mass extinction, which is bound to prejudice the analysis towards a periodic interpretation Indeed, Hoffman argues, if the probabilities of extinction in successive stages are entirely independent, simply looking for peaks in the record of extinctions implies a one-in-four chance that any stage will be recognized as a time of mass extinction and, given the exclusion of the possibility that consecutive stages may be thus described, the analysis is certain to yield the conclusion that, on the average, extinction peaks occur every four stages. This, says Hoffman, is the origin of Raup and Sepkoski's periodicity.

The chief effect of this argument will be to emphasize how tricky is the concept of mass extinction. To be fair, many of the pitfalls were acknowledged by Raup and Sepkoski. The plain truth is that the disappearance of one or several families of genera from the fossil record cannot be counted as proof of a mass extinction, in the ordinary meaning of that term, without much more detailed scrutiny of the data than mere enumeration. How abrupt was the transition? Did all species disappear simultaneously, or can something be learned of the nature of the extinction from the sequence in which they disappeared? And since there are many possible causes of the extinction of species, genera and even whole families, ranging from evolutionary competition and climate change to external influences such as are involved in cometary or other impact, what can be said about the mechanism? The need to give at least partial answers to these questions should now be plain.

Where does this cautionary tale leave the explanations constructed to account for periodic extinctions, particularly for that which seems to have carried off the dinosaurs at the Cretaceous/Tertiary boundary? One conclusion that stands out from Hoffman's argument is what he calls the Maestrichtian stage at the end of the Cretaceous was, by all the different criteria applied, an extinction period.

Thanks to the work of the Alvarezes, father and son, and their associates, this is also recognizable by the world-wide presence of material rich in iridium at the physical boundary between the Cretaceous and the Tertiary The most compelling evidence that this material came from the impact of some extraterrestrial object with the Earth is the finding of cracks caused by explosion stresses in a high-pressure phase of quartz associated with the iridium layer (B. F. Bohor *et al. Science* **224**, 867–8; 1984). The case for this impact is strong (and would be even stronger if there were some trace of where it may have happened), but cautious people will insist that there is as yet only circumstantial evidence that the impact caused the extinction.

By contrast, the explanations of periodicity in the pattern of extinctions assume a somewhat academic character, which is not the same as saying they are uninteresting The most plausible source of material hitting the surface of the Earth is the distant ring of comets called the Oort cloud, from which material may be ejected towards the inner Solar System either by the perturbations of an unseen companion of the Sun (called Nemesis) or by perturbations of the Solar System caused by its periodic passage through the plane of the Galaxy.

Last year's rush of explanation has indeed thrown light on questions such as the stability of the Oort cloud, and of distant (and hypothetical) elements of the Solar System But the case for Nemesis has now wilted. And those who are attached to the notion that extinctions are caused by the oscillations of the Solar System above and below the plane of the Galaxy, among whom Clube and Napier (see *Nature* **283**, 455, 1979) seem to be the first, might now usefully turn the problem around, and ask what happens to the Solar System on these excursions through the galactic plane

John Maddox

Transgenic animals

New advances in the field

from Robin H Lovell-Badge

INJECTION of new genetic material into fertilized mouse eggs followed by development of the eggs into transgenic mice in foster mothers has become an almost routine tool in the analysis of a wide range of biological problems[1]. If the same technique could be used for farm animals it could be of great practical and economic importance, allowing the transfer of characteristics from one breed to another much more rapidly than by conventional breeding. More dramatically, it could allow genetic transfer between species. The paper by Hammer *et al.*[2] on page 608 of this issue is the first demonstration that it is indeed possible to use microinjection to obtain integration into the genome, and in some cases expression, of exogenous DNA in large mammals — namely rabbits, pigs and sheep.

To work with large domestic animals rather than mice requires a considerable investment, particularly in the number of animals required While it is normal to obtain up to 40 eggs from a single superovulated mouse, a ewe gives on average only three or four. Reimplantation of manipulated embryos may pose an even greater problem. Even though most will not survive, mice can tolerate up to 20 offspring, whereas sheep and cattle will not give birth to more than two. The occurrence of freemartins would be an additional problem with cattle.

These considerations have presumably influenced the selection of species used by Hammer *et al.* Pigs offer advantages because they are multiparous, sheep are an example of a difficult species and rabbits, while a reasonably convenient laboratory animal (and many a farmer's nightmare), are similar to pigs and sheep in many aspects of their early embryonic development. It is also essential to choose techniques that are efficient. It is clear from microinjection of tissue culture cells[3] and mouse embryos that transformation frequency is very low unless DNA is injected into the nucleus. But the eggs of many domestic animals have such an opaque cytoplasm that it is impossible to see their pronuclei or nuclei with conventional light microscopy. DNA-specific fluorochromes have been used to visualize nuclei in early bovine embryos[4], but the damage induced by ultraviolet light can severely compromise their development.

For sheep embryos, Hammer and colleagues have solved the problem to a large extent (but see below) simply by the use of interference contrast microscopy. More drastic procedures were necessary for the pig[5]. Centrifugation of the embryos at 15,000 *g* for three minutes separates the cytoplasm into three layers. Most droplets and granules, corresponding to the lowest buoyant density but greatest optical density, are found at one pole. The equatorial region contains a band of small clear droplets and the remaining half of the zygote has a translucent appearance. Nuclear structures may be found in either of these regions and are therefore clearly visible. The centrifugation of the pig embryos seems not to affect their subsequent development. It may also be a useful technique for other species, notably cattle, to allow experiments involving nuclear transfer for investigating the possibility of cloning[6], or parthenogenic development[7], of farm animals.

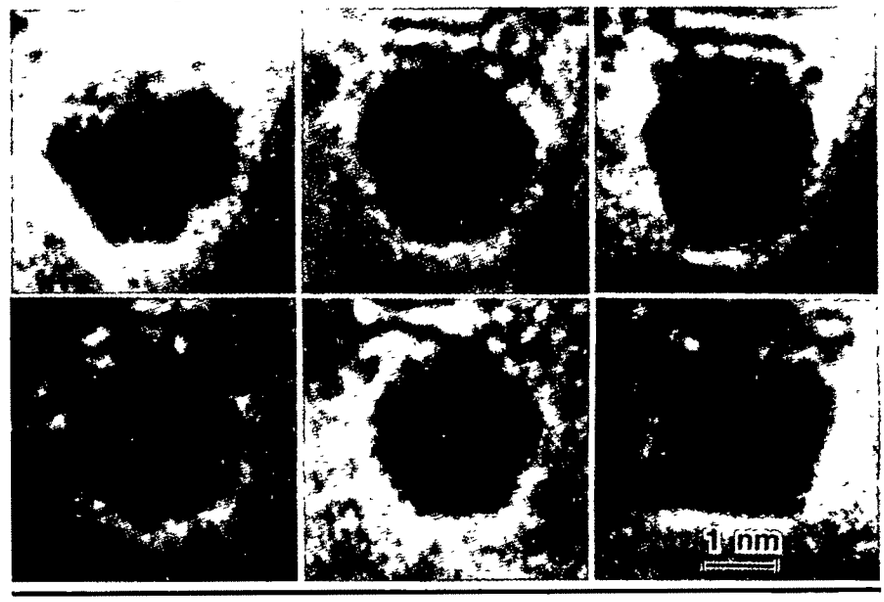
The gene chosen by Hammer *et al.* for injection into the nuclei of embryos of all three species is the mouse metallothionein–human growth hormone fusion gene that has been used in the same laboratory (Ralph Brinster's) for experiments with mice[8,9]. In each case about ten per cent of the injected embryos survived to term after reimplantation into foster mothers. The frequencies of fusion gene integration in the rabbit (12.8%) and pig (11.0%), although lower than the overall frequencies in mice (about 27%), are good, and other workers using these species will be much encouraged.

By contrast, the results with sheep are disappointing. Only one out of the 73 foetuses or newborn carried the injected fusion gene and even then it was obviously deleted or rearranged. It is conceivable that sheep embryos lack the appropriate mechanisms that fortuitously integrate exogenous DNA, but it seems more likely that some aspect of the technique needs refining. Perhaps more copies need to be injected and perhaps the visualization of the pronuclei needs to be improved (nuclear structures were not seen in a proportion of fertilized sheep eggs and these may correspond to more 'receptive' stages). Otherwise it may be necessary to adopt alternative strategies such as the use of retroviral gene vectors that are just beginning to work with mice.

Ovulation and fertilization are not v ry synchronous in the rabbit, pig and sheep, so it was necessary for Hammer *et al.* to inject embryos that ranged from 1-cell to 4-cell stages. For mice, even when the injections were at the pronuclear 1-cell stage, some of the transgenic animals were mosaic, presumably because gene integration was delayed. When this mosaicism was in the germline, few offspring inherited the introduced gene sequence[10].

Japanese gold in atomic motion

Use of a high-resolution electron mircoscope and video recorder has allowed the first real-time recordings of the motion of individual atoms. The pictures above show an ultra-fine particle of gold (18 Å across and containing about 500 atoms) photographed using the new technique by Sumio Iijima, a group leader in Japan's ERATO ultra-fine particles project (see *Nature* 305, 373; 1983). It can be seen that the shape of the particle changes over time, in discrete steps from top left to bottom right with all the gold atoms making up the ultra-fine particle moving cooperatively to form various crystalline structures. The gold atoms in ultra-fine particles thus seem to behave in a 'quasi-solid' manner with some properties like those of a liquid—one gold particle can even 'swallow-up' a smaller one. These phenomena await theoretical explanation.



It will be interesting to see if the same result emerges from breeding studies of the transgenic pigs and rabbits.

Expression of the fusion gene was observed in some of the transgenic rabbits and pigs, with a variation between individuals similar to that in mice and presumably due, at least in part, to different integration sites [8,9]. Further work will be necessary to show that the mouse metallothionein promoter works 'correctly' in rabbits and pigs, although it seems likely that the rules governing expression of exogenous genes in mice will be generally applicable to other mammals. Let us hope so, because it would be useful to derive transgenic mice to test the suitability of particular gene constructs before introducing them into farm animals.

It has long seemed likely that it would be possible to introduce genes into farm animals, and many groups around the world, often with considerable investment behind them, are attempting to do so. What are the potential gains? Some of the most obvious are improved efficiencies in reproductive performances and weight gain, increased disease resistance and changes in wool texture The introduction of extra growth hormone genes would be one way to increase growth but, although the results with mice have been dramatic [8], there has been no effect so far on pigs or rabbits[2]. It may be possible to produce transgenic animals with an increased rate of ovulation by, for example, increasing their gonadotrophin or lowering their oestrogen levels[11]. (Impaired fertility was an unexpected characteristic of some of the growth-hormone transgenic mice[12].) It may also be possible to increase ovulation rate with specific genes such as the *Booroolla* gene of sheep; the action of this gene is not entirely clear, but it may increase the sensitivity of granulosa cells to follicle stimulating hormone so that follicles mature when smaller[13] The association of specific histocompatibility antigens with particular diseases suggests that it may be possible to introduce non-associated alleles to induce resistance. Another possibility is the introduction of keratin genes to alter the properties of wool or hide.

Finally transgenic farm animals could be used to carry novel gene constructs as production systems for biologically and clinically important peptides and proteins. This should be particularly useful where expression in mammalian cells is required to give appropriate post-translational modification, or where the gene products are difficult to purify in other expression systems. It should, for example, be possible to use appropriate promoters to ensure that the introduced genes are expressed in the mammary glands of sheep or cattle, so that the gene products can be isolated from milk. Lest anyone should think this idea as silly as is that of converting the forelimbs of sheep into needles so they can knit, they should be aware that the use of silkworms for the production of interferon has just been reported[14]. □

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Protein folding

. . . yet there is method in't

from Roger Pain

THE mechanism of protein folding has developed from a purely fundamental study into one which is also crucial to the recovery of proteins made by biotechnological means in microorganisms. While the patent literature shows that some degree of success can be achieved by empirical approaches, further improvements in the economy, scale and efficiency of protein recovery can be expected once the refolding pathway is better defined Since the rate at which folding occurs *in vivo* provides one of the major constraints on any theory, *in vitro* kinetics have been studied intensively. Much of this work has been centred on ribonuclease (RNase) and its progress has been followed in these columns[1–3]. But now there are some new twists to the tail

It is because its amino-terminal surface helix should constitute a revealing model for formation and association of a well defined element of secondary structure with a larger protein fragment that RNase has received so much attention. An added advantage is that one can compare RNase A, which is a single polypeptide chain, with RNase S, in which the helix, or "S-peptide", is associated with the remainder, or "S-protein", only by non-covalent forces following cleavage of the chain between residues 20 and 21.

Not surprisingly the observed kinetics of unfolding and refolding are frequently biphasic or more complex but, confusingly, such kinetics cannot be assigned to conformation changes alone. Brandts and colleagues have shown that the existence of at least two unfolded states, which contain different proline isomers and are indistinguishable by accessible spectroscopic techniques, would lead to multiphasic kinetics, even if the actual folding process were simple and monophasic[4]. In the absence of conformational constraints, proline distributes itself between the *cis* and *trans* isomers with half times of a few minutes; folding to a conformation requiring a unique isomer will result in biphasic kinetics from fast and slow refolding forms:

$$U_S \overset{\text{slow}}{\rightleftharpoons} U_F \overset{\text{fast}}{\rightleftharpoons} N \qquad (1)$$

where U_S and U_F are unfolded forms, and N is the native protein.

The characteristic feature of this theory is that proline isomerization occurs in the unfolded state. Subsequent experiments, initially from Baldwin's group, were interpreted as showing that proline isomerization can occur in a collapsed, partially-folded state I according to the scheme:

$$\begin{array}{ccccc} U_S & \rightleftharpoons & U_F & & \\ \uparrow\downarrow & & \uparrow\downarrow & & \\ I_1 & \rightleftharpoons & I_2 & \rightleftharpoons & N \end{array} \qquad (2)$$

where the intermediate form I_1 contains incorrect proline isomers and I_2 contains native proline isomers. In this sequential model, the slow phase of the kinetics arises from the existence of conformational intermediates as well as from proline isomerization[5]. It is further claimed by Baldwin's group[6] that isomerization can be speeded up in the condensed phase ($I_1 \rightleftharpoons I_2$). Schmid has suggested that an enzymically-active folded state, which he showed to be an intermediate on the folding pathway, contained an incorrect proline isomer[7].

Part of the difficulty in resolving these opposing views lies in the lack of methods which enable each intermediate to be fully characterized and each kinetic phase to be assigned to a particular molecular transition. While, for example, NMR has been used to follow the *cis-trans* isomerization in small peptides, it has not yet been successful in proteins In 1983, Brandts (with Lin) returned to the disputation with an elegant method for following the kinetics of proline isomerization: cleavage by certain proteinases of the bond between proline and its neighbour in a polypeptide is dependent upon the isomeric state of the proline, allowing the proportion of *cis* and *trans* isomers to be estimated as a function of time for a particular proline residue[8]. Lin and Brandts show that proline 93 is 100 per cent *cis* in native RNase A but only 70 per cent *cis* in the reversibly unfolded protein. Under refolding conditions, the relaxation time for isomerization from 70 per cent to 100 per cent *cis* is 90 s at 10°C Parallel measurements of regions of ribonuclease activity showed that 30 per cent of the activity is recovered in a slow phase with a relaxation time of

100 s The agreement of amplitude and rate shows that the slow phase in the recovery of activity is rate limited by the isomerization of proline 93, and argues for refolding taking place only from chains in which that proline is in the native *cis* form

Lin and Brandts have now studied two more of the four proline residues in ribonuclease Proline 117 is most probably completely *trans* in both native and unfolded states and therefore will make no contribution towards kinetic complexity. On the other hand proline 114 is completely *cis* in the native protein and 50 per cent *cis* in the reversibly unfolded state, which correlates with the low-amplitude slowest refolding phase This residue must be in the *cis* configuration for the protein to regain activity[9].

The major fastest phase of the refolding kinetics is thus unaccounted for and the isomerization of proline 42 has yet to be investigated — the experiments will be slow and tricky While Lin and Brandts are willing to acknowledge the existence of the non-native active intermediate their view is that, if it accounts for the major refolding phase, it is unlikely to involve proline isomerization. In support of this, they quote unpublished work of Fisher *et al.* This group has purified a new enzyme, peptidylprolyl-*cis, trans*-isomerase[10], which catalyses the kinetic steps associated (by Lin and Brandts) with isomerization of prolines 93 and 114 but has no effect on the major fast phase. Does this enzyme require the ribonuclease to be unfolded in order to catalyse the isomerization of proline, offering support for the Brandts model? To add to the confusion, Schmid, Buonocore and Baldwin[11] have not only further confirmed their sequential model but have also strengthened the evidence that the major phase shows the characteristics of proline isomerization.

On a slightly different tack, a further advance in assigning kinetic phases to distinct regions of the ribonuclease structure has come with the discovery by Labhardt that the S-peptide and S-protein fragments of RNase S can be distinguished by circular dichroism[12]. From the kinetics of conformational changes of both the S-peptide and the S-protein during their association and refolding reactions, Labhardt comes out firmly in support of a sequential type of folding pathway. Intriguingly, his experiments show that the S-protein folds (to a form as yet uncharacterized) in a fast phase before the S-peptide folds to its native helical structure. This fast phase corresponds to 65 per cent of the total change, greater than the proportion of fast folding species U_F in scheme 2. The folded form must correspond in part at least to I_1.

Since Labhardt has already shown that recombination between S-peptide and S-protein occurs fast and very early in the folding pathway of RNase S[13], we have to envisage, contrary to most previous expectations, that the association between these two regions occurs at a stage when the S-peptide, and possibly the S-protein, possess no native-like conformation This would be in keeping with the poorly hydrogen-bonded associated species detected by NMR[14]. If Labhardt's results, obtained with a relatively 'simple' system, are of general application, the model of 'flickering clusters' of secondary and super-secondary structure, which associate to be stabilized in the native conformation, will have to be considerably revised.

The circular dichroism results have also raised a problem relating to the unfolding kinetics. The unfolding rates for RNase S and S-protein, which have been followed by monitoring changes specifically in the S-protein moiety, parallel the formation of the *slowly* refolding species U_S which contains non-native proline isomers (scheme 2). The random isomerization of proline to form U_S had previously been thought to occur in the spectroscopically 'silent' reaction ($U_F \rightleftarrows U_S$) but Labhardt's results suggests that, in terms of the model of scheme 2, there must be a rate-limiting proline isomerization $I_2 \rightarrow I_1'$ which is faster than $I_2 \rightarrow U_F'$, followed by a rapid conformational unfolding $1_2 \rightarrow U_s$. Alternatively, the rate determining step is a conformational unfolding $I_2 \rightarrow U_F'$ followed by a rapid isomerization to U_S. Neither alternative fits easily with the expected relative rates of these processes.

The frustrating aspect of the whole question of proline isomerization is that, however the issue is resolved, it will tell us nothing about the actual *mechanism* of protein folding. Though it is becoming possible to see some of the detailed processes at work in the folding of ribonuclease, the methods have not yet overcome the kinetic madness of the system. □

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100 years ago

AN excellent opportunity of observing the aerial means of propulsion in the flying fish was afforded me during a six days' calm lately when crossing the Bay of Bengal This must be my excuse for again touching this subject I watched day by day some hundreds rise under the bows of the ship The water surface was a glassy calm As each fish rose it spread its wings at once, apparently beating the surface with them two or three strokes before they steadied out. The tail which, of course, under water was in rapid motion, to escape from the ship, now gave ten or a dozen rapid beats, which could be counted by the ripples on the still surface, and the fish was off in aerial flight As each fish lost the impetus of the first rise, which generally happened at about forty yards, the binoculars showed us the anal fins, which had till now been fully extended, drooping to feel the water As soon as the surface was felt the tail was quickly introduced, and five or six smart strokes, also indicated by ripples, brought the impetus up again and carried the fish about another thirty yards, when another droop sent it on again, and so forth, some of the older fish travelling in this way 400 to 500 yards.

From *Nature* **32** 147, 18 June 1885

Astronomical spectroscopy

Interstellar space contains the largest encountered atoms

from William D Watson

OBSERVATIONS published on page 647 of this issue[1], in conjunction with recent Soviet publications[2,3] highlight the presence of extremely high Rydberg states — in which the outer electron of an atom has been excited to very high energies — in the interstellar medium. These results confirm and significantly extend recent identifications[4,5] of spectral lines at radio frequencies an order of magnitude lower than those at which lines had previously been detected. Discoveries of new lines at radio wavelengths have until now tended to be associated with the high-frequency (millimetre and submillimetre) end of the spectrum.

Because of the appearance of several lines in the low-frequency series, it is clear that they result from radiative transitions involving extremely high Rydberg states (or radio-recombination lines) and changes of one unit in the principal quantum number of the state (alpha transitions). The lowest frequency at which these lines have been detected corresponds to the 732 alpha transition, which

indicates that the largest atoms that have yet been directly encountered in any environment are present (the radius of the Bohr orbit for a principal quantum number of 732 is 0.003 cm). These spectral lines are almost certainly due to atomic carbon.

There is evidence of pressure broadening in the new observational data. This phenomenon has not been detected previously at any wavelength in spectral lines associated with the interstellar gas clouds. Taken together for the first time, pressure broadening and the change from emission to absorption which is evident in recombination lines provide new diagnostic methods to delineate physical conditions of interstellar gas clouds. These clouds provide information about the process of star formation.

Radiofrequency transitions of atoms between adjacent or nearby Rydberg states with large principal quantum numbers have commonly been observed in astronomy since their first detection in the 1960s. Studies of these radio-recombination lines have had an impact on several astronomical issues[6] including, for example, the abundance of helium (which has cosmological importance) and the physical state of the star-forming gas (molecular cloud). They are observed primarily in the diffuse gas of our Galaxy, in either the hot, ionized gas of H II regions where lines of hydrogen and helium are seen or in the mostly neutral, cold gas of molecular clouds where the lines of carbon and sulphur are found. Before the prediction in 1959 by N. S. Kardashev that radio-recombination lines should be detectable, it was not appreciated that transitions between states with very high principal quantum numbers could be observed without overlap because of line broadening. High Rydberg states of atoms are also of interest to atomic physicists (see, for example, ref. 7).

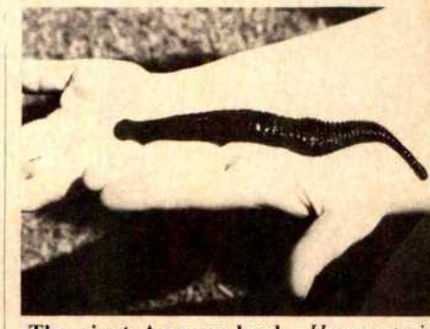
Until recently, the principal quantum numbers of the radio-recombination lines ranged from about 40 to 300. As confirmed by the new observations, the range of principal quantum numbers that can be involved and the characteristics of observable transitions have been dramatically expanded. The current data substantiate the earlier proposal[4] that spectral features detected at frequencies near 26 MHz in the direction of Cassiopeia A by A. A. Konovalenko and L. G. Sodin[5] should be indentified as radio-recombination lines (alpha-transitions) involving the principal quantum numbers 630–640 of neutral atoms of an element other than hydrogen or helium. Except for these two elements, the isotope shifts are not large enough compared with the breadth of the lines to make a unique identification based on frequency alone. General considerations[4,8], as well as the association of these features with concentrations of neutral gas, strongly suggest that the atoms in question are carbon atoms. Carbon is the most abundant element for which the ionization potential is lower than that of atomic hydrogen. Thus, it can occur primarily as C^+ because of ionization by starlight in regions where hydrogen is in neutral form. The high Rydberg states involved in radio-recombination lines are populated primarily by the recombination of free electrons with the ions.

The recent observational studies[1–3] report the detection of the series of recombination lines far beyond the 26 MHz region described previously. In the direction of the radio source Cassiopeia A, the series is found to continue down to 16.7 MHz (732α) and up to 68 MHz (456α). Analogous, but more limited, sequences of such radio-recombination lines have now also been detected in several other directions in the Galaxy.

The idea of such 'large' atoms is, in itself, fascinating. As a result of the very weak binding of these Rydberg electrons, they interact strongly with their environment — that is other particles and radiation. One consequence is the observation of line breadths which exceed that due to Doppler broadening, the broadening presumably resulting from collisions with free electrons.

With increasing principal quantum number, the rate for collisionally induced transitions increases rapidly, whereas the loss rate from the Rydberg states due to spontaneous, radiative decays decreases at a comparable rate. The net effect is a greatly enhanced tendency for the excitation temperature of the very low-frequency recombination lines to approach the kinetic temperature of the gas. As a result, this new class of recombination lines has only been detected as absorption features in the spectrum of background, continuum radio emission. In contrast, at the lower principal quantum numbers ($\lesssim 300$) the populations of the states are expected to be inverted. Before the recent studies involving principal quantum numbers greater than about 450, radio-recombination lines had, in fact, always been detected as emission features. Thus, there is a change from emission to absorption that occurs with increasing principal quantum number. For alpha-transitions and representative physical conditions, the predicted transition tends to occur near 400 (ref. 8). The critical principal quantum numbers at which the change occurs do depend upon the physical conditions; hence, additional information for delineating the physical conditions of interstellar gas clouds can be obtained.

Although the first detection of pressure broadening and the change from emission to absorption by recombination lines are the most striking features of this new class of spectral lines, more detailed theoretical and observational studies may elucidate additional effects of diagnostic value for interstellar gas clouds. The relative importance of various physical effects associated with Rydberg atoms tends to increase rapidly with increasing principal quantum number. For example, pressure broadening by the free electrons increases approximately as the fifth power of the principal quantum number in certain relevant regimes. There is also evidence[2,3] that the strength of the lines is influenced by a resonance in the recombination[8,9] which is related to the fine-structure excitation of C^+. Rough assessments[4] of the expected strengths suggest that these recombination lines are likely to be widely observable in the gas of the Galaxy — a prediction tentatively supported by detections in several directions in the Galaxy other than towards Cassiopeia A. In the absence of a conveniently located compact source of continuum radiation, the diffuse synchrotron emission of the Galaxy is bright enough at the very low frequencies to provide an adequate continuum source against which the absorption can be observed. Finally, the occurrence of spectral lines at these very low frequencies provides a new opportunity for radiotelescope observers and investigators with an orientation towards very low frequencies. □



The giant Amazon leech, *Haementeria ghilianii*, which grows up to 0.5 metres in length. Its salivary gland secretes the specific fibrinolytic enzyme hementin and other biologically active substances of potential scientific and medical use. The gland provides a unique preparation for studying excitation–secretion coupling in single cells, because the cells are very large and are not electrically coupled; see the paper by P. G. Jones, R. T. Sawyer and M. S. Berry on page 679 of this issue.

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Ocean Drilling Program

Rise and fall of carbonate platforms in the Bahamas

*from the Leg 101 shipboard scientific party**

LEG 101, the first international voyage of the Ocean Drilling Project, began on 31 January, 1985 in Miami and ended there on 14 March after 11 sites had been drilled in the Bahamas archipelago. The decision to begin this new phase of deep-sea drilling with a study of carbonate platforms reflects the growing interest of earth scientists in the anatomy of large sedimentary bodies.

The drilling revolved around two themes: the origin of the present array of platforms and basins, and the study of platform flanks, their facies patterns and their response to sea-level fluctuations that entail flooding and exposure of the banks. The origin of Bahamian banks and basins continues to be the subject of intensive debate because the the pattern is unusual for passive ocean margins. In the past decade, the discussion has been governed by two fundamentally different concepts. One of them, the graben hypothesis, calls for strong fault control of the bank-and-basin pattern. It assumes that the present deep-water basins originated as grabens in the rift stage of the Atlantic and have remained topographic lows ever since[1]. On the other hand, the megabank hypothesis[2] assumes that rift structures and other inherited tectonic features were soon smothered by sediments during the spreading phase of the ocean and that for most of the Jurassic and the early Cretaceous, Florida, together with the northwestern Bahamas and northern Cuba, formed a single, flat-topped carbonate platform – the 'megabank'. In the Cretaceous, this platform gave way to the present array of banks and basins.

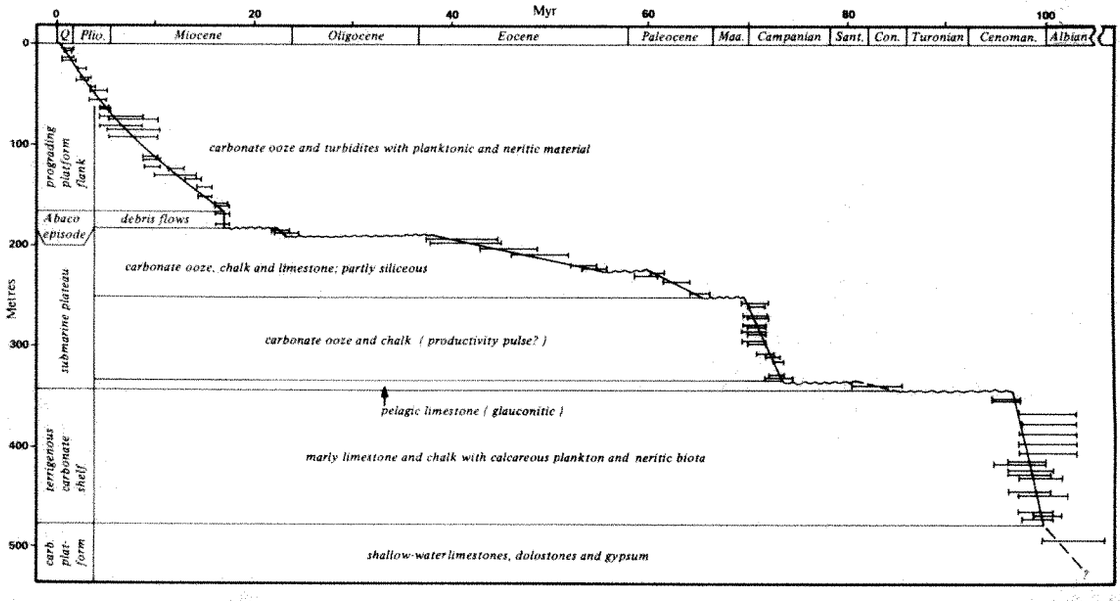
Debate continues in the wake of drilling. Most of us feel that Leg 101 and the preceding seismic-site surveys have greatly strengthened the megabank hypothesis. However, at least one of us (Mullins) considers the evidence inconclusive, mainly because we failed to reach the relevant seismic horizon in the Straits of Florida and Northeast Providence Channel, and prefers to emphasize that deepwater early Cretaceous sediments were recovered on Leg 77 of the Deep Sea Drilling Project, in dredge hauls from Samana[3], and also on this leg from site 635. On the basis of this evidence multiple bathyal environments may already have been established at that time.

Leg 101 recovered shallow-water carbonates and evaporites of the megabank under the southern Blake Plateau. In the Straits of Florida, the carbonate platform was not reached but a close stratigraphic tie was established with an exploration well on Great Bahama Bank, 60 km to the north-east. The continuity of seismic records between site 626 and the Great-Isaac 1 well strongly suggest that the entire Straits of Florida is underlain by a continuous carbonate platform. Furthermore, the two holes indicate that rather than remaining stationary since the Mid-Cretaceous, the margin of Great Bahamas Bank has alternately retreated and prograded tens of kilometres during the past 100 Myr. Blake Plateau and Little Bahama Bank display a similar rhythm of mid-Cretaceous drowning and retreat followed by Late Tertiary expansion of the platforms (see figure). The evidence for wide horizontal migration of the platform margins and for shallow water carbonates underneath some Bahamian basins is not easily reconciled with the graben hypothesis of the Bahamian troughs[2].

Mid-Cretaceous disintegration of the megabank does not seem to be related to local tectonic deformation but rather to a crisis in the depositional system. The drowning of the Bahama platform is part of a world-wide extinction of carbonate platforms that coincides with a tendency towards oxygen deficiency in the oceans[4]. Exploring links between anoxia and platform drowning remains one of the most challenging tasks for the shipboard party.

While for most of us the drilling tipped the balance in favour of the megabank-hypothesis, some modifications of that concept are called for. The stratigraphy of the Leg 101 sites, along with recent dredging results, indicates that mid-Cretaceous platform drowning was not synchronous throughout the archipelago and that it predates the mid-Cenomanian sea-level cycle of Vail *et al.*[5]. Drowning seems to have progressed in steps from the south eastern perimeter of the megabank to the centre. The oldest known deep-water sediments at the southeastern tip of Great Bahama Bank (Samana) are Neocomian in age[3]; 350 km farther northwest,at site 635, they are Albian; at site 627 and in the Great-Isaac 1 well, in the centre of the archipelago, they are Cenomanian in age.

Platform flanks were studied in two three-hole transects with the hydraulic piston corer. The response of platform flanks to sea-level fluctuations has recently become the focus of a lively discussion [6–8] and was one of the principal study objectives of Leg 101. Vail *et al.*[5] assume that sediment is fractionated between shelf and basin in only one way during a sea-level cycle: during high stands of sea level most sediment is trapped on the inner shelf; during low



Age versus depth profile and geological history of site 627 on the southern Blake Plateau. Drowning of the carbonate platform during the mid-Cretaceous crisis is followed by partly terrigenous deposition on a shelf, and by 80 Myr of slow pelagic deposition on a submarine plateau. In the past 20 Myr, the area has been influenced by the prograding Bahama platform again. "Abaco episode" is a wide spread incidence of debris flow deposition in the middle Miocene documented in the Blake Bahama-Basin DSLP Leg 44 (ref 9).

stands, when rivers carry their load right to the shelf edge, most material escapes to the deep sea. Results of Leg 101, along with a growing number of data on the late Pleistocene indicate that carbonate platforms do not fit this trend.

Preliminary comparisons of stratigraphy on the Bahama banks and in the basins strongly suggest that periods of low sea level (and bank exposure) 3 Myr BP and 5-6 Myr BP correspond to intervals of slow deposition and scarcity of turbidites in the basins. When sea level was high and the banks were flooded, sedimentation rates in the basins increased dramatically. This observation supports the concept of 'high-stand shedding' of platforms as opposed to the generally accepted phenomenon of 'low-stand shedding' of terrigenous continental margins.

The fine sediment exported by the platforms consists largely of aragonite, a form of calcium carbonate that is metastable in most burial environments and is rarely found in pre-Quaternary pelagic sediments. On the flanks of the platforms, bank-derived aragonite mixes with the remains of calcitic plankton. Leg 101 drilling provided the first glimpse of the burial history of this peculiar type of carbonate ooze The first-order trend shows a downhole decrease of aragonite and its replacement by calcite cement. Whereas calcite cementation is more rapid than in pelagic carbonate ooze, aragonite dissolution was found to be unexpectedly slow: 7 Myr-old limestones were found to contain 15–20 per cent aragonite. Superimposed on this first-order trend are cyclic variations in aragonite content. In the Quaternary, these have been shown to reflect climatic cycles. Further study of the Leg 101 cores is expected to reveal the Neogene climatic history of the platforms

Hydrocarbons were encountered at several sites and led to abandonment of two holes. Oil stains and wet gas were found in Cretaceous dolomites on southern Blake Plateau, and tar impregnations appeared in Miocene chalks in the Straits of Florida and in turbidites in Exuma Sound All these are interpreted as hydrocarbons that have migrated from deeper parts of the section. In accordance with ODP practice, these holes were plugged with cement.

Leg 101 has added a dimension of Earth history to the numerous studies on modern platform flanks. The modern sediments of the Bahamas, often used as a standard for the interpretation of the geological record, have provided the key to their own past. This part is more varied and turbulent than previously believed. Periods of crises and of rapid expansion figure prominently besides intervals of steady growth. These changes seem to be governed by a complex interplay of oceanic productivity, eustatic sea level and tectonics that we have barely begun to document and understand □

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Astrochemistry

Aluminium clues to the formation of the Solar System

from Donald D Clayton

WHEN groups in Australia and at the California Institute of Technology first showed that aluminium-rich minerals from the Allende meteorite contained more ^{26}Mg than expected, it was argued that a supernova must have exploded beside the forming Solar System. The argument went that the explosion peppered the solar cloud, from which Allende formed, with the short-lived and radio-active ^{26}Al, and that the excess ^{26}Mg in the meterorite is the result of decay of ^{26}Al within the present meteoritic minerals. This conclusion has now been shaken by the detection of surprisingly large amounts of radioactive ^{26}Al in the interstellar medium. As a result, alternatives to both the theory of injection from an exploding supernova and the theory of decay within the actual meteorite are being seriously considered.

The original detection of ^{26}Al — the first ever detection of interstellar radioactivity—was made by the HEAO 3 spacecraft (Mahoney, W.A. *et al Astrophys J* **286**, 578; 1984) which recorded a measurable flux of 1,809 keV gamma rays striking the Solar System These rays are a well known electromagnetic radiations product of the beta decay of the ^{26}Al nucleus. HEAO 3 measured about 4.8 such gamma rays per square meter per second, coming from the general direction of the centre of our Galaxy, but at an unknown distance. Any lingering doubts about the reality of this astonishing discovery have since been removed by a gamma-ray spectrometer on board the Solar Maximum Mission satellite which, since February 1980, has taken unintentional periodic looks at our galactic centre The spectrometer team (Share, G. H. *et al.*, submitted to *Astrophysics J.*) confirm that there are about 40 gamma rays per square metre per second coming from the general direction of the galactic centre, so that it can now be asserted without reasonable doubt that a source of radioactive ^{26}Al lies in that general direction.

In an analysis of the magnitude of this gamma-ray flux and its implications for both the origin of the elements in explosions of stars and for the origin of the Allende minerals (Clayton, D. *Astrophys. J.* **280,** 144; 1984) I came to two unexpected conclusions. First, if the ^{26}Al is spread uniformly throughout the interstellar gas, its concentration of about 10 parts per million of aluminium is rather close to that provided by Allende minerals, suggesting that it is unnecessary to invoke the injection of ^{26}Al into the forming Solar System by a supernova trigger Second, supernova explosions are not adequate to maintain the average level of observed interstellar radioactivity, so that nova explosions or gas streaming away from giant stars are the more likely origins of the radioactive aluminium now found in the interstellar medium. This also argues against the presence of a special supernova trigger to Solar System formation. A.G W. Cameron, an early advocate of the supernova trigger theory, has given that idea up. He now argues (*Icarus* **60,** 416; 1984) that wind from an asymptotic giant branch star of about one solar mass carried ample ^{26}Al along with it and participated hydrodynamically in the formation of a molecular cloud core wherein the Sun formed A similar argument could be constructed with winds from Wolf–Rayet stars, which are also now expected to be enriched in fresh ^{26}Al (Dearborn, D. & Blake, J. *Astrophys J. Lett.* **288,** L21; 1985).

Interpretation of the excess ^{26}Mg in Allende aluminium-rich minerals depends on the correct interpretation of the ^{26}Al gamma-ray line, which requires better information on its angular distribution. Because of their wide viewing angles and serendipitous measurements, the HEAO 3 and Solar Maximum Mission teams have been reluctant to be very precise about the angular distribution. Although they describe it as being "consistent with" radioactivity concentrated in the galactic plane and with peak intensity near the galactic centre, they stop short of claiming that the data requires that interpretation

An alternative explanation invokes a

supernova explosion ten thousand years ago and about 20 parsecs from the Sun. This could happen to have been toward the general galactic centre, and would occupy a large circular area on the sky (perhaps a 30° – 90° cone). But it would be most unlikely that the centre would lie in the plane of the Galaxy. Only if the observing teams can successfully show that the latitude distribution is narrow and centered on the plane, will we be forced to accept the observed concentration of ^{26}Al as a general feature of the gas. In what follows, however, let us assume that the ^{26}Al is a general galactic feature rather than a local one.

At 10^{-5}, the interstellar ratio of ^{26}Al/^{27}Al lies squarely between, but distinct from the ratio of 5×10^{-5} for some, but not all, aluminium-rich Allende minerals and that of less than 10^{-6} could be maintained there by supernova explosions. The excess of ^{26}Al in Allende minerals is therefore still too large to be interpreted as being the average interstellar concentration. Thus, if the present ^{26}Al decayed in the present Allende minerals, there must have been some source of ^{26}Al enhancement in the solar cloud as it collapsed to form the Solar System. This is the reinterpretation that Cameron now advances.

There are, however, new doubts that the ^{26}Al was ever 'alive' in Allende, as I discussed at the 16th Lunar and Planetary Science Conference in Houston, during March 1985 Whatever objects serve as the source of the ^{26}Al currently observed by gamma radiation, they are necessarily ejecting $4M_\odot$ of it per million years into the interstellar medium. By contrast, supernova eject $24M_\odot$ of the new stable aluminium, and stars reinject $60M_\odot$ of old stable aluminium over the same million years. If all of these ejecta condense into refractory aluminium-rich solids as they leave their respective sources, and if the ^{26}Al decays to ^{26}Mg within the resulting mixture of well mixed dust grains, the ratio of ^{26}Al within the aluminium-rich interstellar dust will be 0.05, fully a thousand times greater than the correlation in the meteoric aluminium-rich minerals. This high interstellar correlation of excess ^{26}Mg to Al could not have been totally removed by evaporation, because the Allende minerals carry other isotopic anomalies that demonstrate that they were not evaporated totally at any stage prior to their assembly. Thus these arguments indicate that the ^{26}Mg–Al correlation observed in Allende minerals may be a manifestation of a cosmic chemical memory; the observed concentration of interstellar ^{26}Al necessarily creates such a large interstellar ^{26}Al anomaly that the special chemistry invoked for the ^{26}Mg daughter is no longer required by a fossil theory. The challenge now to mineral chemists is to demonstrate how this memory survives the process of the formation of minerals and the substantial extraction of magnesium from them. □

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Nuclear war

Poor outlook for tropical weather

from A. Barrie Pittock

THE most important environmental effect of a major nuclear war in the Northern Hemisphere may be caused by reduced rainfall in the tropics and subtropics. This conclusion was reached at a recent conference* and will be incorporated in a report later this year (see *Nature* **315**, 534; 1985).

SCOPE (the Scientific Committee on Problems of the Environment) has run a series of scientific workshops, this being the first to be held in the Southern Hemisphere. New results from computer simulations were reviewed, incorporating explicit modelling of smoke dispersion from target areas in North America, Europe and the Soviet Union, and fully interactive smoke (that is, smoke that modifies the atmospheric temperature and wind structure and is then moved around by the modified circulation). This overcomes two of the major weaknesses of the earlier simulations, which assumed an initial uniform smoke distribution over Northern Hemisphere latitude zones and did not allow for smoke movements.

A common feature of the results from simulations performed by the three different laboratories in the United States (Los Alamos, Livermore National Laboratories and the National Center for Atmospheric Research) and in the Soviet Union, was that smoke was 'lofted' from its initial height of injection to around 10 to 20 km in altitude and then moved south, because of heating by absorption of solar radiation. This effect occurs only in the northern spring and summer seasons when the solar radiation is sufficient to cause the lofting to take place. In winter the smoke tends to move towards the Arctic and is more quickly removed.

This lofting effect and the increased stability of the lower atmosphere (which reduces precipitation) quickly separates the smoke from the washout processes in the spring and summer. Such results make it seem more likely that the smoke will last long enough and move far enough south to affect seriously the tropics and the Southern Hemisphere.

In mid-northern latitudes, full-blown nuclear winter effects will probably occur but, as this is the zone where most damage will be done by the direct effects of the nuclear explosions, this 'lofting' effect will be irrelevant. But as the smoke spreads southwards it will stop the solar heating of continental land surfaces that drives the south-west monsoon, halting the rainfall necessary for survival over most of southern and south-east Asia. This could be far more disastrous than temperature decreases in the tropics of 10 – 15°C, even though rice is particularly sensitive to this degree of cooling.

Further south, rainfall over mainland Australia is expected to decrease, initially because of strengthening of the descending arm of the Hadley Circulation in the southern sub-tropics, and later from a general decrease in the hydrological cycle. A pre-'nuclear winter' study by B. Hunt (*Mon. Weather Rev.* **81**, 3677; 1976), where an 18-level primitive equation model was run with the solar insolation turned off, showed a rapid decline in the water vapour content of both the atmosphere and the eddy kinetic energy (strength of the synoptic weather disturbances). As Australian agriculture is mostly limited by precipitation, this effect will have a major effect on productivity, whereas the relatively modest temperature reductions may be less serious. New Zealand and Tasmania, on the other hand, are further south, so temperature is more limiting and even modest cooling may significantly reduce production.

Australia, New Zealand and Argentina currently have food surpluses, so that even large reductions in production may not lead to mass starvation in these countries, unlike much of southern Africa and the tropics. Thus, the greatest impact of the 'nuclear winter effect' may result from reduced rainfall in the highly populated countries of Asia and Africa rather than in countries in the mid-northern latitudes.

Several major uncertainties have been given more adequate treatment in recent, as yet unpublished, work discussed during the workshop. Areas still to be investigated are the rates of smoke washout in the initial plume-rise phase, the optical properties of aged smoke (especially at infrared wavelengths) and the possible smoke removal processes in the stratosphere. Better knowledge of effects on the Southern Hemisphere also requires computer simulations to be run for longer periods and to consider the effects of a war fought in the northern winter. □

* The workshop on the environmental effects of nuclear war was held in Melbourne, 28–29 March 1985, sponsored by the Australian National Committee for the Environment and the New Zealand Committee for the Scientific Committee on Problems in the Environment

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Physiological role of Ca^{2+} transport by mitochondria

SIR—We cannot but whole heartedly agree with D J.Miller[1] that the systems which transfer calcium across the inner membrane of mitochondria are of physiological importance. However, we are very surprised that he completely ignores the strong evidence [2-8] which indicates that the major role of these systems is to regulate the concentration of Ca^{2+} within the mitochondrial matrix rather than, as Miller suggests, the concentration of Ca^{2+} in the cytoplasm. When extramitochondrial Ca^{2+} is raised within the physiological range, a parallel increase in intramitochondrial Ca^{2+} will occur and this may activate three important dehydrogenases which occupy key regulatory sites in oxidative metabolism, namely pyruvate dehydrogenase, NAD-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. In this way, the formation of reducing power for oxidative phosphorylation can be accelerated under the very conditions where there is likely to be an increased requirement for ATP. Increases in cytoplasmic Ca^{2+} generally stimulate energy-requiring processes such as muscle contraction, secretion or ion-pumping. It is worth emphasizing that these key dehydrogenases have so far only been found to be sensitive to activation by Ca^{2+} in vertebrate tissues[9] and only mitochondria from vertebrate tissues appear to take up Ca^{2+} at an appreciable rate at μMolar concentrations.

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The electrostatic charge really is on the surface

SIR — A. A. Berezin recently discussed the question· "What will be a minimum energy configuration of a system of N equal point charges q placed inside a circle?" (see *Nature* **315**, 104; 1985). He considers two configurations, one (A) with all N charges equally spaced around the circumference and the other (B) with one charge at the centre and the remaining $N-1$ equally spaced around the circumference. Assuming a three-dimensional Coulomb potential q^2/r between pairs of charges, he finds numerically that $W(A)<W(B)$ for $N\leqslant 11$ but that for $N\geqslant 12$ (up to at least $N=400$) $W(A)>W(B)$, where W represents the total electrostatic energy of the system. Whilst this answer to the question posed is doubtless true and has some curiosity value, the subsequent suggestion that these results provide an example where charges in a conductor at equilibrium will not reside on its surface is quite wrong.

The fallacy lies in the use of three-dimensional electrostatics for a two-dimensional system. Berezin's circle should properly be regarded as a thin disk and then the charge at the centre of the circle in B is actually at the centre of one of the flat surfaces of the disk. Thus, in the correct three-dimensional interpretation, all of the charges are on the surface of a thin conducting disk for both configurations A and B. (When N is very large, most of the charges will, at equilibrium, reside on the flat surfaces.)

It is of some interest to consider whether any anomalous behaviour arises in the solution of the above problem when it is regarded as strictly two-dimensional. The relevant Coulomb potential is now $-q^2\ln(r/L)$, where L is an arbitary length determining the zero of the potential. It is convenient to choose for L the radius of the circle Then a straightforward analytic calculation shows that $W(B)-W(A)=(q^2/2)[N\ln N-(N-1)\ln(N-1)]>0$ for all $N\geqslant 3$. Thus, in the strictly two-dimensional problem the 'obvious' result is correct and our faith in classical electrostatics is restored.

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The complexity and stability of ecosystems

SIR—I read Pimm's[1] article on stability and complexity within ecosystems with great interest. I would not, as an old-fashioned geologist-palaeontologist, pretend that I understood all of the logic involved, nor that I had been able to follow all of the mathematics employed in the May[2] and MacArthur papers to which Pimm refers. There is completely independent evidence from the fossil record supporting Pimm, as well as the earlier work by May[2] to which Pimm refers. Ecologically complex structures such as reef complexes appear historically to have been less stable than level bottom communities If one concludes that the global marine ecosystem has been subjected to stress sufficient to generate large-scale extinction events, such as occurred at the end of the Permian, then it follows that a comparison can by made between the resultant behaviour of the more simple level bottom communities and faunas as contrasted with the trophically far more complex reef community complexes. The nature of the stress(es) is (are) unknown, but it is reasonable to conclude that the trophically different community types reacted differently. Should one or the other, or at least elements of one or the other, survive such a major environmental perturbation it could be concluded that the surviving items were more stable

There are five major intervals during the Phanerozoic when reef community complexes were in existence—including the present (Fig. 3 in ref. 3) In the Lower Cambrian some of the archaeocyathids occur in what many feel are reef community complexes. Such complexes disappear totally at the end of the Lower Cambrian concurrent with an important extinction event, whereas some level bottom taxa, particularly among the trilobites, survive to later radiate in the Middle Cambrian. The second interval of reef community complex is present in the Middle and Upper Ordovician. It disappears totally in the major, terminal Ordovician extinction event, together with much, but by no means all, of the level bottom fauna grouped into many communities. A third reef community complex, with antecedents differing from those of the Middle and Upper Ordovician, appears about one-third of the way through the Upper Silurian and persists until the middle of the Upper Devonian. The major mid-Upper Devonian extinction totally wipes out the reef community complex together with the majority of the level bottom taxa and many of the communities. A fourth reef community complex occurs in the Upper Carboniferous and Permian, derived from totally different antecedents (presumably, as always, level bottom taxa). The terminal Permian extinction event eliminates the Permo–Carboniferous reef community complex, and the bulk of the level bottom taxa and communities. The level bottom survivors ultimately provide the material for a re-radiation which is responsible for the different reef community taxa of the later Triassic.

There are significant taxonomic differences between the Triassic reef taxa and those of the Jurassic–Cretaceous, which suggests that the terminal Triassic extinction event was involved here. At the end of the Cretaceous the tropical–subtropical reef community complex was almost totally wiped out, and it does not reappear in any abundance until the Eocene. The reef community complex then persists until the present, although there are some major changes, such as the addition of the algal ridge complex in the Miocene. Large numbers of coeval

Jurassic–Recent level bottom taxa persist to the present.

In view of the above it is likely that following severe environmental stress capable of generating major extinction events preserved in the fossil record the reef community complex normally is eliminated *in toto*, but that elements, sometimes a large percentage, of the level bottom fauna and its communities persist. Supporting this conclusion is the fact that during the highly provincial Ordovician, Permian, and later Cretaceous, the biogeographically distinctive reef community complexes in different parts of the world terminate at the same time

I suspect that other trophically complex community types such as pelmatozoan thickets, bryozoan thickets and sponge forests, behave in a fashion similar to the reef community complex rather than to the trophically simpler level bottom communities.

It seems clear that the geological-palaeontological evidence strongly supports Pimm[1], May[2], and the others in their conclusions based on a variety of non-geological evidence.

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Icosahedra in aluminium / manganese alloy

Sir — The experimental observations of regions of icosahedral order in a splat-cooled alloy of Al/Mn[1,2] have excited considerable comment on a novel type of structure which hitherto had been purely conjectural[3]. One way of considering the proposed structure is as the packing of two kinds of rhombohedral units cells, both having the same cell side but one being acute with $\alpha = 63.43°$ and the other obtuse with $\alpha = 116.57°$ These are repeated according to what has become known as the three-dimensional Penrose pattern, and in a ratio of τ acute units to each obtuse unit, where τ is the golden number 1.618 . . The pattern is intrinsically recursive or hierarchic. I wish to point out further similarities with structures already known. There are at least three types of structures entailing local icosahedral symmetry which may be extended hierarchically for various ranges through the material, according to the balance of the demands of local and of long-range ordering. These include:

(1) Close-packed metal structures where, as pointed out by Frank in 1952[4], 12 spheres will pack icosahedrally around a thirteenth which has 95% of their diameter. This is well known in alloys, most clearly in the structure[5] of $Mg_{32}(Zn,Al)_{49}$ which builds four icosahedral shells before the structure goes periodic Most atoms in it have local icosahedral coordination Probably the driving force behind the structure found by Shechtman *et al* is of this type We might note that "decagonal snowflakes" seem first to have been reported by Adam and Hogan and co-workers[6,7] for the corresponding Al/Fe alloy, but were then not so fully characterized.

(2) The boron compounds, summarized graphically by Sullenger and Kennard[8], where many boron compounds, notably α- and β-boron and $B_{12}C_3$, have rhombohedral unit cells with α about 65 5°, closely corresponding to the acute quasi-unit-cell of the Penrose pattern. Here the symmetry results from the icosahedral clusters of boron atoms produced by the directed bonding requirements of boron to which Berezin[9] has drawn attention. It might be noted that in β-boron the packing is hierarchic, 12 icosahedra being themselves packed icosahedrally around a thirteenth:

(3) Tetrahedrally connected networks, where dodecahedral cages can also appear because of the closeness of the tetrahedral angle of 109.47° to the vertex angles of a pentagon (108°). These occur in clathrasils (cage silicates) and in clathrate hydrates.

Using the structural elements which occur in the zeolite ZSM-39[10] and in the type I gas hydrate[11] we have constructed hypothetical unit cells which would correspond to those required for the Penrose pattern. The acute unit cell is composed of Si–O polyhedra as in the ZSM-39 sheets and these polyhedra are the duals of those in the β-boron structure. The unit cell edges consist of alternating dodecahedra sharing faces (two per edge, so that the cell edge length is 4.45408 times the edge length of the dodecahedron). The corresponding obtuse cell consists of the same faces (arranged with $\alpha = 116.57°$ instead of its supplement) but has, at its centre, a polyhedral cage of 18 faces with two dodecahedral caps (of six pentagons each) and a belt of six hexagons equatorially

Next to this cage the dodecahedra are dislocated and have each two hexagonal and two square faces as well as eight pentagonal faces. Thus, the Penrose pattern reported for alloys might also occur in silicates or hydrates, the dodecahedra of the open tetrahedral structure being dual to the icosahedra of the close-packed metallic structures.

The same two acute and obtuse units may also be assembled in various crystalline ways with, for example, the ratio of 5 acute to 3 obtuse In view of the occurrence of the two kinds of unit cells in the numerical ratio τ in the Penrose pattern, it might be timely to look back at the electron compounds of Hume-Rotheray[12,13], some of which are locally icosahedral, whose electron/atom ratios are 21/13 (these often with an atomic ratio of 8/5 as for Cu_5Zn_8) and 3/2 for CuZn, which might seem to be convergents to the golden number (and also 7/4 which is not), to see where else the Fibonacci series may be detected.

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Loess in the Bering Land Bridge

Sir — In his *News and Views* article on the Bering Land Bridge, Colinvaux[1] reported that the *yedoma* deposits of Siberia — long described as alluvium or lacustrine silt — are actually loess, emplaced by wind action when "dry, cold and windswept" conditions prevailed in that part of Siberia. The climatic consequences of this conceptual change are profound and Academician Shilo and co-workers are to be congratulated in bringing about this revolution. Once an idea is in place (for example that a particular deposit is lacustrine silt), it can be very difficult to change; witness the remarkably long time it took to recognize the widespread loess in southern England. But, if the Siberian story is to be fully told there should be a nod of acknowledgement in the direction of Troy Péwé and his proposals that Siberian "silts" were loess[2,3] Péwé and Journaux, admittedly working west of the immediate Bering Strait area, showed that loess was widespread in Yakutia and made a good case for the existence of significant Siberian loess deposits[4].

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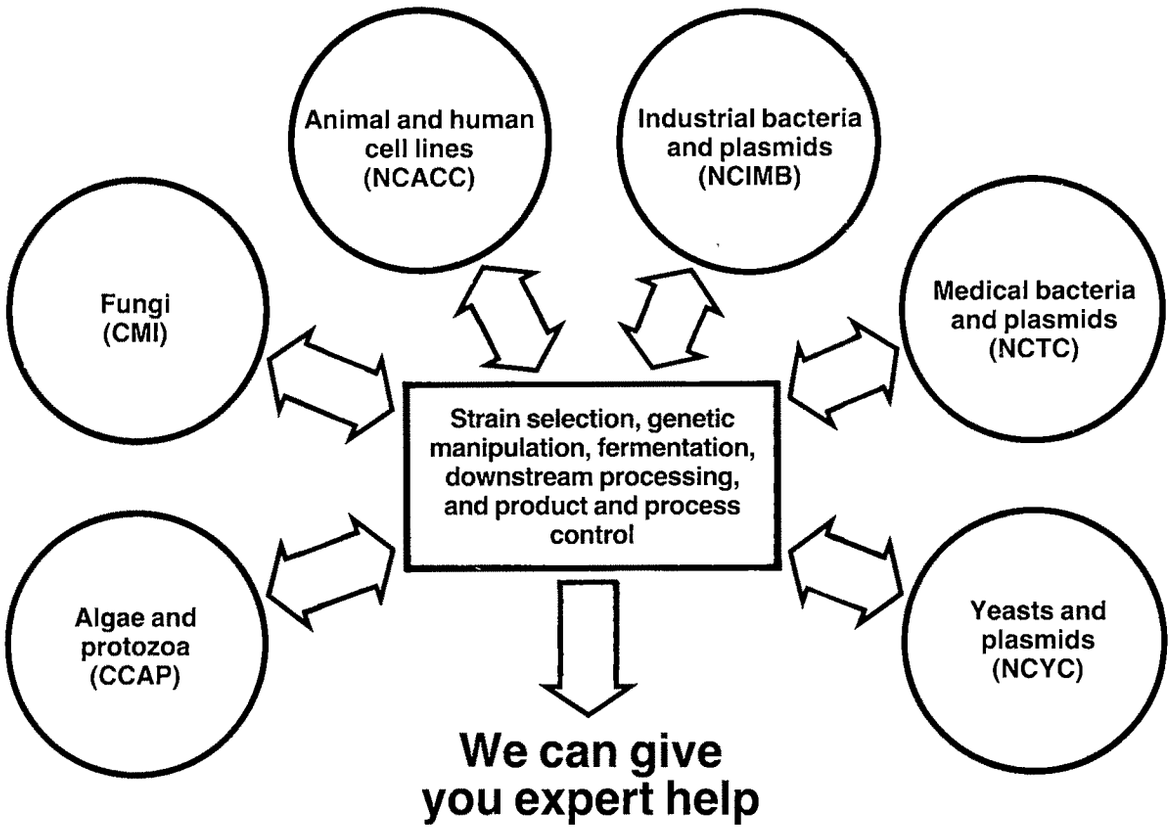
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Refraction scattering as origin of the anomalous radar returns of Jupiter's satellites

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Three satellites of Jupiter, Callisto, Ganymede and Europa, give anomalous radar returns. We show here that this behaviour would arise as a consequence of 'refraction scattering' rather than the familiar 'reflection scattering' and discuss mechanisms that lead to the predominance of such scattering on these satellites.

THREE of the Galilean satellites of Jupiter, Callisto, Ganymede and Europa, have been shown to have anomalous radar reflection properties in several respects (refs 1-3, D. B. Campbell and S. J. Ostro, in preparation). Although all other planetary bodies that have been investigated by radar, including many different areas on the Earth, give a reflection of a circularly polarized radio wave in which the sense of polarization is reversed, these jovian satellites return a wave that shows predominantly the same sense as the incident one: the shorter the wavelength, the more similar the sense of the waves. If a linearly polarized illumination is used, fully 50% of the energy is returned in the orthogonal polarization, whereas the normal for other bodies is <10%.

Although all other planetary bodies seem to have a total radar cross-section (measuring the sum of returned power in two orthogonal polarizations) of the order of 0.1× the geometrical cross-section, the total cross-section at 12.6-cm wavelength for the galilean satellites are 2.6 for Europa, 1.5 for Ganymede and 0.6 for Callisto[4].

All other planetary bodies give a quasispecular return (glare) from the subearth point, but no such effect occurs for the three galilean satellites in the wavelength region in which they have been studied (3.5-70 cm). In fact, the scattering from these satellites is intermediate between that giving a uniformly bright disk and the slight limb-darkening associated with Lambert scattering[4].

In addition to their remarkable visual appearance[5,6], Callisto, Ganymede and Europa are best characterized as bizarre radar objects. Ostro[4] discusses two possible mechanisms which might explain the peculiar polarization properties. One, which is discarded as unlikely, involves double reflection within hemispherical craters, whereas the other involves multiple internal total reflection on flat boundaries between ice and less dense ice (crushed ice, possibly snow) The polarization results, studied by Monte Carlo calculations, involve boundaries of random and uncorrelated orientations. There is no good explanation of the enormous cross-section in this model

We explore here the alternative possibility that the effect results from what we term 'refraction scattering', a type of scattering which has received little attention and which, has not been encountered previously in radar work with solid materials.

In refraction scattering, one measures a wave that penetrates into the body and whose propagation vector is bent as a result of gentle variations of the refractive index in the medium. In the case of sufficiently large variations of the refractive index, the penetrating wave may be deflected so as to emerge again from the surface. In the geometrical optics limit, the polarization of the wave is retained and we shall show that the unusual polarization properties of the three satellites are then reproduced. For refraction scattering to be dominant one requires: (1) The surface reflection must be small. This requires that the surface material has a low refractive index, n, or that there is a 'matching' transition layer on the surface; (2) the bulk medium has sufficiently low absorption of radio waves to allow long pathways within the medium; (3) the patterns of the variations in n are such as to redirect the propagation vectors by large angles, including 180°; (4) the gradients of n are sufficiently small so that internal reflections will not have a dominant influence.

We describe below internal refractive index structures that satisfy the above four requirements and can reproduce naturally all the bizarre radar properties of the three outer galilean satellites.

Internal refraction

The study of the propagation is based on geometrical optics. A set of equations which describes the rays is:

$$\frac{d\vec{r}}{ds} = \vec{s} \tag{1}$$

$$\frac{d\vec{s}}{ds} = \vec{s} \times \left(\frac{\nabla n}{n} \times \vec{s} \right) \tag{2}$$

where s = arc length along ray and $\vec{s}$ = unit vector along ray. If the medium is horizontally stratified and n is higher in the medium than outside, the angle between the ray direction $\vec{s}$ and the vertical must always be less there than at incidence. Therefore, the ray cannot emerge in the return direction and it is necessary that the surface material is laterally inhomogeneous. Surfaces of constant n might have an appearance as shown in Fig. 1.

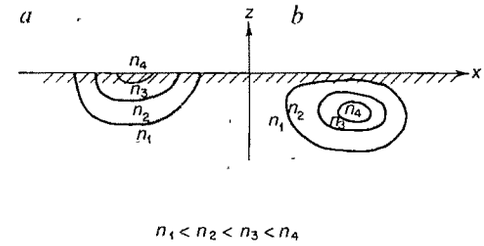


Fig. 1 Contours of constant refractive index: *a*, partly buried; and *b*, fully buried scatter.

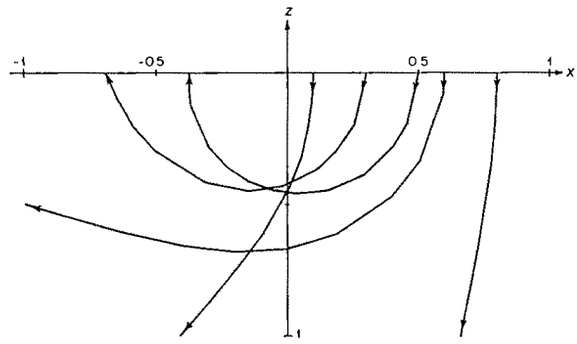


Fig. 2 Rays in gaussian refractivity profiles Normal incidence.

What seems to be required are convex surfaces of constant n surrounding a region of higher n. Further, the curvature of the rays $|\nabla n/n \times \vec{s}|$ must be comparable with the curvature of the equi-refractive index surfaces. These regions of excess refractive index may be quasi-spherical or ellipsoidal, or they may be torus or cylinder shaped with quasi-circular or ellipsoidal cross-sections. Whatever the case may be, such regions, possessing a sufficiently large total range of n, are capable of bending the incoming rays so that they emerge from the surface in all different directions, including the backscatter direction.

We have numerically constructed rays on the basis of equations (1) and (2) for surfaces of several types of n, spherical, ellipsoidal and cylindrical, where n varies either as a cosine, a gaussian or according to the function given below. The refractive index was taken to vary from n_0 at the centre to the value n_1 of the background medium at a distance from the centre, r. Various degrees of submersion of the blobs were considered. The special function mentioned is of the form:

$$n(x,z) = \frac{n_0}{1+(x^2+(z-z_c)^2/a^2} \quad \text{when} \begin{cases} z<0 \\ \sqrt{x^2+(z-z_c)^2} < a\sqrt{n_0/n_1-1} \end{cases} \quad (3)$$

$$= n_1 \quad \text{when} \begin{cases} z<0 \\ \sqrt{x^2+(z-z_c)^2} > a\sqrt{n_0/n_1-1} \end{cases}$$

$$= 1 \quad \text{when} \quad z>0$$

An example of rays traced in a gaussian profile with $z_c = 0$ (Fig. 1*a*), $n_0 = 3.5$, $n_1 = 1$ and $L_x = L_z = 0.4$ (the e-folding distances in the x and the z directions, respectively) is shown in Fig. 2, for normal incidence.

We find no evidence of any qualitative difference between the different models and, therefore, discuss the special case as defined in equation (3) in greater detail, as this is amenable to straightforward analytical treatment. This particular profile, referred to as Maxwell's 'fish eye', has been discussed elsewhere[7].

Consider a spherical blob of excess n of this type fully embedded in a medium of refractive index n_1. We wish to determine the deviation angle between the incoming and existing rays as a function of x (analogous to the impact parameter in collision theory). This relationship can best be derived by reference to Fig. 3. Suppose the radius where the excessive refractive index equals that of the surrounding medium (that is, n_1) is r_1, and suppose the z-axis is aligned with the incoming ray. The ray enters at A. In such refractivity distributions the curvature of the ray is constant and the ray must describe a circle, the centre of which must lie on a line AE normal to the z-axis. A ray going through point A which is a distance r_1 from the centre will cross the diameter through A at a point B on the opposite side of C at a distance $BC = r_2$. In this particular distribution one has.

$$r_1 r_2 = a^2$$
$$a = r_1/\sqrt{n-1} \quad (4)$$
$$n = n_0/n_1$$

The centre of the circular ray is found at the intersection of the bisector of AB with AE, and is thus D. From the construction of Fig. 3 we find that the total deflection angle θ is given by:

$$\theta = 2\arctan\frac{2(n-1)\cdot x\cdot\sqrt{1-x^2}}{n-2x^2(n-1)} \quad (5)$$

where $x \Rightarrow x/r_1$ is the normalized 'impact parameter'.

Figure 4 shows the deflection angle for this particular model plotted against normalized x for various values of n. The actual values of x required to give backscatter (that is, $\theta = 180°$) are given by:

$$x_{180} = \sqrt{\frac{n}{2(n-1)}} \quad (6)$$

and are plotted in Fig. 5*a*. It is clear from this diagram that the rays that are turned back by 180° enter and leave the scatterer near the circumference where n is small. As they will never go through the centre of the scatterer it is not necessary that n actually assumes the value n_0 for backscattering to occur, but it is sufficient that n increases to n_{max}, which it assumes at that point of the ray where it is the closest to the centre of the scatterer. The actual value of this n_{max} can be shown to be:

$$n_{max} = \frac{n}{n-\sqrt{n(n-2)}} \quad (7)$$

Figure 5*b* shows n_{max} as a function of n. It is clear from this diagram that the actual values of $n(r)$ need not in practice reach the peak value at the centre of the scatterer to be able to turn the rays round by 180°.

It can be seen from Fig. 4 that there is a substantial range of x which, for normal incidence, turns the rays back into the hemisphere they came from. If the inner and the outer limits for x are denoted by x_{90} and x_{270}, the fractional energy returned into the hemisphere centred on the incoming wave is $x_{270}^2 - x_{90}^2$. This fraction is plotted as a function of n in Fig. 5*c*.

Finally, we consider the radar backscattering cross-section of one of these spherical enhancements of refractivity. This cross-section is defined as:

$$\frac{\text{Power scattered within } \Delta\Omega}{\text{Incident power density}/4\pi} = \sigma \quad (8)$$

In the geometrical optics approximation discussed above we have found a unique mapping between the area ΔA of the wave front and the solid angle $\Delta\Omega$ onto which this power is scattered. Therefore, the cross-section σ can be expressed as:

$$\frac{S\cdot\Delta A(\Delta\Omega)/\Delta\Omega}{S/4\pi} = 4\pi\cdot\frac{\Delta A}{\Delta\Omega} \quad (9)$$

For an arbitrary direction we have: $\Delta A = x\cdot\Delta x\cdot\Delta\Psi$ and $\Delta\Omega = \Delta\theta\cdot\sin\theta\cdot\Delta\Psi$. Hence we obtain:

$$\sigma = 4\pi\frac{x}{\sin\theta}\cdot\frac{\Delta x}{\Delta\theta} \quad (10)$$

From equation (5) it is easy to show that $\Delta x/\Delta\theta$ remains finite for backscatter, and because x remains near unity σ becomes infinite as $\sin\theta \to 0$. This apparent paradox is resolved if we remember that $\Delta\Omega$ cannot approach zero with ΔA because diffraction eventually starts $\Delta\Omega$ increasing again for small ΔA. We should also note that rays entering and leaving along a circle of radius x will only occur when there is perfect spherical

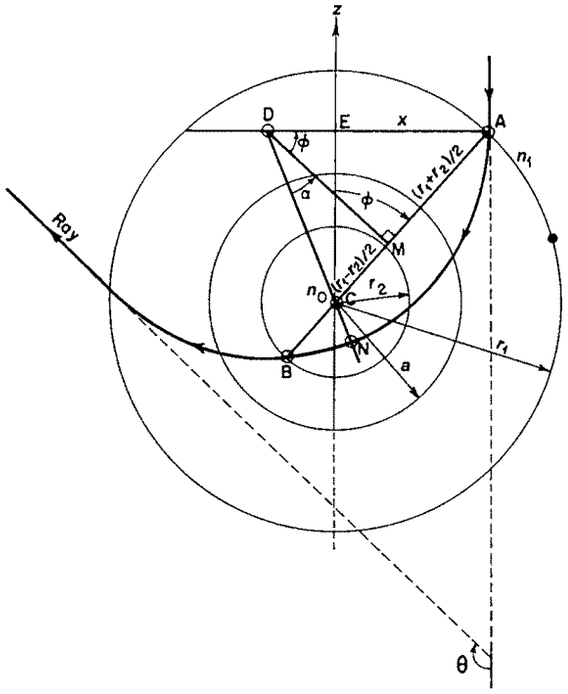


Fig. 3 Derivation of the deflection angle for 'fish eye' distribution of n

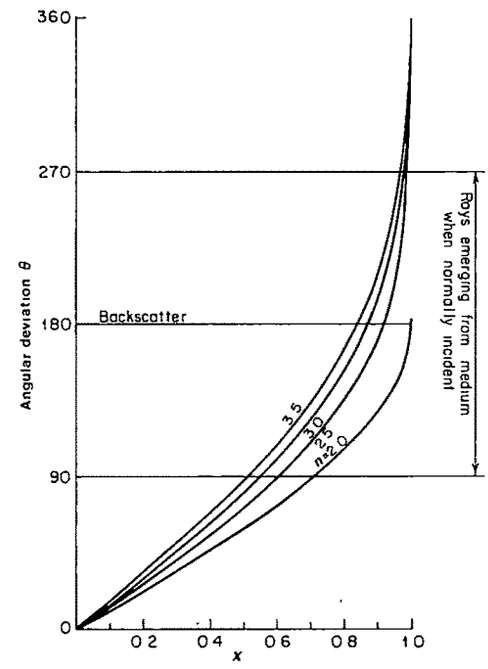


Fig. 4 Angular deviation of rays plotted against 'impact parameter' x with $n = n_0/n_1$ as parameter.

geometry. As soon as we allow for deviations from the perfect spherical symmetry the circular ring will be broken up into disjointed areas and the tendency for backscatter probably decreased. Nevertheless, we suspect that there will also be a distinct preference for backscatter in the real surface

Application to observations

Let us now see how the concept of refraction scattering can be applied to explain the observed radar reflection properties of Callisto, Ganymede and Europa. We must assume that the presence of refractive scatterers is universal on these satellites. The description of the return as a refraction phenomenon, with little reflections from abrupt refractivity changes, implies that a circularly polarized wave will retain its polarization and emerge with the same sense of circular polarization, as required. Our model predicts circular polarization to be maintained fully, in the original sense, whereas the data show this to be only partly so. This may be due to residual surface backscattering, or some internal reflections and/or failure of the geometrical optics approximation to be perfect. Suppose we assume that the backscattering arises as a superposition of two distinct mechanisms, one being the refraction scattering and the other a more usual mechanism, which turns the incoming circular polarization completely into the opposite one in the same manner as an ideal reflecting surface. For a wavelength of 12.6 cm, Ostro[4] quotes for the ratio of 'same circular' (SC) to 'orthogonal circular' (OC) values of 1.5 for Europa and Ganymede and 1.2 for Callisto. It follows that 60% of the power returned from Europa and Ganymede and 55% from Callisto must result from refraction scattering.

The linear polarization results follow from the refraction model as follows: suppose we consider a ray in the xz plane as in Fig. 3. If the linearly polarized E-field is normal to the xz plane, the emerging E-field will be in the same direction as the incoming field. If, however, the E-field is in the incidence plane xz, it will emerge in that plane, but 180° out of phase with a corresponding field normal to xz. This means that a field making

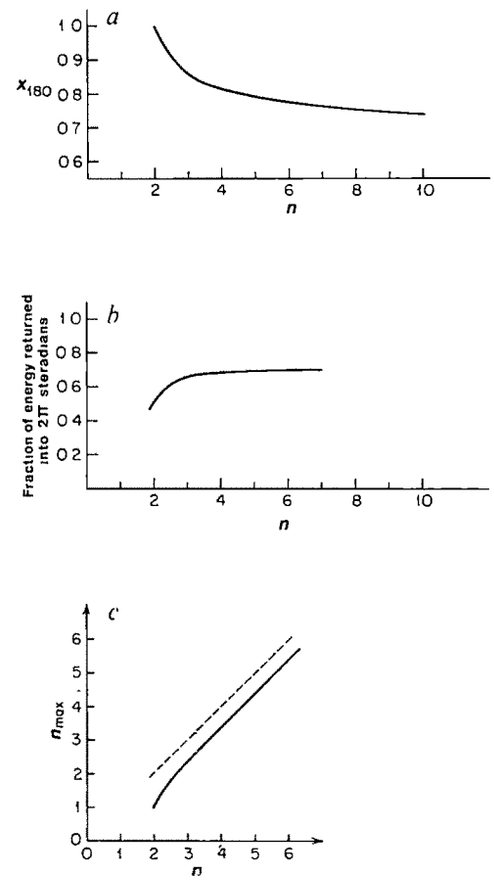


Fig. 5 *a*, Plot of x_{180} against $n = n_0/n_1$ *b*, Plot of maximum refractive index along a backscatter ray against n at the centre *c*, Plot of fraction of normally incident energy emerging from the surface against n.

45° with the xz plane will emerge with its E-field normal to the incoming field. In general, for a field making an angle γ with the plane of incidence, one must have: $E_{SL} = E_0 \cos 2\gamma$ and $E_{OL} = E_0 \sin 2\gamma$ where the indices signify 'same linear' and 'orthogonal linear' as in ref. 4. If powers rather than fields add, and because γ must occur with equal probability between 0 and 360°, one obtains $\sigma_{SL} = \sigma_{OL}$ or $\sigma_{OL}/\sigma_{SL} = 1$. Hence, our ideal model predicts complete depolarization, whereas the data show this ratio to be 0.5. In addition, the assumption of power addition may break down if there is coherency between rays entering and leaving individual blobs, as indeed there is if they are perfectly spherical. Again it seems reasonable to suppose that surface reflections, internal reflections and/or failure of the geometrical optics approximation to decrease the predicted polarization ratio from that of the model. If we imagine that the returned powers be split between refraction scattering and a reflection scattering mechanism as above, and try to reproduce the ratio of orthogonal linear to same linear of 0.47 for Europa and Ganymede and 0.55 for Callisto as observed at 12.6-cm wavelength by Ostro[4], we conclude that 64% of the power returned from the former two can be ascribed to refraction scattering, whereas 70% must be assigned to that mechanism in the case of Callisto. For Europa and Ganymede the consistency is excellent between the deductions made from the linearly and circularly polarized data in view of the limited applicability of our arguments. For Callisto, however, there is an inconsistency between the deductions made, which we suspect results from the failure of the geometrical optics approximation to apply as well as it apparently does on Europa and Ganymede.

We have shown that refractivity inhomogeneities are capable of scattering a substantial fraction of incoming radiation back into space. Such behaviour is consistent with the low emissivity observed by de Pater[8]. The extremely high radar reflectivity is also compatible with the low emissivity and is probably augmented by a preferential backscattering of the individual scatterers, discussed above.

For refraction scattering, or for scattering by internal reflections, the case of normal incidence at the surface has no particular significance; this will account for the lack of a specular reflection highlight near the sub-earth point on these satellites.

Finally, the variation with wavelength referred to earlier receives a natural explanation, in that the geometrical optics approximation will become increasingly poor the larger the wavelength. If this is the explanation of the wavelength dependence, then the size of typical scatterers must be of the order of 1 m.

Discussion

It is noteworthy that the anomalous properties occur just on the only three bodies that have been investigated, which are thought to be composed mainly of ices. Water ice, thought to be the dominant component, is a dielectric of very low dielectric loss, with $n = 1.78$. The other component of the surface material of these bodies is believed to be rock powder whose detailed dielectric properties are not yet known. Lunar rocks and rock powders have been extensively investigated[9] and have values of n ranging from 1.3 for loosely packed material to >3.2 for the dense rock. If there is a tendency for rocks in the domain of the outer planets to be less oxidized, then substantially higher values for the refractivity are to be expected.

Therefore, the physical model we propose is one of a patchwork of rock powders and ices in which n may vary from just over 1 to >3. The uneven distribution of these two components may have resulted from an initial accretion of layers of rock and ice particles, which, together with impacts of larger objects, would have turned a neatly layered system into one that is both laterally and vertically erratic. In particular, it may be that the overturning of material in an impact could create the torus-shaped structures referred to above. Also, impact on a loosely packed but cohesive matrix of rock powder left behind by evaporation of volatiles could be compacted and form the semi-spherical volumes of excess n discussed above. Evaporation of volatiles from the surface may have helped to concentrate rock powders. Outpouring of liquid water from deeper and warmer regions may have laid down 'clean' ice. Both these processes would contribute to the creation of a heterogeneous medium.

The requirement of low dielectric loss may well be satisfied because of the very low temperatures. Ice well below its melting point leads to absorption lengths of thousands of wavelengths. The lunar rocks gave results for the absorption length between 100 and 300 wavelengths. At the low temperatures of these icy satellites it is likely that the low dielectric loss of these rock powders would be maintained despite the presence of water in solid form. (At the higher temperatures prevailing on earth, hydrated rocks dominate and exhibit a high dielectric loss.)

The condition of low surface reflectivity may be met by having a very tenuous medium (therefore of low n) in which the regions of excessive refractivity are embedded. If the medium is not so tenuous, it may be that a low density transition layer, as found on the Moon, can provide the proper match and serve to suppress the reflection at the surface. An extremely fluffy outer surface of rock powder or snow may have resulted from the partial evaporation of ices from the pore spaces in the material. In addition it may be that the interesting mechanism suggested by Spencer and Maloney[10], which explains the migration of water on Callisto as sublimation and capture in cold traps, may have played a role in creating the heterogeneous conditions required. This mechanism alone would apparently create depletions in refractivity rather than enhancements, and, therefore, must have been modified by some other mechanism, such as impact cratering, to create the favourable conditions for refraction scatter to occur.

Also within the material, sharp discontinuities of n must be sufficiently rare for refraction to remain the dominant cause of backscatter. If rock powder and ices make up the bulk of the material, this implies that although variations of the mixing ratio of these two substances are responsible for the refraction, they are generally not too abrupt on the scale of the radio wavelengths used.

Conclusions

We have shown that refraction scattering from convex regions of excess n has all the properties required to explain the observed radar returns from Callisto, Ganymede and Europa. We conjecture that the conditions which prevail on these satellites are probably ideal for the creation of the refractivity structure required to make refraction scattering important. Therefore, we conclude that refraction scattering is the most likely explanation for the bizarre scattering properties of these satellites.

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Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs

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Two distinct but distantly related complementary DNAs encoding proteins sharing human interleukin-1 (IL-1) activity (termed IL-1α and IL-1β), were isolated from a macrophage cDNA library. The primary translation products of the genes are 271 and 269 amino acids long, although expression in Escherichia coli *of the carboxy-terminal 159 and 153 amino acids produces IL-1 biological activity.*

INTERLEUKIN-1, a protein produced by activated macrophages, mediates a wide range of biological activities, including stimulation of thymocyte proliferation via induction of interleukin-2 (IL-2) release, stimulation of B-lymphocyte maturation and proliferation, fibroblast growth factor activity and induction of acute-phase protein synthesis by hepactocytes[1]. IL-1 has also been reported to stimulate prostaglandin and collagenase release from synovial cells, and to be identical to endogenous pyrogen[1].

The question of whether this array of activities resides in a single protein has excited much discussion. The conventional approach to its resolution has been to purify IL-1 activities from the culture supernatants of macrophages activated with lipopolysaccharide (LPS), but the results have been ambiguous. Apparently homogenous protein preparations display characteristic IL-1 activities, including IL-2 induction and pyrogenicity. However, many reports associate IL-1 with proteins of relative molecular mass (M_r) ~15,000 and 30,000 and isoelectric points ~*p*H 5 and 7 (refs 1-8). These studies suggest that either a considerable amount of protein processing leads to the appearance of active polypeptides of differing sizes from a single protein, or that activities characteristic of IL-1 are shared by several different proteins.

To clarify these issues, we attempted to clone the gene(s) responsible for IL-1 activity using two different strategies. First, we screened macrophage complementary DNA clones by hybridization to macrophage RNA, followed by *in vitro* translation of the selected RNA and detection of IL-1 biological activity using a sensitive assay that measures its capacity to induce IL-2 synthesis by a cloned T-cell line[9]. Second, we purified to homogeneity a protein having IL-1 activity[2], performed partial amino-acid sequence analysis, constructed an oligonucleotide probe based on this sequence and used the probe to screen a macrophage cDNA library.

These experiments led to the isolation of two distinct cDNAs encoding proteins with a characteristic IL-1 activity, defined by the induction of IL-2 synthesis by a T-cell line or by thymocytes. These proteins, which we term IL-1α and IL-1β, showed only distant homology to each other. Both molecules seem to be synthesized as larger precursors that are processed to smaller forms. Here we discuss the relationship of these findings to recent reports of other IL-1 cDNAs[10,11].

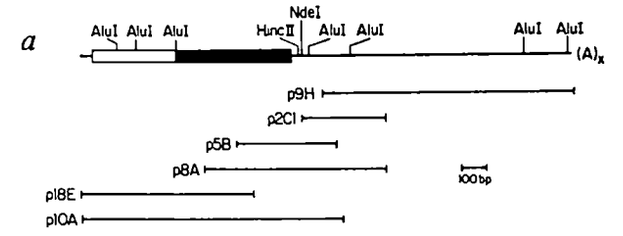
Isolation of IL-1 messenger RNA

Optimal conditions for the production of IL-1 messenger RNA from peripheral blood monocytes were established. Initially, mRNA was injected into *Xenopus* oocytes and the supernatants assayed for biological activity. Low levels of IL-1 could be detected using an assay that measures the capacity of IL-1 to stimulate IL-2 release from a phytohaemagglutin-stimulated cloned murine lymphoma cell line (LBRM-33-1A5) (termed the 1A5 conversion assay)[9]. This assay is ~1,000-fold more sensitive than the standard IL-1 assays of direct thymocyte mitogenesis or co-mitogenesis[2], and was used as the basis for the molecular cloning of IL-1. Stimulation of the monocytes with 10 μg ml^{-1} *Escherichia coli* lipopolysaccharide (LPS) for 12 h gave the highest levels of IL-1 mRNA. Unstimulated monocytes contained little IL-1 mRNA.

Table 1 Identification of IL-1α cDNA clones by hybrid selection of biologically active mRNA

First screen Pools of 48		Second screen Pools of six		Third screen Single colonies	
Filter Starting RNA	IL-1 (U ml^{-1}) 1,195	Filter Starting RNA	IL-1 (U ml^{-1}) 1.5×10^4	Filter Starting RNA	IL-1 (U ml^{-1}) 1.8×10^4
1	ND	2A	ND	2C1	257
2	59	2B	ND	2C2	ND
3	ND	2C	1,006	2C3	ND
4	10	2D	ND	2C4	ND
5	ND	2E	ND	2C5	ND
6	ND	2F	ND	2C6	ND
7	ND	2G	ND		
8	ND	2H	ND		
9	ND	11A	ND		
10	ND	11B	ND		
11	72	11C	ND	11F1	ND
12	ND	11D	ND	11F2	ND
13	ND	11E	ND	11F3	ND
14	ND	11F	440	11F4	ND
15	ND	11G	ND	11F5	ND
16	39	11H	ND	11F6	1,072
Control	ND	Control	ND	Control	ND

Single bacterial colonies were picked into LB-broth plus 15 μg ml^{-1} tetracycline and grown overnight, then 48 1-ml cultures were combined and plasmid DNA isolated according to standard procedures[27] with the modification that the plasmid DNA was ribonuclease treated and separated from small-M_r nucleic acids by chromatography on a Sephacryl S-400 column. 10 μg plasmid DNA was digested with *Bam*HI, denatured and bound to nitrocelluose filters (Schleicher and Schuell)[28]. Each filter was hybridized to 20 μg of LPS-stimulated macrophage RNA overnight at 50 °C in 50% formamide, 100 mM PIPES *p*H 6.8, 0.4 M NaCl, 10 mM EDTA, then washed 10 times at 65 °C in 1×SSC, 0.2% SDS and five times in 0.2×SSC at 65 °C. The bound RNA was eluted in boiling water containing 6.6 μg μl^{-1} yeast transfer RNA and recovered by ethanol precipitation, then translated in rabbit reticulocyte lysates[29,30] and an aliquot of the lysate was assayed for IL-1 activity in the 1A5 conversion assay[9]. ND, not detected. No biological activity over the background control was observed at a sample dilution of 1:50. Control, 10 μg pBR322 DNA was bound to the filter as a negative control



b

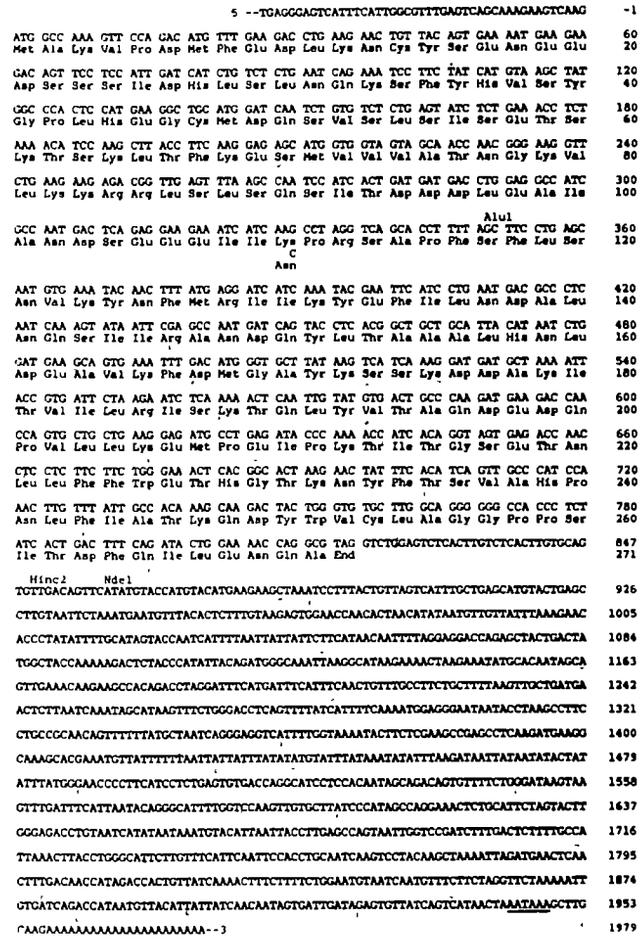


Fig. 1 *a*, Restriction endonuclease map of human IL-1α Coding sequences are boxed. Shaded boxed region represents the presumptive mature form of IL-1α Below are shown the cDNA clones used for sequencing. One clone, p10A, contains the entire coding region Each cDNA clone begins and ends with *Pst*I sites, generated by the cloning procedures. *b*, Nucleotide sequence and predicted amino-acid sequence of IL-1α. Nucleotides and amino acids are numbered from the presumed initiator codon The star under Ser 113 indicates the position of the presumed mature NH_2 terminal. The nucleotide sequence differs between p18E and p10A at base 330 (G→C) which would cause an amino-acid change, Lys 110→Asn The 3′ maturation signal[36] is underlined Coordinates of cDNA clones shown in *a* are: p18E, nucleotides −45–652, p10A, −35–1,075; p8A, 447–1,195; p5B, 591–995; p2C1, 860–1,194; p9H, 928–1,979.

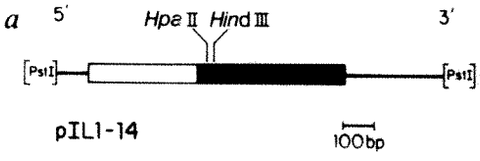
Methods. *a*, Size fractionation of LPS-stimulated macrophage RNA Human peripheral blood mononuclear cells were isolated as described previously[2]. Macrophages were purified further by adherence to plastic tissue culture flasks and stimulated with 10 μg ml^{-1} LPS for 12 h. Total RNA was isolated by a modification of the guanidinium thiocyanate and guanidine hydrochloride methods[12,31]. Cells were solubilized in 4 5 M guanidinium thiocyanate containing 50 mM sodium citrate, *p*H 7 0, 5% β-mercaptoethanol and 0 5% sodium sarcosinate Following sonication, 0 6 vol ethanol was added and RNA precipitated at −20 °C overnight. The pelleted RNA was resuspended in 8 M guanidine hydrochloride containing 50 mM sodium acetate, *p*H 6.0, extracted with phenol/chloroform/isoamyl alcohol (25:24·1) and reprecipitated with 0.6 vol ethanol, as above Following a wash with 80% ethanol, the RNA pellet was dissolved in water and chromatographed on an oligo(dT) cellulose column to select polyadenylated RNA[32]. Polyadenylated RNA (250 μg) was then fractionated on 1.5% ultra-low-melt agarose gels (Marine Colloids) containing 10 mM methyl mercuric hydroxide[12]. Gel slices were melted at 65 °C in binding buffer (0.5 M NaCl, 10 mM Tris-acetate *p*H 7.5, 1 mM EDTA) and applied to small columns of NACS-52 resin (BRL) The columns were washed with binding buffer, RNA eluted with 6 M guanidine hydrochloride, 10 mM Tris-acetate *p*H 7.5, 1 mM EDTA, mixed with 10 μg yeast tRNA and purified by several ethanol precipitations and washes to remove the guanidine hydrochloride. RNA was then redissolved in 15 μl water for injection into *Xenopus* oocytes. For each fraction, 40 stage VI oocytes were each injected with 50 nl RNA and incubated overnight at 19 °C in Barth's medium. Undamaged oocytes were transferred to 300 μl of 1 2 complete Click's medium containing 1.5 mM $CaCl_2$ and 100 μg ml^{-1} gentamycin and incubated for 2 days at 19 °C. Supernatants were assayed for IL-1 activity using the 1A5 conversion assay[9] (see text) Double-stranded cDNA was synthesized from the size-fractionated RNA using standard techniques[12,13]. The cDNA was size-fractionated on a Sephacryl S-400 column and fractions >500 bp long were pooled and tailed with dCMP residues[33] The tailed cDNA was annealed to dGMP-tailed, *Pst*I-cut pBR322 (NEN) and used to transform *E. coli* strain MM294 according to the procedure of Hanahan[34], ~20,000 transformants were obtained from 25 ng cDNA. 5,000 transformants were picked into microtitre plates and grown to saturation in LB-broth containing 15 μg ml^{-1} tetracycline. The 5,000 selected transformants were replica-plated onto nitrocellulose filters and hybridized to ^{32}P-labelled cDNA synthesized from polyadenylated, unstimulated macrophage RNA[12]. Colonies hybridizing strongly were eliminated from further consideration and 768 of the remaining colonies screened by the hybrid selection procedure described in Table 1, resulting in the isolation of p2Cl. The insert from p2Cl was ^{32}P-labelled by nick-translation and used to screen the whole library, resulting in the identification of p9H. An oligonucleotide was synthesized from the sequence of p2Cl, 5′GTTGGTTCCACTCTTAC3′, complementary to nucleotides 959–975 (*b*). This was ^{32}P-labelled by T_4 polynucleotide kinase and [γ-^{32}P]ATP and used to rescreen the library[35], resulting in the isolation of clones p5B and p8A A 324-bp *Pst*I/*Hae*III fragment isolated from p8A was then used to probe the library, resulting in the isolation of p18E and p10A. *b*, Restriction endonuclease fragments from the cDNA clones were subcloned into M13mp18 or mp19 (ref 37) and sequenced by the chain-termination method[38] as described previously[39,40]

The low levels of IL-1 activity secreted by the oocytes raised the possibility that IL-1 might be sequestered inside the oocyte. However, oocyte homogenates were highly inhibitory to the 1A5 conversion assay because, when 4,000 U of purified IL-1 was mixed with homogenates from uninjected oocytes, no IL-1 activity could be demonstrated. Subsequently, we found that mRNA translated in rabbit reticulocyte lysates could generate much higher levels of IL-1 activity, so we used this system.

Polyadenylated RNA showing IL-1 activity was size-fractionated on denaturing agarose gels and fractions assayed for IL-1 mRNA content (data not shown). The fraction having the highest activity was used to construct a cDNA library of ~20,000 transformants in plasmid pBR322 by established techniques[12,13].

IL-1α-containing plasmids

The production of high levels of IL-1 activity in reticulocyte lysates allowed the identification of IL-1 cDNA clones by hybrid selection of biologically active IL-1 mRNA. Starting with 5,000 transformants from the library, we eliminated from consideration those clones that were resistant to ampicillin (which presumably lacked a cDNA insert) and those that contained cDNAs corresponding to abundant mRNA common to both unstimulated and LPS-stimulated macrophages. Such clones were identified by their strong hybridization to ^{32}P-cDNA synthesized from unstimulated macrophage RNA. Of the remaining 2,000 clones, 768 were screened in pools of 48 for IL-1 activity using a hybrid selection procedure (Table 1). Two pools of plasmid DNA were positive in this assay and were broken down further until two individual cDNA clones (p2C1 and p11F6) were obtained that selected RNA yiedling IL-1 biological activity. Although the IL-1 activity selected by p2C1 in the third screen was not as great as that selected by p11F6, it was significantly above background values. In subsequent experiments, the two plasmids selected comparable levels of IL-1 activity (several thousand U ml^{-1}), and were found to cross-hybridize (data not shown).



b

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-37  5' - TTCGAGGCACAAGGCACAACAGGCTGCTCTGGGATTCTCTTCAGCCAATCTTCATTGCTCAAGTGTCTGAAGCAGCC   -1

ATG GCA GAA GTA CCT GAG CTC GCC AGT GAA ATG ATG GCT TAT TAC AGT GGC AAT GAG GAT    60
Met Ala Glu Val Pro Glu Leu Ala Ser Glu Met Met Ala Tyr Tyr Ser Gly Asn Glu Asp
                                10                                              20

GAC TTG TTC TTT GAA GCT GAT GGC CCT AAA CAG ATG AAG TGC TCC TTC CAG GAC CTG GAC   120
Asp Leu Phe Phe Glu Ala Asp Gly Pro Lys Gln Met Lys Cys Ser Phe Gln Asp Leu Asp
                            30                                              40

CTC TGC CCT CTG GAT GGC GGC ATC CAG CTA CGA ATC TCC GAC CAC CAC TAC AGC AAG GGC   180
Leu Cys Pro Leu Asp Gly Gly Ile Gln Leu Arg Ile Ser Asp His His Tyr Ser Lys Gly
                            50                                              60

TTC AGG CAG GCC GCG TCA GTT GTT GTG GCC ATG GAC AAG CTG AGG AAG ATG CTG GTT CCC   240
Phe Arg Gln Ala Ala Ser Val Val Val Ala Met Asp Lys Leu Arg Lys Met Leu Val Pro
                            70                                              80

TGC CCA CAG ACC TTC CAG GAG AAT GAC CTG AGC ACC TTC TTT CCC TTC ATC TTT GAA GAA   300
Cys Pro Gln Thr Phe Gln Glu Asn Asp Leu Ser Thr Phe Phe Pro Phe Ile Phe Glu Glu
                            90                                              100
                                                                *
GAA CCT ATC TTC TTT GAC ACA TGG GAT AAC GAG GCT TAT GTG CAC GAT GCA CCT GTA CGA   360
Glu Pro Ile Phe Phe Asp Thr Trp Asp Asn Glu Ala Tyr Val His Asp ALA PRO VAL ARG
                        HpaII   110                                             120

TCA CTG AAC TGC ACG CTC CGG GAC TCA CAG CAA AAA AGC TTG GTG ATG TCT GGT CCA TAT   420
SER LEU ASN Cys THR LEU ARG ASP SER GLN GLN LYS SER LEU VAL MET SER GLY PRO TYR
                            130                                             140

GAA CTG AAA GCT CTC CAC CTC CAG GGA CAG GAT ATG GAG CAA CAA GTG GTG TTC TCC ATG   480
GLU LEU LYS ALA LEU HIS LEU GLN GLY GLN ASP MET GLU GLN GLN VAL VAL PHE Ser Met
                            150                                             160

TCC TTT GTA CAA GGA GAA GAA AGT AAT GAC AAA ATA CCT GTG GCC TTG GGC CTC AAG GAA   540
SER PHE VAL GLN GLY GLU GLU Ser ASN ASP LYS Ile Pro Val Ala Leu Gly Leu Lys Glu
                            170                                             180

AAG AAT CTG TAC CTG TCC TGC GTG TTG AAA GAT GAT AAG CCC ACT CTA CAG CTG GAG AGT   600
LYS ASN LEU TYR LEU SER Cys Val Leu Lys Asp Asp Lys Pro Thr Leu Gln Leu Glu SER
                            190                                             200

GTA GAT CCC AAA AAT TAC CCA AAG AAG AAG ATG GAA AAG CGA TTT GTC TTC AAC AAG ATA   660
VAL ASP PRO Lys Asn Tyr Pro Lys Lys Lys Met GLU LYS ARG PHE VAL PHE ASN Lys ILE
                            210                                             220

GAA ATC AAT AAC AAG CTG GAA TTT GAG TCT GCC CAG TTC CCC AAC TGG TAC ATC AGC ACC   720
GLU ILE ASN ASN Lys LEU GLU Phe Glu SER ALA GLN PHE PRO ASN TRP TYR ILE Ser Thr
                            230                                             240

TCT CAA GCA GAA AAC ATG CCC GTC TTC CTG GGA GGG ACC AAA GGC GGC CAG GAT ATA ACT   780
Ser Gln Ala Glu Asn Met PRO VAL PHE LEU GLY GLY Thr Lys Gly Gly Gln Asp Ile Thr
                            250                                             260

GAC TTC ACC ATG CAA TTT GTG TCT TCC TAA AGAGAGCTGTACCCAGAGAGTCCTGTGCTGAATGTCGAC   849
Asp Phe Thr Met GLN PHE VAL SER SER End
                        269

TCAATCCCTAGGGCTGGCAGAAAGGGAACAGAAAGGTTTTTGAGTACGGCTATAGCCTGGACTTTCCTGTTGTCTACAC   928

CAATGCCCAACTGCCTGCCTTAGGGTAGTGCTAAGAGGATCTCCTGTCCATCAGCCAGGACAGTCAGCTCTCTCCTTTC  1007

AGGGCCAATCCCAGCCCCTTTTGTTGAGCCAGGCCTCTCT -3'   1047
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Fig. 2 *a*, Restriction map of pIL-1-14. *Pst*I sites in brackets are those generated by the cloning procedure. Box, protein coding sequence; open box, N-terminal portion of the primary translation product removed during proteolytic processing; closed box, M_r 17,500 biologically active portion of IL-1β isolated from activated macrophage supernatants. *b*, Nucleotide and predicted amino-acid sequence of the IL-1β primary translation product. Amino acids in capitals indicate those residues confirmed by protein sequencing analysis. Numbering of nucleotides is negative for the 5′ non-coding region and positive from the presumed initiator methionine codon. Protein numbering begins at the initiator methionine. Underlined amino acids refer to the first cycle of different protein sequence analyses. Star, NH_2-terminal residue of the M_r 17,500 form of IL-1β (Ala 117). The *Hpa*II site was used in construction of plasmids used for expression of IL-1β (Fig 4, Table 2).

Methods. *a*, A cDNA library was constructed from size fractionated, polyadenylated human macrophage RNA as described in Fig. 1*a*. A 62-nucleotide probe was constructed using the amino-acid sequence data corresponding to amino acids 133–158 (see below). The probe (5′ ACTTGTTGTTCCATGTCTTGGCCTTGCAGG TGCAGGGCTTTCAGTTCGTAGGGGCCGGACAT 3′) was labelled to high specific activity with T_4 polynucleotide kinase and [γ-^{32}P]ATP and used to screen the library[35]. Positive colonies were analysed by restriction mapping using standard techniques[12]. *b*, Nucleic acid sequencing: Various restriction fragments of pIL-1-14 were cloned into M13mp18 or M13mp19 and sequenced as described in Fig. 1. Protein sequencing: amino-terminal sequencing: ~12 μg (680 pmol) unmodified IL-1β was placed in a protein sequencer (Applied Biosystems Model 470A) and analysed according to manufacturer's specifications. Phenylthiohydantoin amino acids were identified by HPLC as described previously[41]. Residues 117–123 and 125–136 were identified and tryptic peptides prepared as follows: 10.5 μg (600 pmol) IL-1β was partially digested with 250 ng trypsin for 2 h at 25 °C, reduced, carboxymethylated and injected onto a Vydac C18 Model 218TP54 4.6 × 250 mm HPLC column and fractionated by a 0.5% min^{-1} linear gradient of acetonitrile in 0.1% (v/v) trifluoroacetic acid at 0.7 ml min^{-1}. Fractions (0.7 ml) were concentrated and analysed by sequencing. Residues identified were 121–123 and 125–126; 133–158, 162–167 and 169–171. *S. aureus* V8 protease-specific peptides were obtained as follows: 10.5 μg (600 pmol) IL-1β was digested with 250 ng V8 protease for 8 h at 25 °C, reduced, carboxymethylated and fractionated as described above. Residues identified were 181–186, 200–203, 213–218 and 230–238. Fragmentation by CNBr was performed on the same sample that had been used to obtain the N-terminal sequence. After 20 cycles of NH_2-terminal sequencing, the filter in the glass block holder was removed and placed into a small beaker. The filter was saturated with 30 μl of a solution containing 15 mg CNBr in 100 μl 70% (v/v) HCOOH. The remaining solution was placed open in the beaker, the beaker sealed with Parafilm and placed in the dark. After 20 h at room temperature, the filter was dried under vacuum and placed back into the sequencer. Five simultaneous sequences were seen, differentiated from each other by their wide range of relative yields. We identified clearly residues 153–158; 161–167 and 169–171; 212–218, 220–224 and 225–226; 247–252; and 265–269. All these amino acids confirmed the sequence obtained from the cDNA.

Nucleotide sequence analysis of p2C1 showed that it contained a non-coding region of IL-1 mRNA. Therefore, nick-translated and oligonucleotide probes derived from p2C1 were used to isolate further homologous cDNA clones from the library (Fig. 1). 5′ probes from these new clones were then used to identify further cDNA clones, each able to select biologically active IL-1 mRNA (data not shown). The nucleotide sequence of IL-1 mRNA defined by the overlapping cDNA clones is shown in Fig. 1. It predicts an mRNA size of at least 2,002 nucleotides, excluding the poly(A) tail. Primer extension analysis indicated that the IL-1 mRNA extends ~15 nucleotides upstream of the sequence shown here (data not shown). A single, large open reading frame of 271 amino acids was found at the 5′ end of the mRNA encoding a protein of predicted M_r 30,606 (designated IL-1α). Although IL-1 is thought to be a secreted protein, the predicted sequence does not contain a classical N-terminal signal sequence of largely hydrophobic amino acids such as are found in other lymphokines[14–17], nor does it have any long internal hydrophobic amino-acid sequences.

While this work was in progress, Lomedico *et al.* published the cloning and sequencing of a murine IL-1 cDNA[10]. Comparison of the two predicted amino-acid sequences reveals that they are 62% homologous, suggesting that the gene cloned here is the human homologue. They have also defined the N-terminus of a subset of biologically active murine IL-1 molecules at Ser 115 (ref. 10). By matching the sequences of murine IL-1 and human IL-1α, this corresponds to Ser 113 in the human sequence. The relationship between different IL-1 molecules will be discussed below.

Cloning of IL-1β

In a separate approach to the definition of IL-1, we have reported previously the purification (from activated macrophage supernatants) of a M_r 17,500 protein with IL-1 activity[2]. We attempted to sequence this molecule to design oligonucleotide probes to identify IL-1 cDNA clones. However, amino-terminal sequence analysis of this purified protein yielded ambiguous results because of a low absolute yield in the first cycle, followed by a very sharp decline in repetitive yield. Therefore, we fragmented the molcule enzymatically. We performed a partial tryptic digest on 600 pmol protein and, after reduction and carboxymethylation, fractionated the resulting peptides by reversed-phase HPLC. A M_r 13,500 peptide was isolated in high yield. Sequence analysis of this fragment showed a continuous stretch of 26 amino acids followed by 10 identifiable residues in the subsequent 14 cycles. The 26 amino-acid stretch (Ser 133–Phe 158; Fig. 2) was used to design (based on published human codon frequencies) a synthetic, 62-base oligonucleotide probe (see Fig. 2 legend) which was ^{32}P-labelled and used to screen the size-fractionated macrophage cDNA library. Several positively

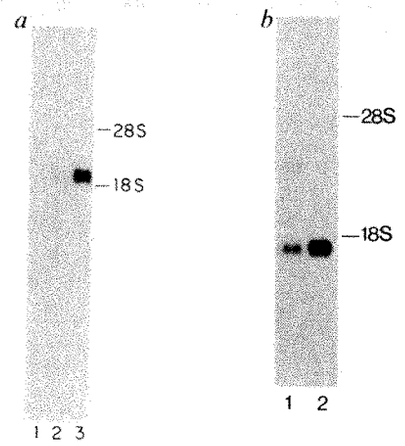


Fig. 3 Northern blot analysis of: *a*, IL-1α; *b*, IL-1β mRNA. *a*, Lane 1, 5 μg total RNA from mitogen-stimulated peripheral blood T cells; lane 2, unstimulated peripheral blood macrophages; lane 3, LPS-stimulated peripheral blood macrophages. *b*, Lane 1, 5 μg total RNA from unstimulated peripheral blood macrophages; lane 2, LPS-stimulated peripheral blood macrophages.

Methods. Total RNA was extracted from LPS-stimulated and unstimulated macrophages as in Fig. 1, and from peripheral blood T cells stimulated with the mitogens concanavalin A (20 μg ml^{-1}) and phorbol myristic acetate (10 ng ml^{-1}) as described previously[35]. RNA was electrophoresed on 1.5% agarose gels containing formaldehyde and transferred to nitrocellulose filters[12]. Blots were hybridized with ^{32}P-RNA probes synthesized by SP6 polymerase as described previously[42]. The IL-1α probe used was derived by subcloning a 1,020-bp *Pst*I/*Hin*dIII fragment from p9H into pSP64 (Promega Biotec). The IL-1β probe was derived by subcloning the insert from pIL-1-14 into the *Pst*I site of pSP64. Hybridization was for 16 h at 63 °C in Stark's buffer[43]. Blots were washed in 0.1 × SSC for 30 min at 68 °C and autoradiographed with intesifying screens. The position of large (28S) and small (18S) ribosomal RNAs indicated were determined by direct visualization of the unbaked blot with a short-wave ultraviolet lamp.

hybridizing clones were isolated, one of which, pIL-1-14, was chosen for nucleotide sequencing (Fig. 2). The clone, 1,124 base pairs (bp) long, contained the probe sequence with 12 mismatches out of 62 nucleotides and encoded the protein sequence obtained from the M_r 13,500 tryptic fragment.

The sequence of pIL-1-14 defined a single large open reading frame of 269 amino acids encoding a protein with a predicted M_r of 30,749 (Fig. 2). We found a presumptive initiator methionine codon flanked by sequences closely conforming to the consensus sequence suggested for mammalian translational initiation[18] 78 bp from the 5′ end of the pIL-1-14 cDNA sequence. The cDNA insert contained 240 nucleotides of 3′ non-coding sequence but did not extend far enough to include the polyadenylation site and poly(A) tail. Primer extension analysis indicated that the message extended ~10 nucleotides 5′ of pIL-1-14 (data not shown). The predicted amino-acid sequence of the protein encoded by pIL-1-14 (designated IL-1β) showed only limited homology to that of IL-1α (see below).

To establish the location of the M_r 17,500 form of IL-1 in the 269 amino-acid open reading frame, we sequenced a larger preparation (~680 pmol) of purified IL-1β, and could distinguish a major signal corresponding to the sequence starting at Ala 117, and which agreed with the sequence predicted from pIL-1-14 for the subsequent 20 amino acids (Fig. 2). Although a yield of only 266 pmol (39%) was obtained for the N-terminal sequencing run, no other discrete N-termini were found. These results were confirmed by additional runs on the same, and other, preparations of IL-1 protein. A similar result was reported for murine IL-1 (ref. 10). Sequence analysis of peptides generated from a *Staphylococcus aureus* V8 protease digest and from *in situ* cyanogen bromide cleavage of IL-1β (Fig. 2 legend) yielded additional confirmation of protein sequence predicted by the cDNA sequence at 96 positions of the 153 amino-acid sequence (capitalized amino acids in Fig. 2) predicted for the M_r 17,500 IL-1β, including definition of the carboxy-terminus at Ser 269. A potential *N*-glycosylation site is present at Asn 123, although there is no previous evidence that IL-1β is a glycoprotein[2]. Direct protein sequencing detected asparagine at this position, confirming the absence of carbohydrate. Thus, the M_r 17,500 form of IL-1β extends from Ala 117 to Ser 269 and represents a carboxy-terminal segment of the primary translation product. The predicted M_r of this form of IL-1β is 17,377, in close agreement with the M_r of 17,500 determined previously[2].

Similar to IL-1α, the amino-acid sequence predicted for the primary translation product of IL-1β does not contain any N-terminal or internal stretches of hydrophobic amino acids that resemble conventional secretory signal sequences.

IL-1 mRNA expression

We analysed IL-1 mRNA levels in stimulated and unstimulated macrophages by Northern blotting using IL-1α and IL-1β-specific probes (Fig. 3). In both cases, we detected a single predominant species of RNA much more abundant in LPS-stimulated than unstimulated macrophage RNA. The low levels of IL-1 RNA in unstimulated macrophages resulted from partial activation during culture as cells cultured under pyrogen-free conditions do not have detectable IL-1 RNA (K.G. and A.L., unpublished). The sizes were estimated to be 2,100–2,200 nucleotides for IL-1α mRNA and ~1,800 for IL-1β. Neither IL-1α mRNA (Fig. 3*a*) nor IL-1β mRNA (data not shown) was detectable in RNA extracted from peripheral blood T cells activated with the mitogens concanavalin A and phorbol myristic acetate, even on longer exposure of the autoradiogram.

Interestingly, IL-1β mRNA seemed to be ~10-fold more abundant than IL-1α mRNA. IL-1α cDNA clones were present at a frequency of ~1 in 1,000 in the size-fractionated cDNA library, whereas IL-1β cDNA clones were present at ~1 in 100. Given an estimated enrichment for IL-1 mRNA by agarose gel electrophoresis of ~10-fold, this would predict abundances of ~0.01% and 0.1% of the poly(A)$^+$ RNA from LPA-stimulated macrophages for IL-1α and IL-1β mRNAs, respectively.

Although IL-1α and IL-1β mRNAs are induced by the same stimulus (LPS), it is not known whether they are produced by the same cell type in the peripheral blood monocyte population. The availability of specific nucleic acid and antibody probes for IL-1α and IL-1β should clarify this issue.

Expression of IL-1α and IL-1β activity

Evidence presented here for IL-1β, and by Lomedico *et al.* for the murine homologue of IL-1α[10], suggests that the biological activity of the two IL-1 molecules resides in their C-terminal 153 and 159 amino acids, respectively. To prove this, we constructed plasmids designed to direct synthesis by *E. coli* of these portions of IL-1α and IL-1β. We used synthetic oligonucleotides that added a ribosome binding site and initiation codon to the C-terminal 159 amino acids of IL-1α (beginning at Ser 113) or to the C-terminal 153 amino acids of IL-1β (beginning at

Table 2 Expression of IL-1 biological activity

Plasmid	IL-1 in culture (U ml^{-1})	
	30 °C	43 °C
pILPα	3.2×10^3	5.1×10^6
pILPβ	4.1×10^2	1.4×10^6
pILPc	ND	ND

Cells were grown at 30 °C to an $A_{720} = 1.0$ in induction broth (M9 medium supplemented with 32 g l^{-1} tryptone, 20 g l^{-1} yeast extract and 0.4% glucose). Cultures were then grown at 43 °C for 1 h. Cells from 1 ml culture were pelleted and quick-frozen on dry ice/methanol. Before assay the cells were dissolved in 50 μl 7 M guanidine hydrochloride. IL-1 biological activity was assayed as described for Fig. 1*a*. Results are expressed as IL-1 units per ml *E. coli* culture. ND, not detected. No biological activity over background control at a sample dilution of 1 : 50 (1A5 assay).

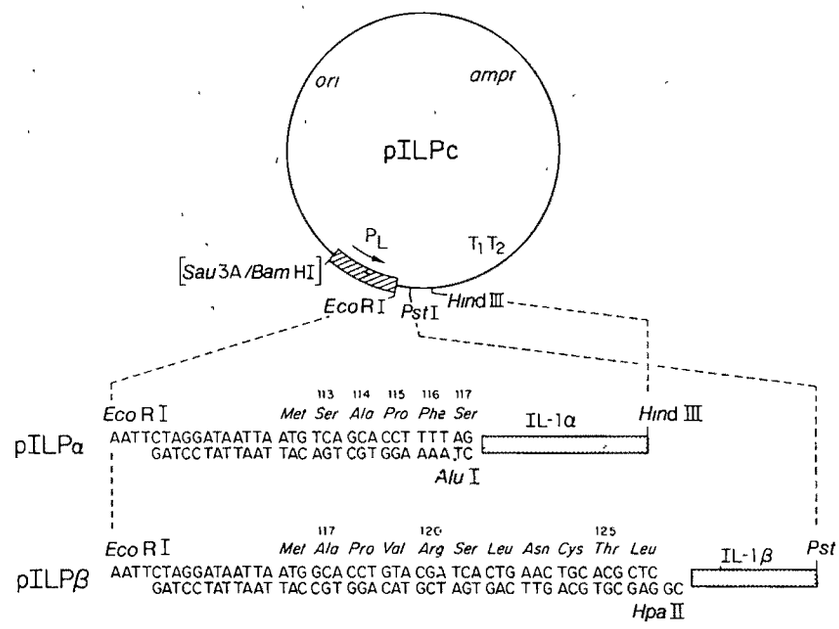


Fig. 4 The expression plasmid, pILPc, was constructed by replacing the *Bam*HI/*Eco*RI fragment of pKK223-3 (Pharmacia), containing the Tac promoter, with a *Sau*3A/*Eco*RI fragment from pPLc28 (ref. 44) that contains the λ P_L promoter. Synthetic oligonucleotides were used to provide an *E. coli* ribosome binding site, a translational initiation codon and the codons for amino acids Ser 113–Ser 117 for IL-1α or Ala 117–Leu 126 for IL-1β. To construct pILPα, the oligonucleotides were ligated together with a 720-bp pair *Alu*I/*Hin*dIII fragment into *Eco*RI/*Hin*dIII-cut pILPc. This fragment was derived from the yeast expression plasmid, pYαfIL-1α (V.P., unpublished), and specifies amino acids 118–271 of IL-1α followed by 200 bp of sequence originally derived from the 3′ non-coding region of the human *IL-2* gene. To construct pILPβ, the oligonucleotides were ligated together with a 669-bp *Hpa*II/*Pst*I fragment from pIL-1-14 into *Eco*RI/*Pst*I-cut pILPc. This fragment specifies amino acids 127–269 of IL-1β. T_1T_2, transcriptional termination sites from the rrnβ operon pILPc, pILPα and pILPβ transformed into *E. coli* strain ΔHI (ref. 45) that produces a thermolabile repressor of the P_L promoter.

Ala 117), and placed this under transcriptional control of the λ phage P_L promoter (Fig. 4). The resulting plasmids, pILPα and pILPβ, were introduced into cells containing a thermolabile repressor of P_L transcription. On heat induction, the cells produced high levels of IL-1 activity that could be extracted from the cells by 7 M guanidine hydrochloride as detected in the 1A5 conversion assay (Fig. 4, Table 2) or thymocyte co-mitogenesis assay[2] (data not shown). No IL-1 activity was found in cells transformed with a control plasmid lacking IL-1 sequences.

Interleukin-1 relationships

To determine the degree of homology between the IL-1β sequence and the related sequences for human IL-1α and murine IL-1[10], we aligned them as shown in Fig. 5*a*, with several insertion/deletion gaps to obtain the maximum number of identical residues. The amino-acid sequences for human IL-1α and murine IL-1 are closely related, having 167 out of 271 (62%) identical positions, whereas IL-1β is only distantly related to either of the other molecules, with 70 out of 271 identical positions (26%) with human IL-1α and 80 out of 270 (30%) with murine IL-1. Slightly over half of the identical positions between the IL-1 proteins occur in the M_r 17,500, C-terminal biologically active portion of the molecules, and a large proportion of these occur in the middle and C-terminal regions (Val 164–Ile 266 of IL-1α) of the M_r 17,500 forms, suggesting that these areas comprise portions of the molecules more critical for their biological activities. In contrast, very few residues are shared between the three proteins in the N-terminal third (Ser 113–Ala 163 of IL-1α) of the processed IL-1 sequences. This sequence diversity suggests that the N-terminal region of the M_r 17,500 form either is not involved in a biological role or provides an area for expression of different biological functions in each molecule.

Of the residues common to all three proteins, 47% (28 of 59) occur in the portion of the primary translation product that precedes the active M_r 17,500 IL-1 segment. In this region, 81 out of the 167 identical residues between human IL-1α and murine IL-1 occur, showing that these portions of the molecules are more homologous (81 of 112; 72%) than the M_r 17,500 IL-1 segments (86 of 159; 54%). IL-1β also has more homology with both molecules in this region. Such a degree of homology would not be expected for a non-functional portion of a protein. Therefore, it seems plausible that the N-terminal halves of the IL-1 gene products serve some as yet unknown biological function. Indeed, several biological activities have been associated with high M_r forms of IL-1[8,19], some of which may be related to the conserved N-terminal segment.

Note that although there is only 26% homology between the two human IL-1 protein sequences, significantly more occurs at the nucleic acid level (45%). Thus, it is possible that a gene duplication event gave rise to the two IL-1 molecules. The degree of divergence between the two sequences indicates that the duplication was an early event on the evolutionary scale[20].

The structural features common to all three IL-1 polypeptides are illustrated in schematic form in Fig. 5*b*. All three primary translation products have a higher degree of homology in the amino-terminal 75–80 residues. This segment (N in Fig. 5*b*) does not seem to contain a signal sequence or membrane spanning region. In fact, it has no features that would serve to distinguish it from typical globular protein sequences. Following the N-terminal domain, all three IL-1 molecules have a short section of multiple basic residues (+ in Fig. 5*b*). This segment is reminiscent of known proteolytic processing sites in prohormone precursors[21,22], maturation cleavage sites of the haemagglutinin protein of influenza virus[23] and complement component C3 (ref. 24). Although the mechanism for IL-1 processing is unknown, it is tempting to speculate that this region serves as a proteolytic processing site to yield an intermediate polypeptide from which biologically active fragments are produced by further proteolytic cleavage. The segment following the multiple basic residues is less conserved in the three molecules, but in all cases, this segment (− in Fig. 5*b*) contains many acidic amino acids. No basic residues are found in this region, except just before the amino terminus of the active IL-1 segments. Such a negatively charged region, present in all three molecules, probably has a conserved structural or functional role. The rest of the sequence comprises the processed active M_r 17,500 IL-1 region.

Discussion

We have isolated cDNA clones representing two distinct but distantly related forms of human IL-1, IL-1α and IL-1β, from LPS-stimulated macrophage RNA. Although the nucleotide sequence of the cDNA clones predicts primary translation products of M_r 30,606 and 30,749 for IL-1α and IL-1β, respectively, we have shown that the C-terminal 159 and 153 amino acids are sufficient for the production of IL-1 biological activity. Previous reports have characterized IL-1 activities as having

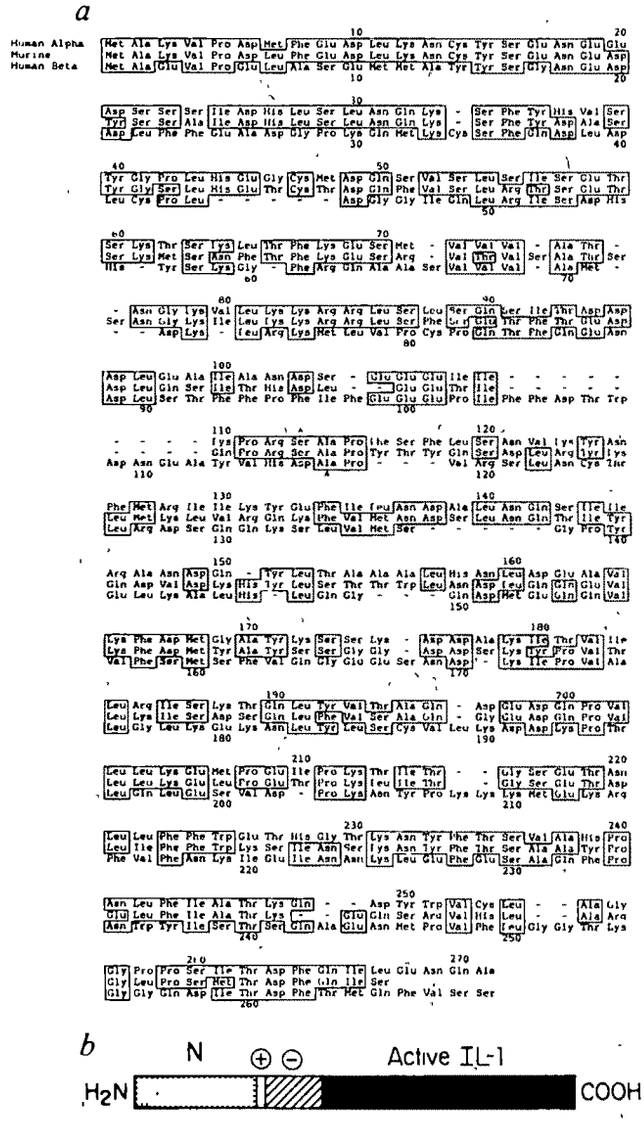


Fig. 5 *a*, Alignment of human IL-1α, murine IL-1[10] and human IL-1β amino-acid sequences. Numbering begins at the presumed initiator methionine; numbering above the top row is for IL-1α; that below the bottom row is for IL-1β. Boxed residues indicate identities between two or more of the sequences. Star above Ser 113, presumed NH_2-terminus of IL-1α; star below Ala 117 of IL-1β NH_2-terminus as defined by protein sequencing. Alignment presented was determined by a combination of computerized methods[20,46], hydrophilicity analysis[47] and visual inspection. *b*, Schematic representation of the common features of IL-1 polypeptides. Stippled box (N), N-terminal conserved region from Met 1 to Gly 78 in IL-1α, or to Asp 72 in IL-1β. Small open box (+), multiple basic region (Lys 79–Arg 85 in IL-1α, or Lys 73–Lys 76 in IL-1β). Hatched box (−), negatively charged region (Leu 86–Arg 112 in IL-1α, or Met 77–Asp 116 in IL-1β). Closed box ('Active IL-1'), C-terminal region of the primary translation product representing the extent of the processed, active M_r 17,500 IL-1 (Ser 113–Ala 271 for IL-1α; Ala 117–Ser 269 for IL-1β).

M_rs of ~30,000 and 17,000. Our results resolve this inconsistency and support the concept that human IL-1s are synthesized as higher M_r precursors that are proteolytically cleaved to M_r 17,500 active forms, as has been proposed for murine IL-1[8]. However, we have not yet defined all of the naturally occurring, biologically active forms of IL-1α and IL-1β; this will require the generation of specific antisera against these molecules.

An interesting difference between IL-1α and IL-1β is that the primary translation product of IL-1α seems to be biologically active, judged by the production of IL-1 activity by hybrid-selected IL-1α mRNA in a rabbit reticulocyte lysate (Table 1). In contrast, hybrid-selected IL-1β mRNA was not biologically active under the same conditions, even though a protein corresponding to the IL-1β primary translation product could be seen clearly when the hybrid-selected IL-1β mRNA was translated in a reticulocyte lysate supplemented with ^{35}S-methionine and analysed on an SDS-polyacrylamide gel (data not shown). It is also possible that the primary translation product of IL-1α is more efficiently cleaved to a smaller active form. This suggestion is supported by the presence of a basic amino acid immediately before Ser 113 that might be a cleavage site for a trypsin-like protease.

Unlike other secreted proteins, IL-1α and IL-1β do not contain a stretch of hydrophobic amino acids, either at the N-terminus or internally, raising the possibility that IL-1 is not actively secreted by macrophages, but is released by damaged cells[25]. This conclusion is supported by the inefficient release of IL-1 activity by *Xenopus* oocytes injected with IL-1 mRNA or by COS-7 monkey kidney cells transfected with IL-1 expression vectors (data not shown). Alternatively, macrophages may export IL-1 by a mechanism distinct from that described for other secretory proteins.

Recently, Auron *et al.* reported the sequence of a cDNA clone that is very similar to IL-1β[11]. There are seven nucleotide substitutions between the two sequences, five of which are silent changes. Plasmid pIL-1-14 has an adenine instead of a guanine at nucleotide 16, changing lysine to glutamic acid, and lacks the upstream, out of frame, potential initiation codon found in the sequence of Auron *et al.* (position −23 in Fig. 2). All the nucleotide substitutions in our sequences have been verified by sequencing other IL-1β cDNA clones, and presumably represent allelic differences or reverse transcriptase errors. Van Damme *et al.* have recently described the N-terminal amino acid sequence of a secreted factor with strong homology to the predicted amino acid sequence of the central region of the IL-1β cDNA clone[48].

It is striking that two distinct molecules should mediate the induction of IL-2 synthesis. Whether they share all the biological activities previously attributed to IL-1 is under investigation. It will now be possible to determine if both IL-1α and IL-1β bind to the same receptor, analogous to the binding of the structurally distinct epidermal growth factor (EGF) and transforming growth factor-α molecules to the EGF receptor[26]. Resolution of these issues should increase our understanding of the biological role of IL-1 in immunity and inflammation.

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LETTERS TO NATURE

Observation of highly excited radio recombination lines towards Cassiopeia A

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The absorption line at 26 MHz observed by Konovalenko and Sodin[1] towards Cas A was interpreted by Blake *et al.*[2] as the 631α recombination line due to carbon or heavier elements. This was confirmed subsequently by Konovalenko and Sodin[3], who detected the 640α line at the same optical depth as the 631α line, within error limits. These lines represent the highest excited states yet detected in interstellar gas and could provide[4] a unique diagnostic of conditions in diffuse clouds if observed over a range of frequencies. We now report new observations of these lines carried out near 26, 38, 52 and 68 MHz using the National Radio Astronomy Observatory (NRAO) 91-m telescope at Green Bank. We detected absorption lines at all these frequencies, with a striking fourfold increase in width towards low frequencies due to collisional or radiation broadening. Assuming that these are recombination lines of carbon, the observed radial velocity corresponds well with that of the H I, OH, NH_3 and H_2CO absorption features that originate in the Perseus arm in the same direction[5-8].

The 68- and 52-MHz observations were made in July 1984, using the 50-80-MHz tunable dipole on the travelling feed system. The 38- and 25-MHz observations were made in August 1984 using a broadband 20-50-MHz dipole especially made for this experiment and mounted on the travelling feed, which is capable of tracking a source for 76 sec δ minutes, where δ is the declination. In the frequency range of these observations, the system temperature was dominated by Cas A and the galactic background. At each of the four frequencies, two or three nearby transitions in carefully chosen interference-free bands were observed simultaneously. The 384-channel autocorrelation receiver was split into four independent spectrometers of 96 channels each, providing a frequency resolution of 0.98 kHz at the two higher and 0.49 kHz at the two lower frequencies. Reference spectra were measured by switching to a noise source which was adjusted to match the total system temperature when the antenna was pointed to Cas A.

Table 1 Observed line parameters

Frequency (MHz)	V_{LSR} (km s^{-1})	T_L/T_C ($\times 10^3$)	ΔV (km s^{-1})	$\int \tau\, d\nu$ (s^{-1})
26	−45±5	−3.8±0.5	32.9±2.1	11.6±0.7
38	−49±2	−3.5±0.4	17.9±0.9	8.4±0.5
52	−49±2	−3.5±0.4	13.0±0.7	8.6±0.5
68	−48±2	−2.9±0.6	8.3±0.9	5.9±0.8

Errors quoted are 1σ values. V_{LSR} is the radial velocity with respect to the local standard of rest, T_L and T_C are the line and continuum antenna temperatures respectively; ΔV is the full linewidth at half maximum intensity; $\int \tau\, d\nu$ is the optical depth integrated over the line.

The spectra obtained after removing a linear baseline at each of the four frequencies are shown in Fig. 1. The recombination line is seen in absorption at all the four frequencies at the velocity corresponding to the Perseus arm. In the spectra in Fig. 1 there are suggestions, at least at the three lower frequencies, that the absorption line may be present at the velocities of both −47 and −39 km s^{-1} at which H I absorption is seen in the Perseus arm[5]. However, we find no evidence in our spectra of a line at 0 km s^{-1} corresponding to the Orion arm absorption, and no hydrogen recombination lines. The line parameters obtained by fitting a single gaussian to each of the observed spectra are given in Table 1. As Cas A dominates the system temperature at these frequencies, the line-to-continuum ratio plotted in Fig. 1 and given in Table 1 is approximately equal to the observed optical depth.

All high-frequency observations (mainly towards H II regions) show recombination lines of hydrogen or heavier elements only in emission. However, there are good reasons for expecting recombination lines to be seen in absorption at very low frequencies particularly from cold gas[2] and in the presence of strong background radiation. At very large n (the principal quantum number) electron collisions dominate (as among free electrons) and therefore maintain thermalization of the populations. It is only at lower n (and correspondingly higher frequencies) that radiative processes begin to dominate and the excitation temperature moves away from the kinetic temperature of

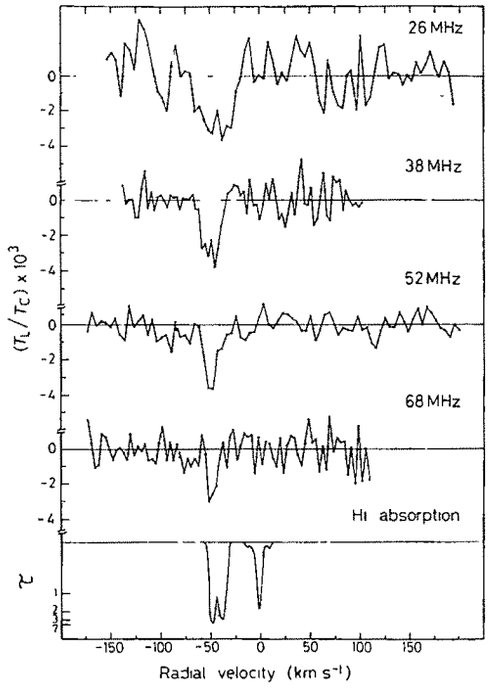


Fig. 1 Recombination line spectra towards Cas A observed at four frequencies At each frequency the spectrum is an average of two or three nearby transitions that were observed simultaneously. These transitions are 26 MHz, 629α and 634α; 38 MHz, 554α and 556α; 52 MHz, 497α, 501α and 503α; 68 MHz, 456α, 457α and 458α. The effective integration times for the four frequencies are 4.4, 10.7, 10.5 and 9.6 h respectively. The H I absorption spectrum is from ref. 5.

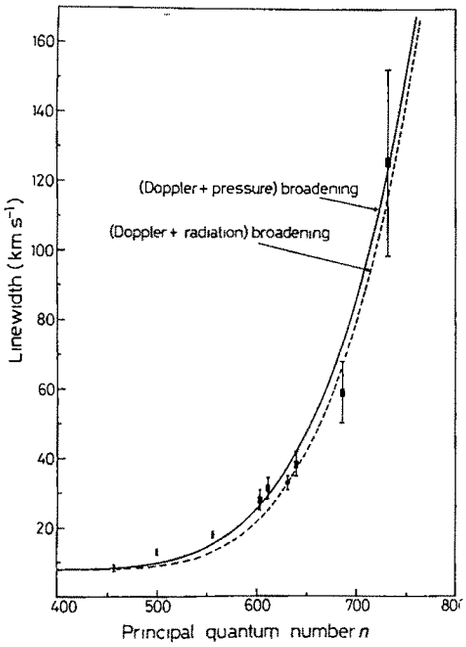


Fig. 2 Calculated and observed variation of line width (FWHM). ●, Present observations; ■, data from ref. 12. Pressure broadening is calculated for $T_e = 50$ K and $N_e = 0.18$ cm^{-3} and for radiation broadening a brightness temperature $T_R = 2{,}000$ K at 100 MHz ($\alpha = -2.6$) was used. A Doppler component of 8 km s^{-1} is assumed for both cases. The relevant formulas are taken from ref. 13.

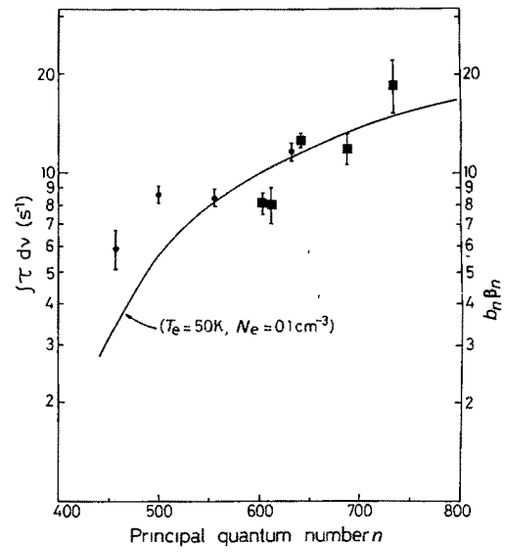


Fig. 3 The observed variation of integrated line intensity. The quantity $b_n\beta_n$ (solid curve) taken from ref. 9 is normalized to a value of 10 at $n = 600$. This quantity which is proportional to the net optical depth was calculated by Walmsley and Watson[9] based on a dielectronic recombination process. ●, Present observations; ■, data from ref. 12.

the gas, towards negative values, so that the line can never be seen in absorption irrespective of the background radiation. The turnover frequency will, however, depend on the density and temperature of the gas and the populating mechanism[9] for the species of atom in question.

As pointed out by Blake *et al.*[2], the identification of the heavy element responsible for the line towards Cas A cannot be made on the basis of frequency alone. It is most likely to be carbon if it originates in cold gas. Alternatively, it could be due to heavier elements such as calcium or magnesium if located in hot gas[2,10,11]. In the present case, however, the agreement in velocity with the H I and molecular absorption features strongly favours cold dense clouds as the site of absorption, and carbon as the element in question.

The observed width of the line at 68 and 52 MHz is similar to that of the H I absorption line. The width, in velocity units, increases by nearly a factor of four at 26 MHz. This line has now been observed down to 16.7 MHz by Konovalenko[12] and the width increases even more dramatically (Fig. 2), which clearly indicates pressure and/or radiation broadening. Using the formula given by Shaver[13] for the collisional width at low temperatures we infer an upper limit of 0.2 cm^{-3} for the electron density of the gas responsible for the observed lines, assuming a temperature of 50 K. If the carbon in this cloud has a cosmic abundance ($C/H = 3 \times 10^{-4}$) and all of it is ionized, then this implies an upper limit to the hydrogen density of 600 cm^{-3}. This is consistent with the upper limit to the H I density obtained from the measured column density and the minimum size of the cloud implied by its high H I optical depth. From consideration of radiation broadening[13], we infer an upper limit of 2,000 K (at 100 MHz) for the ambient brightness temperature for the recombination line gas (see Fig. 2). This further implies a lower limit of 150 pc for the distance of the absorbing gas from Cas A, if we take the flux of Cas A at 100 MHz to be 15,000 Jy (ref. 14) and its distance and linear size to be 4 kpc and 5 pc, respectively.

At temperatures of 50–100 K, which are typical for H I clouds, calculations by Walmsley and Watson[4,9] show that dielectronic-like recombination on to carbon could be an important populating mechanism. This predicts an enhanced absorption at low frequencies and a variation of line strength as a function of frequency which depends on the electron density in the gas. At low electron densities ($N_e \sim 0.01$ cm^{-3}) a steep decrease in line strength from 25 to 100 MHz is predicted. From Table 1, although the observed peak optical depth in the line remains nearly constant in the frequency range 68–26 MHz, the integrated absorption ($\int \tau \, d\nu$) increases roughly by a factor of 2. If the dielectronic recombination process is operating, then the observed variation of the total line strength indicates an electron density of ~0.1 cm^{-3} (see Fig. 3) and rules out much lower densities.

Alternatively, these lines may be formed by carbon associated with dense molecular clouds rather than by carbon associated with more widely distributed H I. Batrla *et al.*[7] have observed two such clouds in H_2CO absorption against Cas A, each with a diameter of about 1 arc min and with velocities of −39 and −47 km s^{-1} respectively. These are foreground clouds in the Perseus arm which cover only a few per cent of the solid angle subtended by Cas A. If the low-frequency absorption lines are formed in the molecular clouds, then the true optical depths of these lines would be 20–30 times higher than the average optical depths that we observe.

It must be possible to observe these highly excited lines in many other directions in the galactic plane. At these low frequencies, the non-thermal galactic radiation can provide the background continuum and the presence of a discrete background source may not be necessary for the detection of the lines. The present observations have indicated that recombination lines below 100 MHz will be an important diagnostic of the interstellar medium as was emphasized by Shaver[13].

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Analytical solution for the effect of increasing CO_2 on global mean temperature

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Increasing atmospheric carbon dioxide concentration is expected to cause substantial changes in climate[1]. Recent model studies suggest that the equilibrium warming for a CO_2 doubling ($\Delta T_{2\times}$) is about 3–4 °C[2–4]. Observational data show that the globe has warmed by about 0.5 °C over the past 100 years[5,6]. Are these two results compatible? To answer this question due account must be taken of oceanic thermal inertia effects, which can significantly slow the response of the climate system to external forcing. The main controlling parameters are the effective diffusivity of the ocean below the upper mixed layer (κ) and the climate sensitivity (defined by $\Delta T_{2\times}$). Previous analyses of this problem have considered only limited ranges of these parameters. Here we present a more general analysis of two cases, forcing by a step function change in CO_2 concentration and by a steady CO_2 increase. The former case may be characterized by a response time which we show is strongly dependent on both κ and $\Delta T_{2\times}$. In the latter case the damped response means that, at any given time, the climate system may be quite far removed from its equilibrium with the prevailing CO_2 level. In earlier work this equilibrium has been expressed as a lag time, but we show this to be misleading because of the sensitivity of the lag to the history of past CO_2 variations. Since both the lag and the degree of disequilibrium are strongly dependent on κ and $\Delta T_{2\times}$, and because of uncertainties in the pre-industrial CO_2 level, the observed global warming over the past 100 years can be shown to be compatible with a wide range of CO_2-doubling temperature changes.

We use an energy balance climate model consisting of two atmospheric boxes, one over land with zero heat capacity, and the other over an oceanic mixed layer coupled to a diffusive deeper ocean (compare refs 7–9). On timescales relevant to CO_2 forcing, the atmosphere may be assumed to be in equilibrium with the underlying surface. The model is then described by

$$\Delta T_L = \Delta T_{aL} = \frac{f\lambda \Delta T_i + k\Delta T_{aO}}{f\lambda + k} \quad (1)$$

$$\Delta T_{aO} = \frac{(f(1-f)\lambda + k)\lambda \Delta T_i + k_{as}(1-f)(f\lambda + k)\Delta T}{(f(1-f)\lambda + k)\lambda + k_{as}(1-f)(f\lambda + k)} \quad (2)$$

$$C_m \frac{d\Delta T}{dt} = \Delta Q - \lambda \Delta T - \Delta F \quad (3)$$

where ΔT_L, ΔT_{aL}, ΔT_{aO} and $\Delta T(t)$ are temperature changes for the land, atmosphere over land, atmosphere over ocean and oceanic mixed layer induced by a change in thermal forcing $\Delta Q(t)$, f is the fraction of the globe covered by land ($f \approx 0.29$), λ is the climate feedback parameter[10], k is a land-ocean heat transfer coefficient, k_{as} is an air-sea heat exchange coefficient, and $\Delta T_i(t)$ is the instantaneous equilibrium temperature change

$$\Delta T_i = \frac{\Delta Q}{\lambda} \approx \Delta T_{2\times} \frac{\ln(C/C_0)}{\ln 2} \quad (4)$$

for CO_2 change from C_0 to $C(t)$ (ref. 11). In equation (3), C_m is the effective bulk heat capacity ($C_m = \gamma \rho c h$) where

$$\gamma = \frac{(1-f)(k+\lambda f)}{k+\lambda f(1-f)} + \frac{\lambda}{k_{as}} \quad (5)$$

and h is the depth of the mixed layer in which the density and specific heat capacity are ρ and c respectively. ΔF is the change in heat flux at the bottom of the mixed layer ($z = 0$)

$$\Delta F = -\gamma \rho c \kappa \left(\frac{\partial \Delta T_0}{\partial z}\right)_{z=0} \quad (6)$$

where κ is the thermal diffusivity of the deep ocean ($z > 0$) whose temperature change due to ΔQ is $\Delta T_0(z, t)$. z is depth below the bottom of the mixed layer. ΔT_0 is determined by

$$\frac{\partial \Delta T_0}{\partial t} = \kappa \frac{\partial^2 \Delta T_0}{\partial z^2} \quad (7)$$

With the initial condition $\Delta T_0(z, 0) = 0$ and the boundary conditions $\Delta T_0(0, t) = \Delta T(t)$ and $\Delta T_0(z, t) \to 0$ as $z \to \infty$, the solution to equation (7) can be approximated to give

$$\Delta F = \gamma \mu \rho c h \frac{\Delta T}{\sqrt{\tau_d t}} \quad (8)$$

where $\tau_d = \pi h^2/\kappa$ is a characteristic time for exchange between the mixed layer and the deeper ocean; τ_d varies between 5 and 10 yr for realistic values of h and κ. The constant μ can be determined by comparing results from an analytical solution of equation (9) below, with those from a full numerical integration of the coupled equations (3), (6) and (7). The value of μ depends to some degree on the assumed forcing ($\Delta Q(t)$), but is insensitive to variations in κ, λ and h. The validity of this approximation is discussed below (see Table 1).

Since $\lambda \ll k_{as}$ (ref. 10), $\Delta T_{aO} \approx \Delta T$ in accord with observations (ref. 6), and the λ/k_{as} term in the expression for γ may be ignored. Two limiting cases give the bounds on γ. For $k = 0$ (no land-sea heat exchange), $\gamma = 1$, which also corresponds to an ocean-covered Earth. For $k \to \infty$ (infinitely fast land-sea heat exchange), $\gamma = 1 - f \approx 0.71$, which corresponds to the fraction of the globe covered by ocean. For realistic values of λ and k, γ lies between 0.72 and 0.75.

Substitution of equation (8) into equation (3) gives an ordinary differential equation describing $\Delta T(t)$:

$$\gamma \frac{d\Delta T}{dt} + \Delta T \left\{\frac{1}{\tau_f} + \frac{\mu\gamma}{\sqrt{\tau_d t}}\right\} = \frac{\Delta Q}{\rho c h} \quad (9)$$

where $\tau_f = \rho c h/\lambda$ is the response time for step-function forcing of a thermally isolated mixed layer with no land-sea heat exchange, that is, $\Delta F = 0$ and $\gamma = 1$. τ_f ranges between 4 and 13 yr for realistic values of h and λ.

Equation (9) can be solved exactly for a number of different forms of the forcing function ΔQ. We will consider $\Delta Q = a$, giving the transient response to a step-function CO_2 change (the 'switch-on' experiment[12]), and $\Delta Q = bt \exp(\alpha t)$; a, b and α are arbitrary constants. Since the radiative response is approximately proportional to the logarithm of the concentration (ref. 11 and equation (4) above), $\alpha = 0$ corresponds to an exponentially increasing CO_2 concentration while $\alpha \neq 0$, for appropriate b and α, corresponds closely to observed changes of CO_2 over the past 130 yr.

For the switch-on experiment, ΔT tends to ΔT_e for large t where

$$\Delta T_e = a/\lambda \tag{10}$$

The solution to equation (9) is then

$$\frac{\Delta T}{\Delta T_e} = 1 - e^{-W} - 2G \tag{11}$$

where $G = G(Z, N) = N[D(Z+N) - e^{-W}D(N)]$, $W = W(Z, N) = Z^2 + 2NZ$, $Z^2 = t/(\gamma\tau_f)$ and $N^2 = \gamma\mu^2\tau_f/\tau_d$. In $G(Z, N)$, D is Dawson's integral[13], $D(z) = e^{-z^2}\int_0^z e^{u^2}\,du$. The parameter N depends on μ which takes the value 1.4 for step-function forcing.

The switch-on case is important because is has been used by a number of investigators to study the transient response characteristics of the climate system. The speed of response, and the influence of oceanic thermal inertia, can be characterized by the e-folding time, τ_e, defined by $\Delta T(\tau_e) = (1 - e^{-1})\Delta T_e$. If $Z_e^2 = \tau_e/(\gamma\tau_f)$, then τ_e is determined by

$$e^{-W_e} + 2G(Z_e, N) = e^{-1} \tag{12}$$

For large N the solution is $Z_e \approx (e-1)N$ (using $D(u) \sim 1/2u$), an excellent approximation (error in Z_e less than 5%) for $N > 1.5$, that is, for almost all values of N likely to be encountered. Hence

$$\tau_e \approx \gamma^2\mu^2(e-1)^2(\tau_f)^2/\tau_d \approx 47.8\gamma^2\mu^2\kappa/\lambda^2 \tag{13}$$

for κ in $cm^2\,s^{-1}$ and λ in $Wm^{-2}\,K^{-1}$. If we consider the case of a step-function doubling of CO_2 concentration, then (from equation (10)) $\lambda = \Delta Q_{2\times}/\Delta T_{2\times}$ where $\Delta Q_{2\times} \approx 4.3\ Wm^{-2}$ (ref. 3). Since $\mu = 1.4$, equation (13) becomes

$$\tau_e \approx 5.08\gamma^2\kappa(\Delta T_{2\times})^2 \tag{14}$$

τ_e is independent of the mixed layer depth, h. This analytical result can be compared with the numerical calculations of Hansen *et al.*[3]. For $\kappa = 1\ cm^2\,s^{-1}$ and $\Delta T_{2\times} = 4.2, 3.0, 2.0\,°C$, these authors obtain $\tau_e = 102, 55, 27$ yr using $\gamma = 1$. Equation (14) gives $\tau_e = 90, 46, 20$ yr, in close agreement. For $\gamma = 0.75$ (see above), τ_e ranges between 6 and 174 yr for $\kappa = 1$–$3\ cm^2\,s^{-1}$ and $\Delta T_{2\times} = 1.5$–$4.5\,°C$. There is thus a considerable range of uncertainty in τ_e resulting from uncertainties in the magnitude of the CO_2-doubling temperature change and in sub-mixed-layer mixing.

The e-folding time demonstrates that oceanic thermal inertia has a significant effect in determining the response of the climate system to external forcing. However, the results of a switch-on experiment are transferable to the case of steadily increasing CO_2 concentration only through a convolution integral[4]. We therefore consider the 'continuous increase' experiment with time-dependent forcing.

Forcing of the form $\Delta Q = bt\exp(\alpha t)$ corresponds to CO_2 variations given by

$$C(t) = C_0\exp(Bt\,e^{\alpha t}) \tag{15}$$

where C_0 is the initial, pre-industrial concentration and $B = b\ln 2/\Delta Q_{2\times}$. Observed CO_2 changes can be fitted to this form. For example, if $C_0 = 270$ parts per million by volume (p.p.m.v.) in 1850[14], and C = 315 p.p.m.v. in 1958 and 338 p.p.m.v. in 1980[15], then $B = 5.59\ 10^{-4}$ and $\alpha = 8.69\ 10^{-3}\ yr^{-1}$.

The solution to equation (9) for $\Delta Q = bt\exp(\alpha t)$ is

$$\frac{\Delta T}{\Delta T_i} = \frac{\gamma\tau_f}{\beta^2 t}[\tilde{Z}^2 - \tilde{N}\tilde{Z} - (1-\tilde{N}^2)(1-e^{-\tilde{W}}) + (3-2\tilde{N}^2)\tilde{G}] \tag{16}$$

where ΔT_i is the instantaneous equilibrium temperature change at time t (equation (4)), $\beta = 1 + \alpha\gamma\tau_f$, $\tilde{Z}^2 = \beta Z^2$, $\tilde{N}^2 = N^2/\beta$, $\tilde{W} = W(\tilde{Z}, \tilde{N})$ and $\tilde{G} = G(\tilde{Z}, \tilde{N})$. The case $\beta = 1$ (that is, $\alpha = 0$, $\tilde{W} \equiv W$, $\tilde{G} \equiv G$) corresponds to linear thermal forcing due to an exponentially increasing CO_2 concentration. The departure of $\Delta T/\Delta T_i$ from unity is a measure of the degree of disequilibrium due to oceanic thermal inertia effects.

Since the initial CO_2 level and the history of past CO_2 variations appear in equation (16) only through the α term in β, and since β is always close to 1, the degree of disequilibrium must be insensitive to the details of past CO_2 changes. This is demonstrated in Table 1. Insensitivity to the initial CO_2 level can be seen by comparing $\Delta T/\Delta T_i$ values for various values of C_0, and insensitivity to the CO_2 history for given C_0 can be seen by the proximity of $\Delta T/\Delta T_{lin}$ (where ΔT_{lin} corresponds to ΔT for $\alpha = 0$) to unity. The degree of disequilibrium, however, does vary markedly with κ and $\Delta T_{2\times}$.

Equation (16) implies that the response $\Delta T(t)$ lags behind the instantaneous equilibrium response by a time-varying amount L which can be defined by

$$\Delta T(t) = \Delta T_i(t - L) \tag{17}$$

It can easily be shown that

$$\frac{\Delta T(t)}{\Delta T_i(t)} = \left(1 - \frac{L}{t}\right)e^{-\alpha L} \tag{18}$$

so that, for $\alpha \geq 0$, L is a maximum in the linear forcing case. For this case ($\alpha = 0$) $L(= L_{lin})$ can be related to the switch-on e-folding time τ_e (equation (14)) by substituting equation (16) into (18) and using $D(u) \sim 1/2u$:

$$L_{lin} \approx 0.91\sqrt{t\tau_e} - 0.84\tau_e(1 - 0.91\xi) + \gamma\tau_f(1 - 1.37\xi) \tag{19}$$

where $\xi = (0.91 + \sqrt{t/\tau_e})^{-1}$. L_{lin} is therefore independent of the initial CO_2 level and the timescale for CO_2 increase (see Table 1).

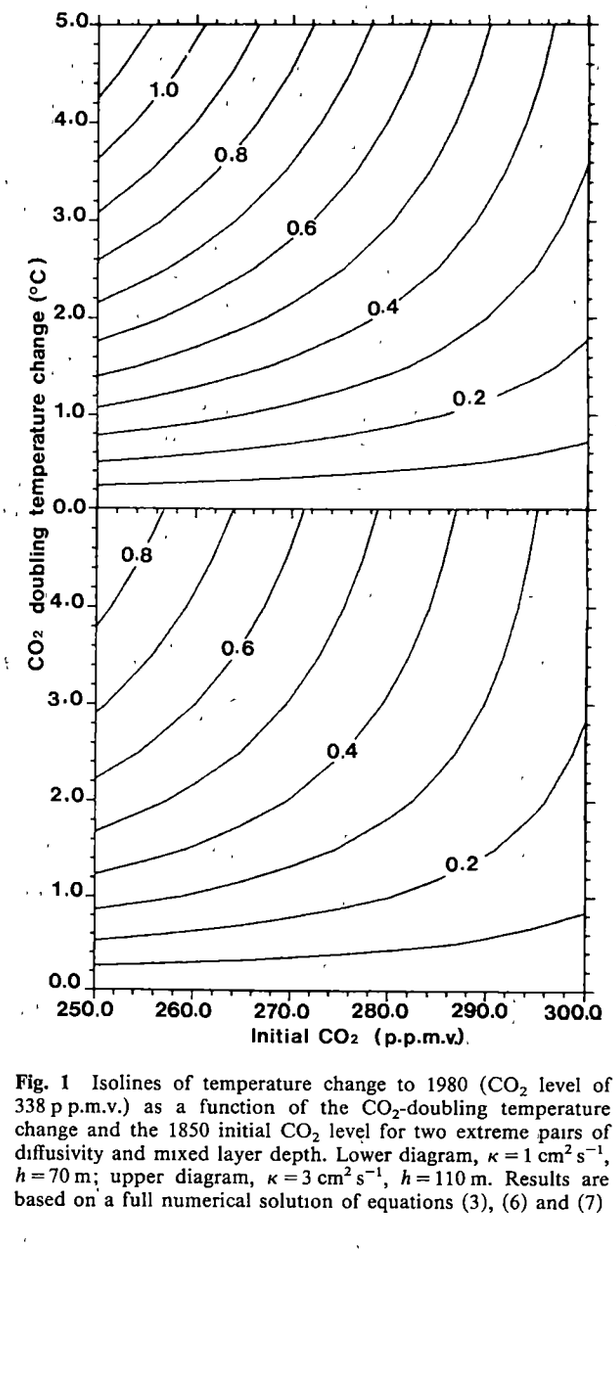


Fig. 1 Isolines of temperature change to 1980 (CO_2 level of 338 p p.m.v.) as a function of the CO_2-doubling temperature change and the 1850 initial CO_2 level for two extreme pairs of diffusivity and mixed layer depth. Lower diagram, $\kappa = 1\ cm^2\,s^{-1}$, $h = 70$ m; upper diagram, $\kappa = 3\ cm^2\,s^{-1}$, $h = 110$ m. Results are based on a full numerical solution of equations (3), (6) and (7)

For $\alpha \neq 0$, however, the lag is strongly dependent on α and C_0, the parameters characterizing the history of past CO_2 variations. The sensitivity to α can be seen by comparing L with L_{lin} in Table 1, while the sensitivity to C_0 can be seen directly in this table. Because of this, and because C_0 is uncertain[14], the lag time is not a particularly good parameter to use as an indicator of oceanic thermal inertia effects. Table 1 also shows that L is strongly dependent on κ and $\Delta T_{2\times}$, the values of which are also uncertain.

The most important result shown in Table 1 is the wide range of uncertainty in the disequilibrium ratio, $\Delta T/\Delta T_1$, 0.42 to 0.79 for $C_0 = 270$ p.p.m.v. $\Delta T/\Delta T_1$ decreases as $\Delta T_{2\times}$ increases, in accord with the results of Siegenthaler and Oeschger[9] (who used $\Delta T_{2\times} = 2.0$ °C and 3.2 °C with $\kappa = 1$ cm^2 s^{-1}), and as κ increases, in accord with Hansen *et al.*[3] (who used $\kappa = 1$ and 2 cm^2 s^{-1} with $\Delta T_{2\times} = 4.2$ °C). Hansen *et al.*'s values of $\Delta T/\Delta T_1$ are noticeably less than ours because they used $\gamma = 1$ If the climate sensitivity is as high as recent model results suggest (refs 2-4) then $\Delta T/\Delta T_1 \approx 0.5$ and a substantial future warming must occur even in the absence of further CO_2 increases, a point already noted by Hansen *et al.*[3] and by Siegenthaler and Oeschger[9].

The overall uncertainty is further illustrated in Fig. 1 which shows $\Delta T(1980)$ as a function of initial CO_2 level and CO_2-doubling temperature change. The warming depends strongly on the initial CO_2 level. ΔT values in Fig 1 are compatible with the observed global mean warming of around 0.5 °C over the past 100 yr, especially when one considers the uncertainties in the observational record[5,6]. For $260 < C_0 < 280$ p.p m.v. (ref. 14) and $1.5 < \Delta T_{2\times} < 4.5$ °C (ref. 1), ΔT ranges between 0.26 and 0.96 °C. If observed temperature changes in the range 0.4–0 6 °C were to be explained solely in terms of CO_2 changes then, for $260 < C_0 < 280$ p.p.m.v., the implied $\Delta T_{2\times}$ range is 1.3–11.5 °C. A consideration of transient response effects, therefore, helps to remove the apparent conflict noted in ref. 16 between recent model results[2-4] and observations[5,6] (a point noted by Hansen *et al.*[3]). The high upper limit to the implied $\Delta T_{2\times}$ value (that is, 11.5 °C, corresponding to $\Delta T = 0\,6$ °C, $C_0 = 280$ p.p.m.v., $\kappa = 3$ cm^2 s^{-1}, $h = 110$ m) requires comment. $\Delta T_{2\times}$ affects $\Delta T(t)$ in two competing ways; through $\Delta T_1(t)$, which increases with increasing $\Delta T_{2\times}$, and through the disequilibrium ratio, $\Delta T/\Delta T_1$, which decreases as ΔT_{2x} increases. Therefore, as Fig. 1 shows, the 1980 value of $\partial \Delta T/\partial \Delta T_{2\times}$ becomes small for large $\Delta T_{2\times}$ (particularly for large κ), so that $\Delta T(1980)$ can be quite insensitive to changes in $\Delta T_{2\times}$. The 11.5 °C upper limit for $\Delta T_{2\times}$ is in this insensitive region, but its value is sensitive to C_0 and κ, and to the chosen history of CO_2 variations. Thus, it is the qualitative conclusion that a large value of $\Delta T_{2\times}$ could be consistent with observed ΔT values which is important, rather than the specific numerical value obtained here.

An additional source of uncertainty becomes evident retrospectively from the analytical solution. Because of the lag in the response due to the thermal inertia of the oceans, we can be fairly sure that the climate system was not initially in equilibrium as we have assumed in our calculations. The subsequent warming does depend on whether the initial global-mean temperature was above or below the equilibrium temperature, that is on the thermal forcing history before 1850. In the absence of information about the degree or even direction of the initial disequilibrium, we must neglect this confounding issue.

We have considered only CO_2 in defining the thermal forcing function $\Delta Q(t)$. Recent increases in the concentrations of other trace gases (such as methane and chlorofluorocarbons) may have added noticeably to the forcing[3,17], contributing perhaps as much as 0.2 °C to the overall warming since 1960[18]. The CO_2 contribution to the observed global mean warming of 0.4–0.6 °C over the past 100 yr would therefore be only 0.2–0.4 °C. For $260 < C_0 < 280$ p.p.m.v., this implies a $\Delta T_{2\times}$ range of only 0.6–3.1 °C (see Fig. 1). Recent ice-core evidence, however, suggests that the 260–280 p.p.m.v. initial CO_2 range may be too low. Neftal *et al.*[19] give new data with concentrations around 290 p.p.m.v. in the late nineteenth century. Initial levels of 270 and 290 p.p.m.v give $\Delta T(1980)$ values which differ by about 0.2 °C, largely compensating for the trace gas effect. This higher initial CO_2 level may, therefore, be necessary in order to ensure compatibility between model results and observations.

These results cannot be construed as proof of the detection of CO_2 effects on climate, although they do help to bring model results more in line with observations. Detection requires a statistically significant warming above the noise level of natural interannual and interdecadal temperature fluctuations[20,21], together with a convincing attribution of at least part of such warming to carbon dioxide. These statistical aspects, together with problems of data reliability and representativeness, and our present inadequate understanding of the processes other than CO_2 that can cause global climate change, make it impossible to attribute the observed warming unequivocally to CO_2. In fact, transient response effects make detection even more difficult because, by lowering the signal expected at any given time, the signal-to-noise ratio is correspondingly lowered. The insensitivity of $\Delta T(1980)$ to $\Delta T_{2\times}$ for large $\Delta T_{2\times}$ means that it may be very difficult to determine $\Delta T_{2\times}$ from observational data.

Table 1 Disequilibrium ratios and lags (yr)

	C_0	250			270			290		
$\Delta T_{2\times}$	κ	1	2	3	1	2	3	1	2	3
1.5	$\Delta T/\Delta T_1$	0.81(0.79)	0.76(0.73)	0.72(0.69)	0 79(0.79)	0 73(0.73)	0.69(0.69)	0.74(0 77)	0.68(0.72)	0.63(0.68)
	$\Delta T/\Delta T_{lin}$	0.97(0.96)	0.97(0.95)	0 96(0.94)	0 94(0 95)	0.93(0.94)	0 92(0 94)	0 88(0.93)	0.87(0.92)	0.85(0.92)
	L	18(19)	23(25)	27(29)	14(14)	19(19)	22(22)	11(10)	14(12)	16(14)
	L_{lin}	22(23)	28(29)	33(34)	22(23)	28(29)	33(34)	22(23)	28(29)	33(34)
3.0	$\Delta T/\Delta T_1$	0 68(0.65)	0.60(0 58)	0.56(0.53)	0 64(0 64)	0.57(0.57)	0 52(0 53)	0 58(0.63)	0 51(0.56)	0 46(0 51)
	$\Delta T/\Delta T_{lin}$	0.96(0 93)	0.95(0.92)	0 95(0.91)	0.91(0 92)	0.90(0.91)	0 89(0 90)	0.82(0.89)	0 80(0 88)	0 79(0.88)
	L	31(34)	39(42)	45(47)	26(25)	32(32)	37(36)	19(17)	24(21)	27(24)
	L_{lin}	38(39)	48(48)	54(54)	38(39)	48(48)	54(54)	38(39)	48(48)	54(54)
4.5	$\Delta T/\Delta T_1$	0.58(0 56)	0.50(0.48)	0.45(0.43)	0.54(0.55)	0.47(0.47)	0.42(0.43)	0 48(0 53)	0.41(0.46)	0.36(0.41)
	$\Delta T/\Delta T_{lin}$	0.95(0.91)	0.94(0.90)	0 94(0.89)	0.89(0 90)	0.88(0.89)	0.87(0.88)	0.79(0 86)	0 77(0.86)	0.76(0.85)
	L	42(45)	52(54)	57(60)	35(34)	43(42)	48(47)	26(23)	32(28)	35(31)
	L_{lin}	51(51)	61(61)	67(67)	51(51)	61(61)	67(67)	51(51)	61(61)	67(67)

Values represent variations in the disequilibrium ratio ($\Delta T/\Delta T_1$) and the lag (L) in 1980 for $\gamma = 0.71$ and various values of the diffusivity ($\kappa = 1$–3 cm^2 s^{-1}, see refs 4, 9, 22, 23), the CO_2-doubling temperature change ($\Delta T_{2\times} = 1.5$–4.5 K, see ref. 1) and the initial (1850) CO_2 level ($C_0 = 250$–290 p.p.m.v., a range which spans the most likely range of 260–280 p p.m.v given in ref. 14) ΔT and L correspond to CO_2 variations given by equation (15) fitted through 315 p.p.m.v in 1958 and 338 p.p m.v. in 1980[15], while ΔT_{lin} and L_{lin} correspond to CO_2 variations given by equation (15) with $\alpha = 0$ fitted through 338 p p.m.v in 1980 (that is, to linear radiative forcing) ΔT and L are relatively insensitive to variations in h; $h = 70$m has been used here (ref. 3). The values shown are based on a full numerical solution of equations (3), (6) and (7), while the bracketed figures are those based on the approximate analytical solution (equation (16)) with $\mu = 2.2$ in the linear case and $\mu = 2.7$ otherwise. In the nonlinear case the optimum value of μ depends on C_0, but we have used the value appropriate to $C_0 = 270$ p.p.m.v. throughout.

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Methane flux from northern peatlands

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The concentration of methane (CH_4) in the global troposphere is increasing. Ambient air measurements document an approximate rate of increase of 1–2% yr^{-1} over the past decade[1–4]. Measurements of CH_4 in air bubbles trapped in polar ice indicate that tropospheric concentrations of CH_4 several hundred years ago may have been ~45% of present levels[5–7]. To understand and assess possible causes of the atmospheric CH_4 increase requires improved quantitative knowledge of global sources and sinks of CH_4. Previous attempts to estimate sources of atmospheric CH_4, based on very few measurements, have suggested that natural and agricultural wetlands are major sources[8,9]. The major wetland regions of the world are in boreal, low Arctic and tropical ecosystems[10]. It is these regions, particularly in peatland habitats where major accumulations of organic materials occur under anaerobic conditions, that should be significant sources of global tropospheric CH_4. The most extensive peatlands in the world occur in the boreal taiga zone between ~45° and 65° N latitude. More than 95% of world peat resources occur in the Soviet Union, Canada, the United States, Sweden, Norway, Finland and the United Kingdom[10]. We report here the first survey of CH_4 flux from northern peatlands of the United States. Emission rates ranged from 0.003 to 1.94 g CH_4 m^{-2} day^{-1}, with half of these values between 0.1 and 0.4 g CH_4 m^{-2} day^{-1}. The frequency distribution is log normal (Fig. 1) and the mean emission rate is 0.337 g CH_4 m^{-2} day^{-1}. Such fluxes are higher than most values reported for other ecosystems, suggesting that northern peatlands may be an important source of global tropospheric CH_4.

In an effort to improve our understanding of wetlands as a source of atmospheric CH_4, which should aid in the interpretation of global distributions and trends, we developed a portable system for rapid, direct measurement of CH_4 flux in remote wetland environments. The system consists of a gas-filter-correlation (GFC) infrared detector[11] coupled to a controlled environment chamber. Flux measurements were made in a closed-chamber mode with air continuously recirculated from the sampling chamber over the surface peat and vegetation, then into the GFC and back to the chamber; CH_4 concentration was measured continuously. Continuous measurement of CH_4 in the chamber enables us to separate 'pulses' related to any human disturbance of the bog from the natural efflux. For a typical 15-min continuous sampling and analysis period, this system has a flux detection limit of 1×10^{-4} g CH_4 m^{-2} day^{-1}. Calibration was carried out in the field using certified CH_4 standards. Details of instrument design, calibration, and field testing are given elsewhere[11,12].

Our survey of CH_4 emissions from northern wetlands was conducted in August 1983, during daylight hours. Four sites were in the Marcell Experimental Forest, north-central Minnesota (47°32′ N, 93°28′ W), where a single watershed often consists of a central peatland surrounded by forested uplands. Characteristic plants of the Marcell fens and bogs are given by Boelter and Verry[13]. Upland soils over slightly calcareous glacial debris constitute 74% of the Marcell Forest surface, peatlands comprise 21%, and open water the remaining 5% (ref. 14). Peat deposits consist primarily of *Sphagnum* peat at the surface with woody, sedge, and aquatic plant residues at depth[15]. Three of our Marcell sites are perched ombrotrophic bogs; their water is derived almost entirely from precipitation (that is, WS-1, WS-2, and WS-4 in Table 1). Site WS-3 is a minerotrophic shrub/sedge fen; its water is derived primarily from groundwater. The hydrology and chemistry of these bogs have been studied in detail elsewhere[13]. The ombrotrophic bogs have low concentrations of dissolved ions, with a conductance 51 ± 13 μS cm^{-1} at 25 °C and an average *p*H of 3.6 ± 0.3. The minerotrophic fen (WS-3) has high concentrations of dissolved ions from reactions of groundwater with soil, conductance 125 ± 48 μS cm^{-1}, *p*H 6.5 ± 0.3 (ref. 14).

We also studied CH_4 flux from 21 separate sites in an area where a weakly minerotrophic fen surrounds a small ombrotrophic bog, 10.5 km west of Zerkel, Minnesota (47°10′ N, 95°24′ W). The vegetation includes a central pond with *Brasenia schreberi*, surrounded by a floating mat of *Carex lasiocarpa* and *Vaccinium macrocarpon*. The sedge mat is being invaded on its landward side by bog mosses such as *Sphagnum angustifolium*, *S subsecundum* and *S. magellanicum* and by the vascular plants *Vaccinium oxycoccus*, *Potentilla palustris*, *Dulichium arundinaceum* and *Scheuchzeria palustris*. Closer to the upland is a zone of low leatherleaf shrubs (*Chamaedaphne calyculata*) and several bog mosses, including *Sphagnum rubellum*, *S. magellanicum*, *S. papillosum*. *S. angustifolium* and *S. fuscum*, together with *Vaccinium oxycoccus*, *Scheuchzeria palustris*, and *Andromeda glaucophylla*. We also sampled ombrotrophic sites at this location inside and outside a small stand of black spruce (*Picea mariana*) associated with *Chamaedaphne calyculata*, *Ledum groenlandicum*, *Carex trisperma*, *Eriophorum spissum* and

Table 1 Methane flux from peatland sites in northern Minnesota

Site	No	CH_4 flux (g CH_4 m^{-2} day^{-1}) Average	Range
Marcell Experimental Forest			
WS-1 (bog)	3	0.156	0.127-0.206
WS-2 (bog)	9	0.047	0.019-0.174
WS-3 (fen)	3	0.004	0.003-0.005
WS-4 (bog)	6	0.194	0.033-0.468
Zerkel fen and bog	18	0.419	0.060-1.943
Wild Rice River sedge meadow	1	0.664	—
Lake Itasca			
Wild rice bed	4	0.493	0.127-0.883
Shoreline fen	2	0.165	0.158-0.171

the mosses *Sphagnum angustifolium, S. magellanicum, S. rubellum, S. fuscum, Aulacomnium palustre, Calliergon stramineum* and *Ptilium crista-castrensis*. This location is more fully described by Joyal[16]. The Zerkel sites ranged in *p*H from circumneutral in the pond and the outer part of the floating sedge mat (*p*H 6.1–7.3) to distinctly acid in the ombrotrophic area of black spruce and *Sphagnum* (*p*H 4.1–4.4). Conductivities ranged from 29 to 98 $\mu S\ cm^{-1}$ in various parts of the pond and floating mat, and from 43 to 108 $\mu S\ cm^{-1}$ in the zone of leatherleaf shrubs In the spruce stand, we have one measurement of 60 $\mu S\ cm^{-1}$.

For comparison with other wetland habitats of the region, we measured the CH_4 flux in the Wild Rice River sedge meadow, located ~5 km west of Zerkel, and dominated by *Carex lacustris* and *Calamagrostis canadensis*, and in a stand of wild rice (*Zizania aquatica*) and shoreline fen adjacent to Lake Itasca, Minnesota (47°15′ N, 95°10′ W), where the *p*H ranged from 6.9 to 7.5. The combined set of sites studied are representative of major types of wetlands found in boreal North American ecosystems.

Our results are summarized in Table 1. Note that average CH_4 efflux rates are much the same across a wide range of peatland sites, from circumneutral lake edges and marginal sedge fens through increasingly acid sedge/*Sphagnum* and leatherleaf/*Sphagnum* communities to an ombrotrophic bog community of black spruce and *Sphagnum* This is particularly interesting because laboratory studies using Minnesota peats indicted that $pH = 6$ is optimal for CH_4 production[17]. This difference between laboratory and field observations may well be explained by all variables except *p*H being held constant in the laboratory. At two locations with significantly lower CH_4 flux (Marcell WS-2 and WS-3) our qualitative field observations suggest that differences in peatland hydrology may be important in controlling CH_4 production. The Marcell fen WS-3, which had by far the lowest average daily CH_4 flux, 0.004 $g\ m^{-2}\ day^{-1}$, was the only site with visible water flow in surface peat derived from active groundwater input Thus, we hypothesize that relatively stagnant conditions were present at the other sites studied (conditions at WS-2 were intermediate) which favoured higher rates of methanogenesis. Methane fluxes from the sedge meadow, wild rice, and shoreline fen were similar to rates measured in the bogs.

The distribution of all CH_4 flux measurements made in Minnesota peatlands (Fig. 1) indicates that emission rates ranged over three orders of magnitude. Approximately half of our CH_4 measurements fell within the limited range of 0.1–0.4 $g\ CH_4\ m^{-2}\ day^{-1}$. The logarithmic nature of the frequency distribution of flux rates is of considerable interest. We speculate that the lowest values represent a combination of low decay rates and largely molecular diffusion from the bog surface, whereas the high values, above ~0.4 $g\ CH_4\ m^{-2}\ day^{-1}$, represent scattered 'hotspots' of decaying, recently dead roots or small animals and the possible buildup of methane bubbles.

The only comparable data of which we are aware are from an ombrotrophic *Sphagnum* bog in subarctic northern Sweden[18] where CH_4 emissions ranged from 0.0003 to 0.058 $g\ CH_4\ m^{-2}\ day^{-1}$, a minerotrophic peatland in Sweden[18] that emitted 0.054–0.95 $g\ CH_4\ m^{-2}\ day^{-1}$, and an acid *Sphagnum* bog in the Pennine mountains of England[19] with a range of 0.004–0.03 $g\ CH_4\ m^{-2}\ day^{-1}$ The generally higher emission rates from Minnesota peatlands are probably due, in part at least, to higher soil temperatures that ranged from 14 to 20 °C at 6 cm, as compared with 8 to 16 °C in Sweden and ~11 °C in the British bog[20] during late summer.

Comparison of our flux data with data on other biogenic CH_4 sources indicates that northern wetlands should be considered a potentially significant source of global tropospheric CH_4. For example, our Minnesota flux data are similar to the average emissions of 0.25 $g\ CH_4\ m^{-2}\ day^{-1}$ from California rice paddies[21] and 0.1 $g\ CH_4\ m^{-2}\ day^{-1}$ from rice paddies in Spain[22], and higher than emissions of 0.01–0.09 $g\ CH_4\ m^{-2}\ day^{-1}$ from undisturbed freshwater cypress swamps[23] Two additional factors that determine the global significance of biogenic sources

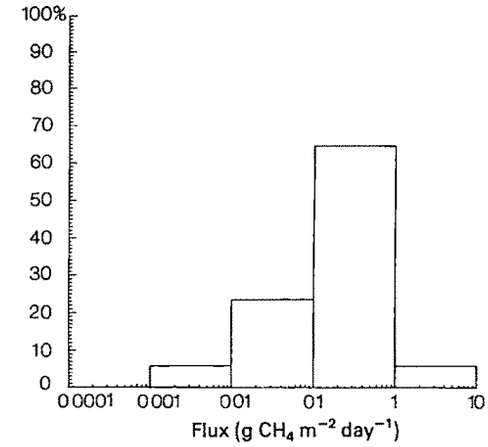


Fig. 1 Histogram of CH_4 flux data from Minnesota peatlands. There were 51 observations which gave a mean flux of 0 337 ± 0 076 $g\ CH_4\ m^{-2}\ day^{-1}$ and a median of 0 173.

of CH_4 are the geographical extent of the source and the duration of emissions. The surface area of boreal and Arctic peatlands and wetlands is not well known. Recently published estimates of these source areas[24] are: boreal bogs and peatland = $1.2 \times 10^6\ km^2$, tundra wet sedge = $1.0 \times 10^6\ km^2$, and tundra tussock = $0.9 \times 10^6\ km^2$. The area of other potentially significant source regions such as wet rice agriculture and Amazonian floodplain are ~$1.1 \times 10^6\ km^2$ and $0.06 \times 10^6\ km^2$, respectively. All of these areas produce CH_4 for 3–6 months a year, depending on climatic and cultivation cycles. We hypothesize that the combination of relatively high emission rates and large areal extent should rank northern peatlands high as a natural source for global atmospheric CH_4. Flux measurements from the great peatlands of Canada and the Soviet Union are needed to test our hypothesis.

Habitats suitable for peat development are largely a result of the most recent glacial period. Extensive peat accumulation began in Minnesota mostly between 3,000 and 4,000 years ago (ref. 25 and E.G., unpublished data). Major peatlands across Canada from British Columbia to the Atlantic Provinces exhibit, as expected, much less synchroneity in 35 basal dates compiled from a variety of sources (E.G., unpublished data). These dates range from 1,600 to 11,200 yr BP, and average 6,500 yr BP. Could the development of boreal peatlands, and their spread by swamping of large areas of surrounding uplands, contribute sufficient additional CH_4 flux to result in a long-term increase in global atmospheric CH_4?

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A lattice statistics model for the age distribution of air bubbles in polar ice

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Measurements of CO_2 in bubbles in polar ice have been used to establish a pre-industrial concentration[1-4]. Similar measurements have been made for other atmospheric constituents[5,6]. However, in order to use ice-core measurements to determine the increase in CO_2 over the last 200 years, it is necessary to consider the time delay between the deposition of the original snow and the bubble trapping and also the distribution of trapping times over several decades[7]. The percolation model from lattice statistics describes the static geometrical aspects of trapping and reproduces various aspects of recent observations. The observations of large seasonal fluctuations in trapped bubble volume reflect the enhanced susceptibility to perturbations near the percolation transition. The critical exponent of the percolation probability largely determines the stability of the deconvolution of observed concentrations, indicating that the bubble deconvolution problem is less poorly posed than typical geochemical source deduction problems.

In order to represent the relation between atmospheric concentrations and bubble concentrations, a negative time variable z (in years) is introduced. The variable z increases with depth and has its origin at the present (that is, the time at which the ice core was extracted). The atmospheric concentration of CO_2 (or other tracer of interest) z years ago is denoted $c(z)$. The mean concentration in bubbles in a layer deposited z years ago is denoted $q(z)$. The trapping function $R(z)$ gives the amount of air in a layer that was trapped z years after the layer was deposited and is expressed as a proportion of the total amount of gas that will be trapped. Thus $R(z)$ is normalized to

$$\int_0^\infty R(z)\,\mathrm{d}z = 1 \qquad (1)$$

From the definition of $R(z)$, it follows that the relation between q and c is

$$q(z) = \int_0^z R(z-z')c(z')\,\mathrm{d}z' \qquad (2)$$

The static geometric aspects of bubble-trapping can be naturally modelled by using the percolation model of lattice statistics[8-10]. This model is usually studied for regular lattices and considers connected clusters in a lattice where elementary steps in possible connecting pathways are broken in a random uncorrelated manner. A key quantity in the theory is the percolation probability, $P(p)$, which gives the probability that a given lattice element is part of an infinite cluster of connected elements with p being the probability that an individual step in the lattice is broken. The main results of percolation theory are that there is a critical probability p_c above which $P(p)$ is zero and that $P(p)$ behaves as $(p_c-p)^\beta$ for $p \to p_c^-$. It was pointed out by Kasteleyn and Fortuin[11] that the percolation model is a limiting case of the Potts models[12], a family of models used in statistical mechanics to describe a range of continuous phase transitions. The property that distinguishes such transitions and makes them difficult to analyse is that the transition behaviour is influenced by fluctuations on all possible length scales.

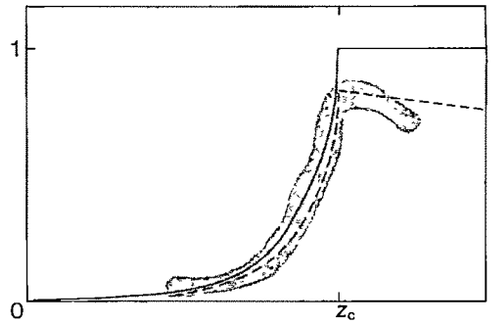


Fig. 1 A schematic representation of the cumulative trapping function as predicted by percolation theory (solid curve). The age distribution function is the derivative of this curve. The dashed curve shows the effect of a correction applied to convert the fraction trapped into bubble volume. Shading shows the range of observed volumes[7], with the horizontal and vertical scales chosen to give agreement at the 50% and 100% points.

Having established the connection with statistical mechanics, the techniques of the renormalization group[13] in statistical mechanics can be used to justify earlier conjectures of universality in percolation models. This implies that the exponent β depends on the dimensionality of the system but will be the same for all three-dimensional lattices and for amorphous three-dimensional systems such as that involved in firn closure. (The exponents will also be the same for the so-called 'site' and 'bond' problems[8-10], which is why the neutral terms 'step' and 'element' were used above.)

As a layered system the firn should be regarded as being two-dimensional. However, it is only very near p_c that the correlations become long enough to detect the essential two-dimensional nature so that the β for two-dimensional systems should apply. Further away from p_c the shorter-range correlations see an essentially three-dimensional structure. In the firn system the crossover[14] from $\beta_{2D} = 5/36$ (conjectured[15,16]) to $\beta_{3D} \cong 0.454$ (series estimate[17]) should be so close to p_c as to be undetectable. The remaining discussion will assume a purely three-dimensional description.

To model firn closure using percolation, the elements in the infinite cluster are those that are still connected to the atmosphere. If we assume that the channel closure probability, p, increases smoothly with age z, then we can consider the percolation probability as a function of z without changing the critical exponents and there will be a critical delay time, z_c, at which point all gas has been trapped. $P(z)$ represents the proportion of gas-containing elements *not* trapped at time z after deposition so $1-P(z)$ represents the cumulative proportion trapped. The incremental trapping function $R(z)$ is thus

$$R(z) = -\frac{\partial P(z)}{\partial z} \sim (z_c - z)^{\beta-1} \quad \text{as } z \to z_c^- \qquad (3)$$

Schwander and Stauffer[7] measured the bubble volume of trapped bubbles as a function of depth. In the percolation model the proportion trapped is $1-P$, which is plotted schematically as the solid line in Fig. 1. To convert this to bubble volume, a depth-dependent factor must be included to allow for the fact that the bubbles are subject to compression once trapped. The result is shown schematically as the dashed line in Fig. 1; the range of values observed by Schwander and Stauffer is shown by the shaded region. As the general theoretical results do not determine the specific relation between the measured ice density and the bond closure, both the horizontal and vertical scales on Fig. 1 have been linearly related to those used by Schwander and Stauffer[7] so as to match the points of 50% and 100% trapping. The percolation model adequately describes the geometrical aspects of bubble trapping, but the assumption of proportionality between z and the compression factor is inadequate.

Both the percolation model and the measurements by Schwander and Stauffer[7] determine the time of bubble trapping relative to the time of snow deposition and do not directly determine the age of the air that is trapped. However, measurements of ^{39}Ar indicate that air in the 'open' volume is well mixed throughout the firn[18], so that the trapping time distribution should be close to the age distribution.

For use in solving equation (1), the percolation model will require an empirical calibration using data such as that plotted by Schwander and Stauffer[7] even if only to determine the amplitude of the singularity. Direct theoretical calculation of $P(z)$ seems impractical mathematically since even highly structured lattice percolation problems do not have closed form solutions. Even an approximate calculation of $P(z)$ would require more detail of the firn structure than is readily available.

Given the singular nature of $R(z)$, it is of interest to note that the ice-core deconvolution problem is less poorly posed than many typical geochemical inversion problems. Equation (1) becomes

$$q(z) \sim \int_{z-z_c}^{z} (z_c - z + z')^{\beta-1} c(z')\, dz', \text{ for } z > z_c \qquad (4a)$$

or putting $t = z_c - z$, $t' = -z'$

$$q(z_c - t) \propto \int_{t-z_c}^{t} (t - t')^{\beta-1} c(-t)\, dt' \qquad (4b)$$

Apart from the variable lower limit (which has little influence on the integral) this is proportional to the expression given by Ross[19] for defining a β-fold integration of $c(-t)$ for non-integer β. The inverse operation of obtaining c from q corresponds to the operation denoted by

$$c(t) \propto \frac{d^{\beta}}{dt^{\beta}} q(t) \qquad (5)$$

Anderssen and de Hoog[20] have characterized the numerical difficulty of unstable inverse problems as being given by the equivalent order of differentiation when this is applicable. Most source deduction problems in geochemistry are essentially equivalent in difficulty to a single numerical differentiation since observed concentrations represent the integrated effects of sources. If the percolation model is valid, the deconvolution of bubble concentrations in ice cores is formally equivalent to about a 0.454th-order differentiation and so is less poorly posed than typical source deduction problems in geochemistry. Thus the resolution which can be achieved for the reconstruction of c should be somewhat better than the time period over which the firn closes.

Since submission of this manuscript we have learnt that the bubble trapping problem has also been considered in terms of the percolation model by B. Stauffer, J. Schwander and H. Oeschger (unpublished data). They performed simulations using a regular lattice approximation and concentrated on determining the critical point rather than discussing the critical exponent.

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Prokaryotic and eukaryotic microfossils from a Proterozoic/Phanerozoic transition in China

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Cherts of the Doushantuo Formation in the Yangtze Gorges, South China, contain a superbly preserved heterogeneous assemblage of bacteria, cyanobacteria, planktonic algae, submillimetre-sized burrows and problematica. The microbiota has a bearing on defining the Precambrian–Cambrian boundary. *Baltisphaeridium* and associated spinose microfossils in the chert suggest an early Cambrian age whereas other acritarchs, vendotaenids and pretrilobite small shelly fossils from the conformably overlying the Dengying Formation, indicate a late Proterozoic age for the Doushantuo. This mixed benthic–planktonic assemblage preserved in silicified, laminated, microbial mat-like fabrics, provides a unique sample of diverse microbiotic elements existing at the dawn of the Phanerozoic and records the earliest evidence of meiofauna.

The fossil record of the latest Proterozoic is best known from Ediacaran and allied soft-bodied animals[1,2], acritarchs[3] and stromatolites[4]. This record, though meagre, is fascinating, for it was during this interval that animals originated and began to diversify[5]. Much of our understanding of pre-700-Myr-old palaeobiology has been based on interpreting fossils preserved in chert[6]; unfortunately such occurrences are not well known from post-700-Myr old deposits

The Doushantuo Formation in the Yangzte Gorges, China, conventionally viewed as late Proterozoic[7,8], contains cherts with well-preserved, abundant and diverse microfossils such as coccoid and filamentous cyanobacteria, bacteria, large spiny organic-walled microfossils, problematic tubular microfossils, other problematica and submillimetre-sized backfilled burrows. In addition to a benthic mat biota, the cherts preserve elements of the phytoplankton that lived in the overlying water column.

The Doushantuo Formation is a dark brown to grey-black dolomite with chert, interbedded with shale. It unconformably overlies tillite of the Nantuo Formation and is conformably overlain by dolomite and limestone of the Dengying Formation[9] (Figs 1, 2).

The Yangtze Gorges region contains the type section for the Chinese Sinian System[10] (composed, in ascending order, of the

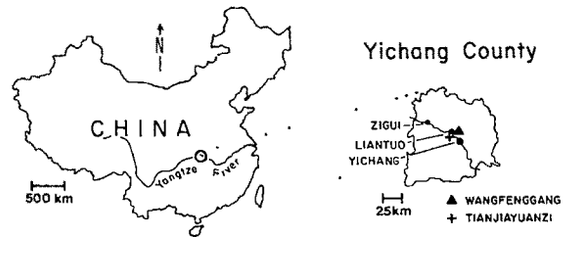


Fig. 1 Index maps showing location of measured sections.

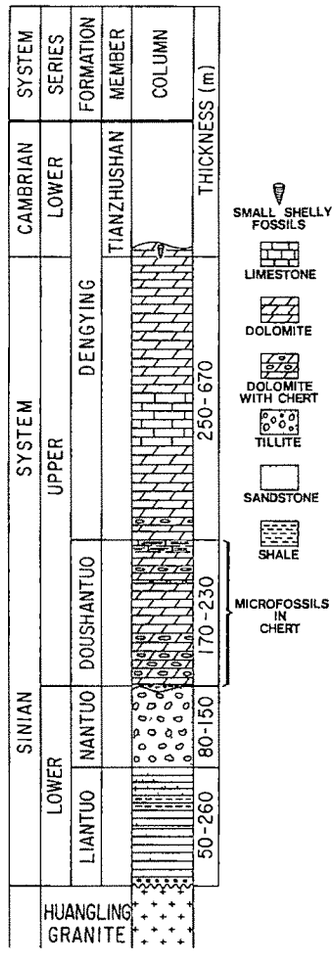


Fig. 2 Generalized stratigraphical column of Sinian System in the Yangtze Gorges, Yichang County, Hubei, China.

Liantuo, Nantuo, Doushantuo and Dengying formations). It would be a potential candidate for any global stratotype section that may be established for a new geological system to embrace the pre-shelly fossil record during which metazoans appeared[11]. Here the Sinian is a thick succession with such lithological and palaeontological curiosities as tillite, phosporite, acritarchs, vendotaenid algae, stromatolites, oncolites, microfossiliferous chert, putative Ediacaran-type metazoans, sponge-like spicules, trace fossils and is conformably overlain by the small shelly fossil-bearing Tianzhushan member (upper Dengying Formation), regarded as signalling the base of the Cambrian[9,12].

Within this stratigraphical framework, the Doushantuo is usually taken to be Late Sinian (Vendian or Ediacarian), probably <700 Myr-old. Although their reliability may be questioned, Rb-Sr whole rock isochron ages from the top of the formation yield 700±5 and 691±29 Myr (ref. 13). Other potential radiometric age constraints include an ion-probe U-Pb age of 740±16 Myr on zircons in tuff from the Lower Sinian Liantuo Formation[13], and ages of 806±6 Myr (Rb-Sr isochron) and 823±7 Myr (K-Ar on biotite) from the crystalline basement underlying the Liantuo[13]. Shale from the upper part of the Tianzhushan member (Dengying Formation) has an inferred isotopic age of 610±10 Myr based on whole rock Rb-Sr isochron analyses[14]. Palaeontologically, acritarchs from acid residues of Doushantuo clastic rocks have been interpreted to indicate a late Proterozoic age (<700 Myr)[7,15]. The overlying Dengying Formation's palaeontology (vendotaenids from the lower part[9]; pre-Cambrian acritarchs from shale[7,9], and small shelly fossils of Meichucunian or Tommotian character at the top[7]) also indicates a pre-Cambrian age for the Doushantuo. Following guidelines set forth by the International Union of Geological Sciences/International Geological Correlation Program Project 29 Working Group on the Precambrian-Cambrian Boundary[16], the small shelly fossils appearing in the Tianzhushan member (Dengying Formation; Fig. 2) would define the base of the Cambrian. Hence, until now, the overall palaeontology, stratigraphy, isotope age data and correlations with other areas suggested a late Proterozoic age for the Doushantuo.

Previously reported fossils from the Doushantuo Formation include stromatolites[17], acritarchs[15] and chitinozoans[17]. Cyanobacteria[18-20] and spiny microfossils[21,22] have been described from chert and sponge-like spicules from dolomite[17]. No megascopic algal, metazoan trace or body fossils have yet been found in the Doushantuo.

The microfossils that we describe here were detected in petrographic thin sections of chert nodules in dolomite from two measured sections of the Doushantuo Formation; one ~150 m east of Wangfenggang Village, the other ~700 m south-southeast of Tianjiayuanzi Village, Yichang County, Hubei, China (Fig. 1). Of the coccoidal microfossils detected, solitary specimens are rare; the more abundant pluricellular varieties are represented by such Proterozoic taxa as *Myxococcoides*, *Palaeoanacystis* and *Tetraphycus*. Among the new taxa present are a *Merismopedia*-like pluricellular form with tightly packed cubic cells and an aggregate of small <1-μm diameter cells that might represent fossilized bacteria. In general, most of the coccoidal morphs resemble cyanobacteria; however, although diverse, the coccoids are only moderately abundant when compared with filaments and large spinose morphs.

The benthic component of the microbiota is dominated by filamentous fossils that resemble cyanobacteria. Bundles, 50–400 μm across, of filaments, 5–45 μm in diameter, are common (Fig. 3*a*) and many superficially resemble the extant multitrichomous oscillatoriacean *Microcoleus* Numerous single filaments, ranging from <1 to as much as 100 μm in diameter, and representing several taxa, occur as solitary strands and in matlike arrangements. Rare examples of an *Obruchevella*-like fossil have been found. Most filaments resemble the empty sheaths of cyanobacteria although others may be bacteria and algae. Preservation at times is so good that a trichome can be found preserved within its sheath (Fig. 3*d*) No heterocystous or branched filaments have been detected.

Cyanobacteria-like fossil diversity is high; >20 species are present. These microfossils occur in rudimentary, stratiform stromatolite-like microstructures not too unlike those seen in Proterozoic microfossiliferous stromatolites[23]. In some instances, this rudimentary lamination is disrupted in a manner suggesting churning of the sediment by organisms that did not leave clear, well-defined burrows.

Planktonic microfossils include a variety of morphotypes. Among the most conspicuous and abundant plankters found in thin section are diverse spinose vesicles, ranging from 30 to 1,200 μm across. Important among these are the spiny acritarchs resembling *Baltisphaeridium* (Fig. 3*b*) hitherto known only from early Cambrian and younger rocks[24,25].

The Doushantuo also contains abundant and diverse, morphologically-complex microscopic fossils of uncertain systematic position. First, there are robust, multilayered and partitioned tubes (Fig. 3*c*). These fossils, 40–100 μm in diameter, occur in variable abundance in numerous thin sections from several sites. At first glance, these tubes resemble large, lamellated, oscillatoriacean cyanobacterial sheaths. However, oscillatoriaceans and other filamentous cyanobacteria, bacteria, algae, and fungi are not known to possess this type of architecture. Superficially they resemble miniaturized coelomates; however, the small size and apparent partitioning of the innermost tube (which would be equivalent to the gut) make this affinity unlikely. This morph might represent an evolutionary novelty in the prokaryotic or eukaryotic line for which there are no modern analogues.

Also, large, 400–900-μm wide and 750–1,500-μm long, morphologically complex, brown coloured vesicles (Fig. 3*f*) have been found in many thin sections from several sites. The fossils are characterized by a tripartate organization consisting of: (1)

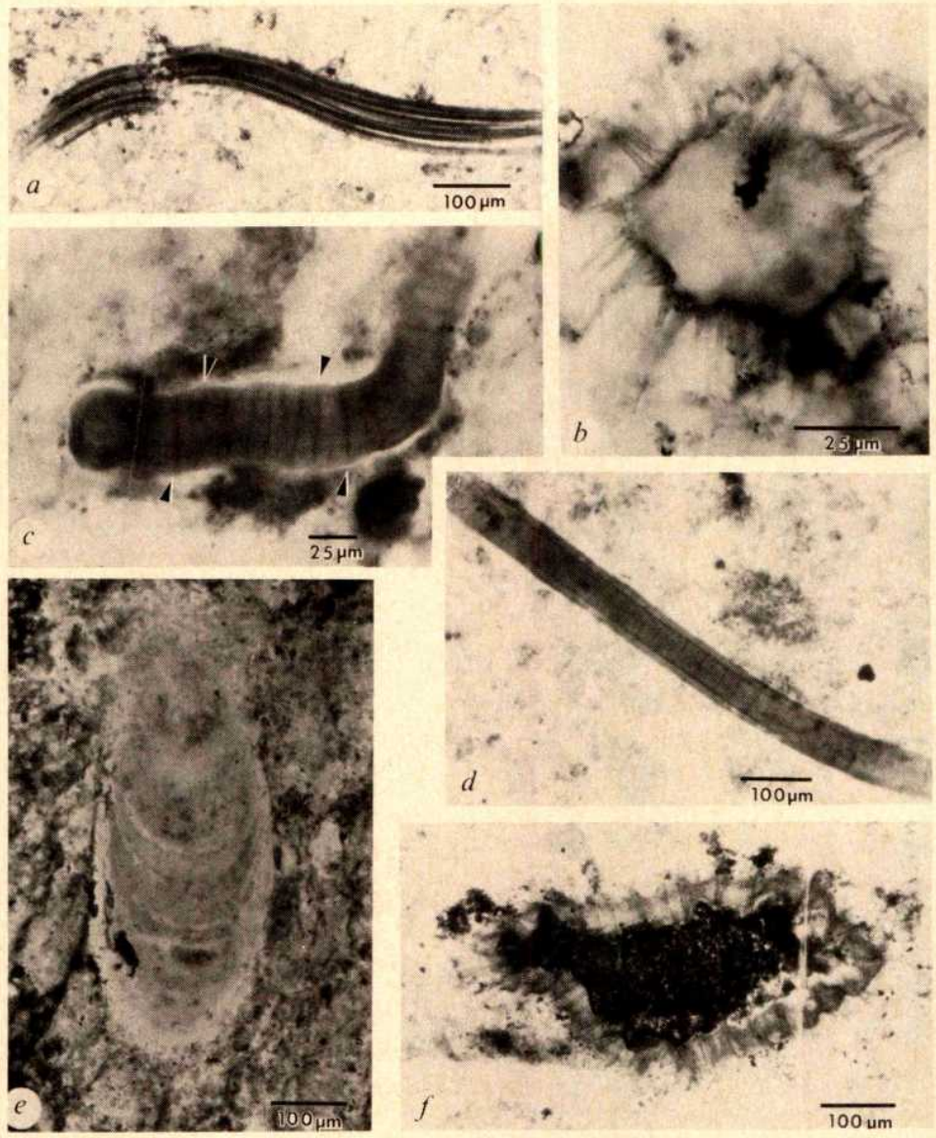


Fig. 3 Microfossils from the Doushantuo Formation. All photomicrographs are from petrographic thin sections housed in the Preston Cloud Research Laboratory. *a*, Bundle of filaments showing possible multitrichomous habit. Sheath is not evident. *b*, *Baltisphaeridium* spiny microfossil; note the closed, tapered ends of the spines. *c*, Partitioned, concentrically-layered tube. Partitions, indicated by arrows, cut the layered cylinder. It is uncertain even to what kingdom this fossil belongs. *d*, Cyanobacterium with trichome preserved within lamellated sheath. The outer sheath is not partitioned and the inner trichome is not layered. *e*, Backfilled burrow. This burrow and others like it (preserved in chert), pre-date previously known bona fide burrows from the Sinian of China. *f*, Complexly organized protistan showing outer layered organic mantle with tube clusters, boundary layer, and interior with particulate contents.

the vesicle interior that typically contains amorphous black particulate organic and pyritic matter and occasionally folded membrane-like material; (2) a well-defined boundary layer or wall that separates an external organic covering from the vesicle interior; and (3) an outer organic covering or mantle that is occasionally layered, as seen in Fig. 3*f*. This organic covering contains clusters of from 3 to 8 small, hollow tubes, each 1–3 μm in diameter. The tubes have closed, tapered ends. Where attached to the boundary, the tubes open to the vesicle's interior. The tubes, wall structure, interior and overall organization of these microfossils are unlike that of previously described spinose acritarchs, although some similarities exist with the Proterozoic morph *Trachyhystrichosphaera*[26]. Superficially they also resemble certain protozoan sporocysts like those of gregarines. The size and complexity of these fossils indicate some kind of eukaryote, probably a protistan. Again the possibility exists that these fossils have no modern analogues.

Structures resembling backfilled burrows, 150–250 μm across (Fig. 3*e*), are known from two collecting sites. Longitudinal and cross-sections of these burrows are found oriented both parallel to, and oblique to, lamination in chert nodules. Although rare (four examples known) and in the case of Fig. 3*e* composed of chalcedony, they probably represent some worm-like meiofaunal animal that peristaltically moved through sediment. These structures cannot be interpreted as laminated constructional features like microstromatolites for the layering is concave down and disrupts chert matrix laminae. The presence of such submillimetre-sized, backfilled burrows in the Doushantuo suggests meiofaunal activity and, if the age of the Doushantuo proves to be late Proterozoic, make them among the oldest burrows known.

Clearly, the discovery of *Baltisphaeridium* and related spinose vesicles in Doushantuo cherts raises questions, not only about the age of the Doushantuo, but also concerning criteria and reliability of criteria used to define the Precambrian–Cambrian boundary.

This precise age controversy aside, the exceptional preservation of the abundant and diverse prokaryotic and eukaryotic fossils in the Doushantuo cherts provide a unique microscopic window on a time during the early stages of animal evolution and the waning of stromatolite-building microbial mat communities[27]. These new data must be considered when defining one of the major boundaries of the geological time scale. The robust nature of many of the cyanobacteria (large size, thick sheaths) as well as some of the problematic morphs may have been an attempt by mat-forming organisms to cope with newly evolved 'meiofaunal' and macrofaunal benthic eukaryotic heterotrophs that ultimately overwhelmed the stromatolite-building biocenoses: an extraordinarily successful biocenose that had flourished for hundreds of millions of years.

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Energy transfer at the surface of clays and protection of pesticides from photodegradation

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Certain potential pesticides are of limited use because of their sensitivity to sunlight. Previous attempts to overcome this limitation have been unsatisfactory because of impairment of insecticidal efficiency or unwanted toxic properties. The photosensitivity results from the balance between the processes, such as energy transfer and chemical reaction, by which photoexcited molecules can release excess energy and return to the ground state. The efficiency of the various energy transfer mechanisms[1-5] depends, in some cases, on the specific spectroscopic characteristics of donor and acceptor molecules, the distance between them and their relative orientations. We present here an approach aimed at protecting compounds of agricultural interest (for example, pesticides) by exploiting the unique surface properties of certain clays in order to build systems in which fast energy transfer occurs before photodegradation starts.

Clays, such as montmorillonite, exhibit outstanding adsorption properties due to their high specific surface areas and high cation-exchange capacities[6]. As organic molecules adsorbed on the clay surface are oriented[7], their intermolecular distances and orientations might fulfil the requirements for an efficient energy transfer process. Measurements of energy transfer on clay surfaces have been described for certain fluorescent cations[8-10]. However, occurrence of such processes between adsorbed non-fluorescent photolabile organic molecules has not been reported.

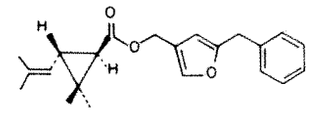
A suitable target for study of photostabilization is the synthetic non-halogenated pyrethroid bioresmethrin (BR) (5-benzyl-3-furylmethyl) (1R)-*trans*-chrysanthemate), a powerful contact insecticide effective against a wide range of insect pests and one of the safest pesticides (its acute oral LD_{50} (50% lethal dose) for rats is 8 g per kg). Despite these advantages, bioresmethrin is not used in agriculture owing to its rapid photodecomposition.

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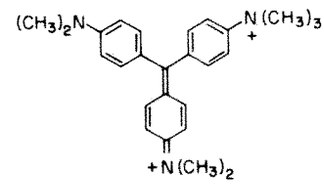
It was found that chemical modification of the pyrethroid structure leads, in some cases, to more stable pesticides[11-16]. Attempts have been made to stabilize the photolabile pyrethroids using various additives (antioxidants and UV-screeners)[17-22]. Recently, the photostabilization of cyphenothrin by dinitroanilines at certain relative concentration ratios has been attributed to possible exciplex or charge transfer quenching[22].

We have achieved stabilization of BR by adsorbing it on montmorillonite (Clay) together with the divalent cation methyl green (MG). Figure 1 shows the results of a typical bioassay using adults of the flour beetle *Tribolium castaneum*. Control samples of free BR exposed to sunlight completely lost their biological activity in <2 h, whereas samples prepared according to our method were still active after several days of exposure to sunlight. BR adsorbed in a complex with 0.2 mmol of MG per g clay was photochemically more stable than BR in a complex with a higher level of dye (0.4 mmol g^{-1}). This indicates that the stabilization effect is due to specific interactions between the two compounds at the surface of the clay rather than to merely a UV-screening effect.

Some details of these interactions at the molecular level can be obtained using Fourier transform IR (FTIR) spectroscopy (Fig. 2). A comparison of spectrum *a* (free BR) with spectrum *b* (BR adsorbed on clay) reveals that the stretching frequency of the C=O bond shifts from 1,722 to 1,709 cm^{-1} on adsorption.



BR



MG

The position of this band in spectrum *c* (BR adsorbed in the presence of MG) clearly shows that the BR molecules in the Clay-MG-BR complex are adsorbed in a similar manner as in the case of Clay-BR. Minor changes in the strong absorption bands of MG at 1,568 and 1,371 cm^{-1} are also induced by the co-adsorption of BR. These changes, although difficult to observe in the measured spectra (Fig. 2*d*, *e*), are evident in the difference spectrum (Fig. 2*c*). In addition, co-adsorption of MG and BR induces broadening of the 1,497 cm^{-1} peak of BR. The fact that vibrational frequencies of one organic molecule are shifted due to the co-adsorption of another one (as detected by the differential FTIR technique) indicates that short-distance intermolecular interactions exist between the molecules of these substances on the clay surface. A change in concentration of the adsorbed molecules results in a modification of the intermolecular distances and/or relative orientations in a manner which evidently affects the efficiency of the photostabilization effect (Fig. 1).

The method we propose for protecting pesticides from photodegradation thus consists of adsorbing the photolabile agrochemical to the surface of a clay to which another organic chromophore has been attached. This second organic chromophore may be selected according to its spectroscopic, geometric and charge characteristics, depending on the type of clay to be used and the pesticide to be protected. If the clay is a smectite, such as montmorillonite, with a negatively charged surface, a cationic protective compound may be used. As a result of its adsorption to the clay, the charge at the adsorption site is neutralized and the surface might become hydrophobic (depending on the size of the organic cation). If a non-ionic presticide molecule is added to this system, it will be adsorbed at these hydrophobic sites or, alternatively, it might be attached

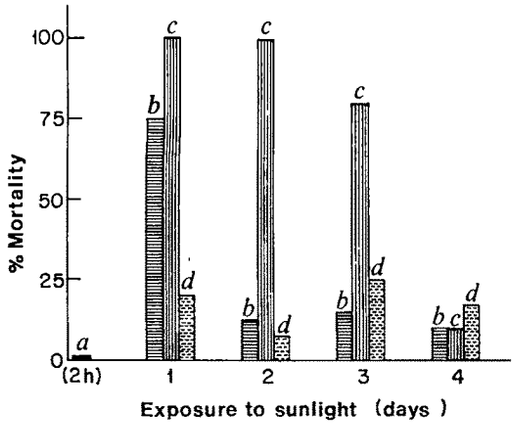


Fig. 1 Bioassay to evaluate protection of bioresmethrin from photodegradation. *a*, Unprotected bioresmethrin, *b–d*, montmorillonite(Clay)-methyl green(MG)-bioresmethrin(BR) complexes with different MG loading. The concentration of BR in all three cases was 0.2 mmol per g clay. Concentrations of MG, relative to clay, were: *b*, 0.1 mmol g^{-1}; *c*, 0.2 mmol g^{-1}; *d*, 0.4 mmol g^{-1}. Clay-MG-BR complexes were prepared as follows: aliquots of a 10^{-3} M aqueous solution of MG were added dropwise to a 0.5% aqueous suspension of Na-montmorillonite (SWy-1 from the Clay Minerals Society repository). After separation by centrifugation at 16,000*g*, the precipitate was washed three times with distilled water, freeze-dried and ground to <50 μm. The appropriate volume of a 10^{-2} M solution of BR in hexane was added to this powder and the solvent was evaporated under reduced pressure. For bioassays, 10 mg of the complex was deposited on the bottom of Petri dishes (5-cm diameter) and exposed to sunlight for various intervals of time; 2-week-old *Tribolium castaneum* (CSbb strain) adults were used. After irradiation, 20 insects were transferred from stock cultures to the Petri dishes and the percentage mortality was recorded. Irradiated controls of Clay-BR complexes (BR adsorbed to montmorillonite in the absence of MG) gave 0% mortality after 4 h of exposure to sunlight. In all cases controls protected from light gave 100% mortality.

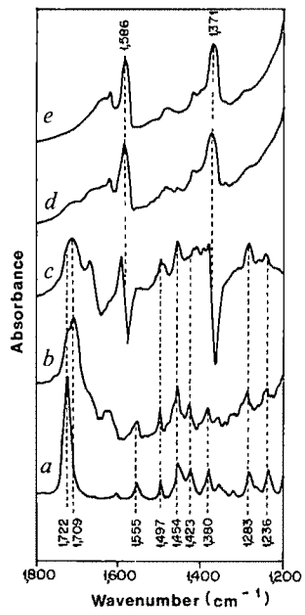


Fig. 2 Fourier transform IR absorption spectra of. *a*, bioresmethrin (BR); *b*, BR adsorbed on montmorillonite. The latter spectrum was obtained by subtracting the spectrum of the clay from that of the complex Clay-BR (none of which are shown). *c*, BR adsorbed in the complex Clay-MG-BR, obtained by subtraction of spectra *e* and *d*. 'Negative' peaks just below 1,586 and 1,371 cm^{-1} result from small shifts in the MG transitions at these positions due to co-adsorption of BR. *d*, Clay-MG-BR, with 0.2 mmol of each of MG and BR per g clay; *e*, Clay-MG (0.2 mmol MG per g clay). All spectra were measured in KBr pellets using a Nicolet MX-S FTIR spectrophotometer.

to the clay surface either directly or through interaction with adsorbed inorganic cations and/or interlayer water molecules. The actual mechanism will depend largely on the structure of the pesticide molecule (nature of functional groups) and on the extent of coverage of clay surface by the organic cation. Following photoexcitation of pesticide molecules, an efficient energy transfer between them and neighbouring organic cations might take place at a high rate, thus preventing the photochemical decomposition of the bioactive compound. In such a system, the protective compound is linked to the clay surface by strong coulombic bonds, whereas the pesticide molecules are retained by much weaker bonds and can be easily detached from the surface, as required for insecticidal activity.

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Patterns of family extinction depend on definition and geological timescale

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The recent claim[1] of an approximate 26-Myr periodicity in the pattern of mass species extinction over the past 250 Myr has already stimulated much astrophysical speculation and debate on causal mechanisms[2–13]. However, as shown here, the evidence for that claim is strongly contingent on arbitrary decisions concerning the absolute dating of stratigraphical boundaries[14], the culling of the database and the definition of what is mass extinction as opposed to background extinction. This evidence becomes insufficient under other plausible geological timescales and other acceptable definitions of mass extinction. Analysis of the non-culled database shows that the reliability of identification of mass extinctions and their timing is at present extremely limited. It also suggests that the apparent periodicity of mass extinctions results from stochastic processes.

The database for recent studies on global patterns of diversity and extinction usually consists of Sepkoski's compendium of stratigraphical ranges of all marine animal families in the Phanerozoic[15]. The stratigraphical resolution is to the level of universally recognizable units called stages (some of them are indeed stages, others are clusters of stages or even series). This compendium is taken at its face value, although the earlier

Table 1 Late Permian to Pleistocene maxima of marine animal family extinction, depending on geological timescale (H–H3 and O–O3) and definition of the maxima (*A–F*)

	Permian		Triassic						Jurassic							Cretaceous												Tertiary								Quaternary	
	Guadelupian	Dzhulfian	Induan	Olenekian	Anisian	Ladinian	Carnian	Norian	Rhaetian	Hettangian	Sinemurian	Pliensbachian	Toarcian	Bajocian	Bathonian	Callovian	Oxfordian	Kimmeridgian	Tithonian	Berriasian	Valanginian	Hauterivian	Barremian	Aptian	Albian	Cenomanian	Turonian	Coniacian	Santonian	Campanian	Maestrichtian	Danian	Late Palaeocene	Early Eocene	Middle Eocene	Late Eocene	Early Oligocene	Late Oligocene	Early Miocene	Middle Miocene	Late Miocene	Pliocene	Pleistocene
A	*		o			*					*	*	*		o			*		*				*					*					*				o			
B	*		*			*	*				*	*	*					*		*				*					*				*				o	o		
C	*		*				*				*	*	*					*		*				*					*				*							
D	*	*				*	*											*						*					*											
E																																										
H	*		*			*						*	o					*			o		o			*			*					o			*	o		
H1	*		*			*	*		o			*	*	*			*				*		*				*								o			*	o		
H2	*		o		o		*					o			*			*			o		o				*								o			*	o		
H3		*	*			*						o				*	o	*	o					o		*		*								o			*	o		
O	*		*		o			*				o	o						*				*			*		*								o	o		*	o		
O1		*	*		o			*				o	o				o		*				*				*		*		o				*	o		*	o		
O2	*				o		*			*			o					*					*				*				o				*	o		*	o		
O3	*				*			*		o		*			o		o				*			o		*			*		*			o			*	*			
F																																										
H	*		*				*					o		o					*			o		o				*								o				o		
H1	*		*			*		o				*		*			*		*		*		o					*								o				o		
H2	*		*		o		*					o			*			*			o		o				*								o				o		
H3		*	*			*						o				*		*						o		*		*								o				o		
O		*	*	*				*				o	o						*				o			*		*								o	o			o		
O1		*	*	*				*				o	o				o		*				*				*		*								*				o		
O2		*		o		*				*									*				*				*										*	o			o		
O3	*			*			*			o		o			o		o				*			*		*			*		*					o				o		

Asterisks denote major maxima, circles denote minor, perhaps insignificant maxima. *A*, Probabilistic rate of extinction per stage, culled data set, maxima defined as local peaks, *B*, number of family extinctions per stage, complete data set, maxima defined as local peaks; *C*, probabilistic rate of extinction per stage, complete data set, maxima defined as local peaks; *D*, probabilistic rate of extinction per stage, complete data set, maxima defined as points more than one standard deviation above the regression line (the same maxima obtain for the number of family extinctions per stage); *E*, number of family extinctions per Myr, complete data set, maxima defined as local peaks (H, timescale given by Harland *et al.*[33]; H1, H2, H3, timescales randomized within the limits of analytical error of absolute dates given by Harland *et al.*; O, timescale given by Odin[32]; O1, O2, O3, timescales randomized within the limits of analytical error of absolute dates given by Odin); *F*, probabilistic rate of extinction per Myr, complete data set, maxima defined as local peaks (timescales as for *E*).

demonstration by Raup[16,17] and others[18–20] that these kinds of data are strongly biased has never been refuted. The data are at the family level of taxonomic hierarchy, and the resultant patterns may not agree with the patterns at the species level[21]. For instance, the Toarcian maximum of species extinction[22–24] is unrecognizable at the family level, whereas the late Eocene maximum of family extinction does not show up at the species level[25–28]. Therefore, the following analysis is, for the sake of consistency, confined to the family level.

The database analysed for periodicity in mass extinction[1] is only a subset of Sepkoski's compendium. This subset is defined by exclusion of extant families and families with imprecise or questionable stratigraphical ranges. The time frame is the latest Permian (Dzhulfian) to middle Miocene. The data culling was intended to remove the noise and the pull of the Recent[16], but it results in data reliability being much lower in the Cenozoic than in the Mesozoic. For instance, the middle Miocene demise of 5 families becomes a possible mass extinction and the late Eocene demise of 10 families is a very pronounced event, whereas 19 families going extinct in the Campanian represents a period of low extinction and the late Permian extinction involves hundreds of families. Clearly, the alleged mass extinction events are incompatible with each other. Furthermore, the removal of extant families causes some extinction peaks to shift; probability of family extinction is higher in the late Eocene than in the middle Eocene only because the family number, that is, denominator, is artificially decreased in the former. The data culling also results in arbitrariness, for there is no criterion to determine which stratigraphical ranges are unquestionable. This must be an arbitrary decision because each range may be extended in either direction by new discoveries (probabilistic methods[29,30] are useless because neither family encounter frequency nor sequence in independent samples can be analysed). The time frame of Raup and Sepkoski's analysis arbitrarily cuts off the Guadelupian (extinction rate and probability higher than in the Dzhulfian) as well as the late Miocene (five family extinctions, as in the middle Miocene) and Pliocene (seven family extinctions). Had the Guadelupian and Pliocene, rather than Dzhulfian and middle Miocene, been accepted as mass extinctions, the 26-Myr periodicity would have to be rejected.

Hence, the following analysis is based on all Guadelupian to Recent taxa recorded in Sepkoski's compendium. Family extinctions with stratigraphical resolution to the series or system level are assigned proportionately to the stages, a procedure which accentuates maxima of extinction. Nevertheless, the culled data set analysed by Raup and Sepkoski shows eight maxima with extinction probability exceeding 0.1 (cutoff level for mass extinctions at the family level[2,31]), whereas the complete data set gives only two events of this magnitude (Guadelupian, Maestrichtian) and only four additional events exceeding the 0.05 level (Olenekian, Norian, Tithonian, Cenomanian). This difference clearly shows the impact of data culling by Raup and Sepkoski.

Analysis of the timing of geological or palaeontological events obviously must depend on the geological timescale. There is much uncertainty in absolute dating of stratigraphical boun-

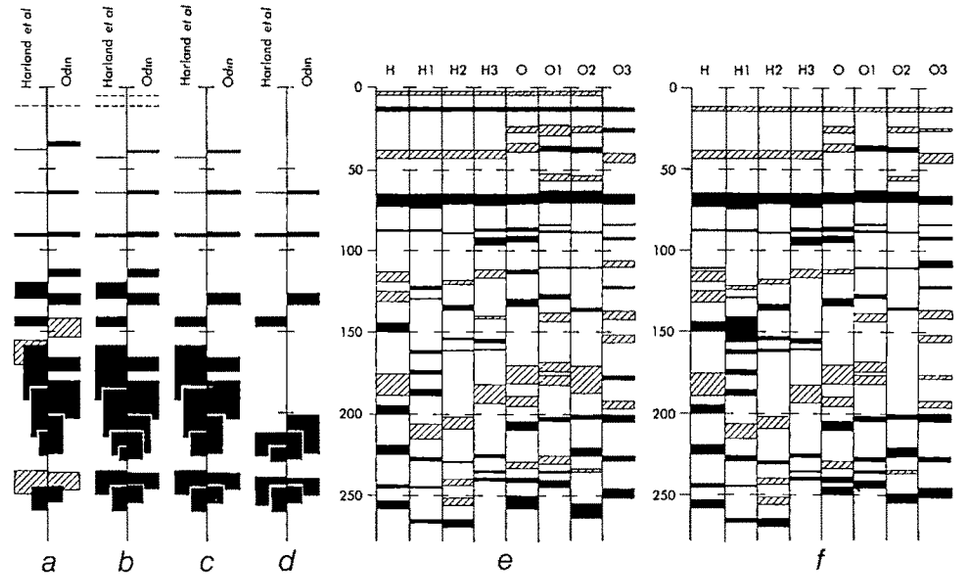


Fig. 1 Time distribution of maxima of family extinction, depending on definition and geological timescale (see Table 1 for explanation and event identification), vertical scale in Myr. Mass extinction events occurred somewhere within time ranges given by the black bars; hatched bars (or dashed lines) denote minor, perhaps insignificant, maxima.

daries. Two recent timescales[32,33] differ by as much as 10–14 Myr for many stratigraphical boundaries in the Mesozoic. This difference might increase to over 20 Myr if the analytical errors of absolute dates, as estimated by the authors of the two scales, run in opposite directions. (The timescale uncertainty is thus not much less than in the post-Cambrian Palaeozoic, where no 26-Myr periodicity in mass extinctions has been discovered[1].) The correlation is only moderate between the two timescales and its statistical significance is largely due to the generally good dating, and hence agreement between the timescales, for the late Cretaceous and Cenozoic. This correlation, however, dramatically decreases for randomized modifications of the timescales, with stage durations determined at random within the limits of analytical error of the absolute dating as given by the authors of the timescales. Such a randomization assumes that, contrary to the timescale of Harland *et al.*[33] accepted by Raup and Sepkoski, stratigraphical stages vary in duration This assumption, however, is strongly corroborated by the evidence from, for instance, late Cretaceous stages ranging from 1 Myr (Coniacian) to 15.5 Myr (Albian)

Analysis of the timing of mass extinctions naturally depends on the choice made from a variety of criteria for distinguishing between mass extinction and background extinction. This is not a trivial matter, for, as noted by Raup *et al.*[34], "it is entirely possible that the initial assumption . . . , that background extinction and mass extinction constitute two discrete populations, may not be correct". Rate or probability of extinction can be taken as extinction metrics. If extinctions are assumed to occur episodically rather than continually, the use of rate or probability per stratigraphical stage is reasonable. This is an *ad hoc* assumption but it may be substantiated by the evidence for catastrophic physical events (for example, extraterrestrial impacts) as the causes of mass extinction and by the absence of correlation between extinction and stage duration[1]. There is, in fact, strong evidence that the terminal Maestrichtian extinction is associated with[35–41], though not necessarily caused by[42–46], a major physical catastrophe, and only disputable evidence for coincidence of the late Eocene extinction peak with a physical catastrophic event[26,47,48]. However, evidence for association of other alleged mass extinctions with major catastrophes is largely absent[46]. On the other hand, the absence of correlation between extinction and stage duration may be caused by the fallacy of equal stage durations; besides, it is irrelevant to individual events. If the assumption that extinctions occur episodically is rejected, extinction rate or probability per Myr should be applied as the appropriate extinction metric, as in the analyses of extinction intensity through geological time[49,50].

Mass extinctions may be defined as peaks of the curve[1]. An arbitrary level of extinction metric may, then, be set to distinguish between true and spurious peaks caused by minor and inevitable fluctuations in background extinction[2,31]. Such a definition is appropriate for extinction metrics normalized for time, although it ignores the possibility of prolonged periods of unusually high extinction. As judged after Sepkoski's data[15], the latter may be the case with (Givetian?–) Frasnian–Famennian, Guadelupian–Dzhulfian and Carnian–Norian–Rhaetian. On the other hand, definition of mass extinctions as peaks of the curve rules out the possibility that two, or even more, consecutive stages were periods of mass extinction. Yet instantaneous mass extinction events may well be separated by one stage only. This may be an alternative interpretation of consistently high extinction in the late Devonian, late Permian and late Triassic if each stage was taken to represent an individual event. Therefore, such a definition is inappropriate for extinction metrics per stratigraphical stage. Instead, all rates or probabilities exceeding a certain arbitrary level can be recognized as mass extinctions. Alternatively, rates or probabilities more than, say, one standard deviation above the regression line over geological time can be identified as mass extinctions. The latter definition takes into account the substantial decrease in extinction intensity through time[49,50].

Table 1 shows the wide array of stages that appear as mass extinctions, depending on definition and timescale. There is some apparent regularity in the pattern of mass extinction events defined as peaks of extinction metrics not normalized for time (Table 1*A–C*), but this may well be pseudoperiodic behaviour of a stochastic system; when the absolute magnitude of alleged mass extinctions is taken into account in addition to their timing, such a model shows a better fit to the empirical pattern[51]. When the absolute magnitude of extinction peaks is disregarded, however, as in Raup and Sepkoski's analysis, such a definition causes the empirical pattern to represent only relative changes in extinction metric over relative geological time. Thus, the conditions for random walk are met. Given a strict random-walk model (0.5 probabilities of both increase and decrease in extinction metric, regardless of what happened at the preceding step), there is 0.25 probability that any particular stage represents a peak of extinction. Peaks are, then, expected to occur approximately every fourth stage, albeit with considerable variance. In fact, their actual distribution in the Phanerozoic does not significantly deviate from this expectation of random-walk model[52]. Given the assumption of equal stage durations for the Triassic to early Cretaceous and given also 6.2 Myr as the average stage duration in the Guadelupian to Quaternary, the 26-Myr periodicity of extinction peaks in that time interval also does not significantly deviate from expectation. (The average stage duration is considerably longer in the Palaeozoic and, therefore, 35–40-Myr periodicity can be expected for this era, which may explain the misfit between Palaeozoic and post-Palaeozoic data reported by Raup and Sepkoski[1].)

On the other hand, the Pliensbachian, Bajocian, Tithonian and Hauterivian rather consistently stand out as significant events in Table 1. These stages, however, also represent peaks of family origination[52], despite the absence of overall correlation between extinction and origination[53]. This may suggest that they were simply longer than adjacent stages, such a hypothesis is especially plausible for the Bajocian, as it also includes the Aalenian in the stratigraphical framework of Sepkoski's compendium.

There are few stages that do not appear as maxima of family extinction in Table 1, regardless of timescale and definition, although some of them might, given other randomizations of the timescale (only three randomizations were made for each of the two timescales[32,33] considered here). Only the Maestrichtian event stands out regardless of timescale and extinction metric. The late Permian maximum shifts from the Guadelupian to Dzhulfian under some timescales, and both of them depart strongly from the regression line of probabilistic extinction rate over time. It is a subjective matter whether the late Triassic maximum is placed in the Carnian, Norian or Rhaetian (in fact, both the Carnian and Norian strongly depart from the regression, whereas the Rhaetian was probably the shortest and, hence, gains prominence if the extinction metric is normalized for time). Under time-normalized extinction metrics, virtually each Jurassic and Cretaceous stage may be a period of mass extinction. If extinction probability per Myr is considered, there seems to be no important event during the 65 Myr of the Cenozoic (the range of variation of the extinction metric does not exceed 0.002 in the Cenozoic, whereas it is an order of magnitude wider in the late Cretaceous); and there is only one late Jurassic event throughout the Jurassic and early Cretaceous (~120 Myr), given some timescales.

When plotted against absolute time (Fig. 1), the pattern of mass extinctions becomes even more ambiguous. Even under the same definition of extinction maxima, there is much uncertainty about their timing. To overstate the case, the Pliensbachian and Bajocian events (if there were such) may be as close to each other as 5 Myr or as far apart as 40 Myr according to Harland *et al.*[33] (this range is somewhat smaller under the Odin timescale[32]). If the extinction metric is normalized for time, there is almost no invariance in time distribution of major events

Thus, the assumption that all major mass extinction events are correctly identified and that no event is either missing from or redundant in the list[2,24] is absolutely unwarranted. Non-parametric testing is, therefore, appropriate. The test developed by Raup and Sepkoski[1] and Rampino and Stothers[12], however, is very liberal, for it is insensitive to the absence of predicted events. Hence, the test is not expected to reject the claim for periodicity even if some minor peaks (for example, Olenekian, Bajocian, Callovian, Hauterivian, middle Miocene) are no longer regarded as true mass extinctions. Some extinction metrics pass that test for various cycle lengths under certain timescales (for example, events defined as in Table 1*D* fit the 26-Myr periodicity under the timescale of Harland *et al.* and no other timescale considered herein). Correlation to various external cycles could, then, be sought and *ad hoc* explanations given for absence of mass extinctions from where they should be (for example, absence of any early to middle Jurassic, early Cretaceous, and Cenozoic events in Table 1*D*). If, however, such absences are considered real, or if minor events are also taken into account, the appearance of periodicity vanishes in most cases.

It is crucial that what is a matter of interpersonal agreement is not accepted as a matter of discovery. There may be periodicity in late Permian to Quaternary mass extinctions, but we only know that there were periods (not necessarily short) of increased extinction in the late Permian, late Triassic, late Cretaceous and perhaps also late Jurassic and mid-Cretaceous. The exact timing of these events and what other events, if any, are to be considered mass extinctions, are subject to arbitrary decisions. It is those subjective decisions that result in the appearance of periodicity of mass extinctions However, even if the objective pattern of mass extinctions did indeed appear periodic, this might be due to combined effects of independent processes rather than to a single causal mechanism[51,52]. Hence, the biological and geological context of each event must be evaluated on its own.

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Outer hair cells in the mammalian cochlea and noise-induced hearing loss

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Hair cells in the mammalian cochlea transduce mechanical stimuli into electrical signals leading to excitation of auditory nerve fibres[1]. Because of their important role in hearing, these cells are a possible site for the loss of cochlear sensitivity that follows acoustic overstimulation[2,3]. We have recorded from inner and outer hair cells (IHC, OHC) in the guinea pig cochlea during and after exposure to intense tones. Our results show functional changes in the hair cells that may explain the origin of noise-induced hearing loss. Both populations of hair cells show a reduction in amplitude and an increase in the symmetry of their acoustically evoked receptor potentials. In addition, the OHCs also suffer a sustained

depolarization of the membrane potential. Significantly, the membrane and receptor potentials of the OHCs recover in parallel with cochlear sensitivity as measured by the IHC receptor potential amplitude and the auditory nerve threshold. Current theories of acoustic transduction[4–6] suggest that the mechanical input to IHCs may be regulated by the OHCs. Consequently, the modified function of OHCs after acoustic overstimulation may determine the extent of the hearing loss following loud sound.

Pigmented guinea pigs (200–250 g) were anaesthetized with pentobarbital sodium, Operidine and Droleptan (Janssen) before the animals were prepared for intracellular recording[1,7]. Acoustic test stimuli were delivered in a closed sound system with a Beyer DT48 earphone and the intense tones delivered by a Bruel and Kjaer 12.5-mm condenser driver (Type 4134). The frequency of the intense tone was fixed at 12.5 kHz (110 dB sound pressure level (SPL), 225 ms), which has been shown[3] to be the most effective stimulus for producing a loss of neural sensitivity in the region of the cochlea (15–20 kHz) from which the majority of IHCs were recorded. For the duration of the experiment cochlear sensitivity was assessed by the threshold response of the auditory nerve to gated tones (1 ms rise time). Identification of the hair cells depended on their electrical properties and their recording locations, which were correlated by intracellular dye marking. After a hair cell had been impaled, normal voltage responses were recorded to either a 600-Hz continuous tone (85 dB SPL), or in the case of IHCs, a gated tone (40–60 dB SPL) close to the characteristic frequency (c.f.) of the cell. The animal was then exposed to the 12 5-kHz tone at 1-s intervals and the responses of the hair cell recorded.

IHC receptor potentials (Fig. 1*a*) generated in response to low-frequency tones can reach 30 mV and demonstrate a depolarizing asymmetry as the intensity increases. As the stimulus frequency increases, the amplitude of the phasic component of the receptor potential decreases at about 6 dB per octave, probably as a direct result of the low-pass electrical properties of the hair cell membrane[1]. For high stimulus frequencies, at or near the c f. of the cell, the receptor potential produced by IHCs is recorded as a direct current (d.c.) component that can reach 15 mV in amplitude (Fig. 1*b*). It is thought that this depolarizing potential generated across the hair cell membrane controls transmitter release at the afferent synapse and subsequent afferent fibre excitation[1,7].

Resting membrane potentials of OHCs in the basal coil of the cochlea are significantly larger than those of IHCs (typically −70 to −85 mV compared with −45 mV) whereas the receptor potentials in response to low-frequency stimuli reach maxima of only 8–10 mV. With low-frequency stimulation the receptor potentials of OHCs are symmetrical at low intensities but, in contrast to IHCs, grow in the hyperpolarizing phase as the stimulus intensity increases (Fig. 1*c*) For tones close to the presumed c.f. of the cell no d c. receptor potential is recorded for stimulus levels less than 90 dB SPL (Fig. 1*d*). A small depolarizing d.c. component (up to 5 mV) may be recorded but only when the intensity of the stimulus approaches 110 dB SPL. It thus appears that the transfer characteristics of the IHCs and OHCs in the basal turn are quite different.

At the onset of the 12.5 kHz exposure tone (Fig. 2*a*), the IHC depolarizes to a level 10–20 mV above the resting potential at a rate that is indistinguishable from the rise of the tone burst (1 ms). The d.c. receptor potential then adapts for the remainder of the exposure. In a number of animals the adaptation demonstrated two separate stages (Fig. 2*d*), an initial rapid phase with a time constant of about 10 ms and then a slower phase where the cell repolarized at a rate of approximately 1 mV s^{-1}. At the offset of the loud tone, the membrane rapidly repolarizes to the pre-exposure resting potential with no obvious sustained elevation of the membrane potential (Fig. 2*d*). The d.c. voltage responses to the test tone at their best frequencies are reduced after the loud tone and recover between the acoustic overstimulations in a manner that can be described by a single exponential function with a time constant of about 200 ms. With multiple presentations of the loud tone these voltage responses show a cumulative decrease in amplitude This loss of sensitivity is also seen as a decrease in the amplitude of the compound action potential recorded from the auditory nerve; that is, with successive loud tones the cochlear shows a progressively greater loss of sensitivity.

The growth of the depolarizing d.c. receptor potentials recorded from the OHC (Fig. 2*b*) during the loud tone differs from the IHC responses in that they demonstrate a considerably longer time constant (4–5 ms) at the onset. In addition, the OHC membrane potential shows no sign of adapting but continues to depolarize during the tone. At the offset the OHC repolarizes, but only to a level above the pre-exposure or control value. With multiple exposures this results in a cumulative depolarization of the cell. For the cell shown in Fig. 2*c*, this depolarization accumulates at the rate of 0.3 mV after each presentation However, the membrane potential does return to the control level if sufficient recovery time is allowed between the 12.5-kHz tones.

The recovery of the IHC d.c. responses to tones close to their best frequencies is closely correlated ($r^2 = 0.97$) with the repolarization of the OHCs following an intense tone (Fig. 3) OHCs in the basal turn of the cochlea do not generate voltage responses to tones at the best frequencies of the adjacent IHCs, so it was not possible for us to compare the recovery in sensitivity of the OHCs with their membrane potentials at these frequencies. However, we were able to make this comparison at low frequencies where it was possible to monitor the phasic voltage responses of OHCs and IHCs to 600-Hz continuous tones before, during and after exposure to intense tones. The voltage responses of OHCs (Fig. 2*b*) were reduced following the intense tones and recovered in parallel with the repolarization of the membrane potential. The simplest explanation for this reduction

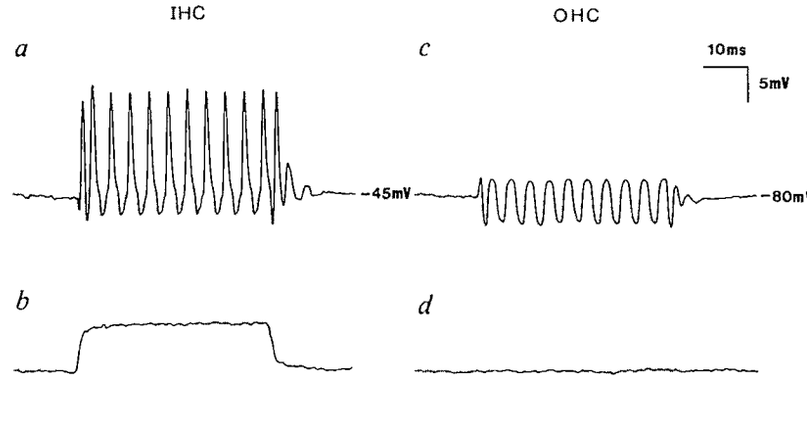


Fig. 1 Receptor potentials of an IHC and OHC recorded in the basal coil of the guinea pig cochlea in the same animal with the same electrode. *a*, *b*, Intracellular recordings from IHCs in response to a gated 200-Hz tone (*a*) and 16-kHz tone (*b*) at intensities of 85 dB SPL and 65 dB SPL respectively In both cases an asymmetrical depolarizing potential is generated during the acoustic stimuli Recordings from identified OHCs at 85 dB SPL show hyperpolarizing potentials during the 200-Hz tone (*c*) and no depolarizing potential during the 16-kHz (65 dB SPL) (*d*) presentation OHC responses are in phase with the cochlear microphonic recorded at the round window while the response of IHC phase leads the OHCs by about 90° for a stimulus frequency of 200 Hz. The transients associated with the onset and offset of the hair cell response are derived from transients in the gated tone. All records are single traces and are not averaged.

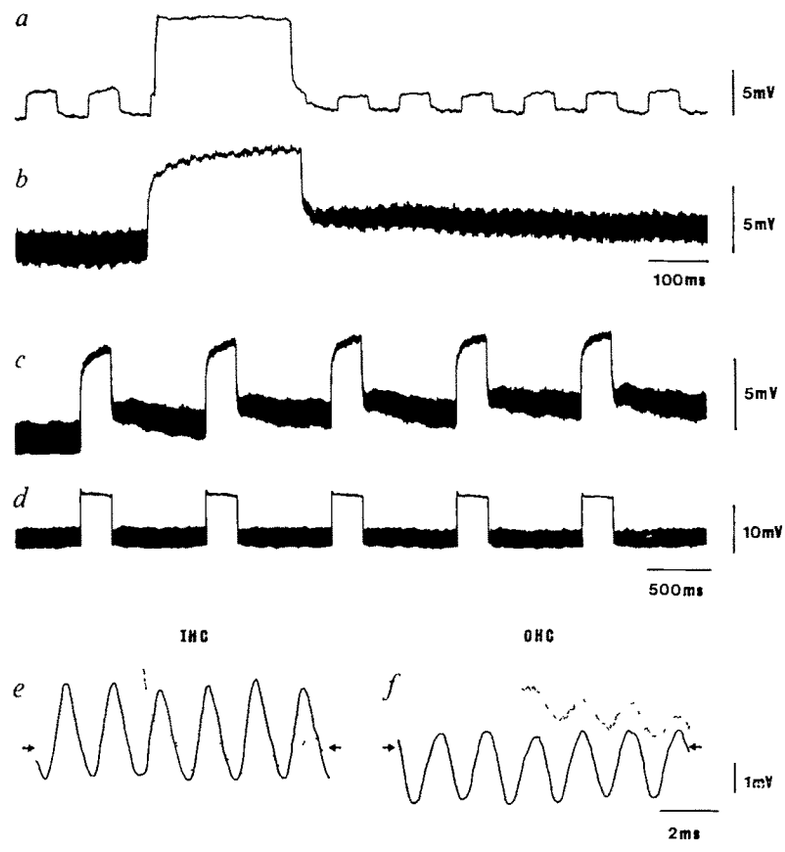


Fig. 2 *a*, Receptor potentials recorded from an IHC in response to a 16-kHz test tone (60 dB SPL) and a single 225-ms 12 5-kHz exposure. The voltage responses of the IHC to the 16-kHz tones decrease immediately following the loud tone and then recover with a time constant of approximately 200 ms. *b*, OHC voltage responses to a continuous 600-Hz tone (85 dB SPL) and a single presentation of the 12.5-kHz tone. The OHC continues to depolarize during the loud tone and at the offset, the phasic component of the receptor potential is reduced in comparison with the pre-exposure amplitude. It then recovers with a time course similar to that of the IHC. *c*, *d*, Voltage responses recorded in OHC and IHC respectively following five successive exposures to the loud tone. The IHC (*d*) demonstrates no sustained elevation of the membrane potential while the OHC (*c*) is progressively depolarized with each exposure *e*, IHC receptor potential evoked by a 600-Hz (85 dB SPL) tone before (continuous line) and immediately after acoustic overstimulation (dotted line) The latter demonstrates a decrease in amplitude and an increase in symmetry of the receptor potential around the resting membrane potential (arrow) and a phase lag when compared with pre-exposure recordings The most significant decrease in amplitude is in the depolarizing phase This decrease is not associated with a change in the resting membrane potential *f*, OHC receptor potential recorded before (continuous line) and immediately following (dotted line) the loud tone. The decrease in amplitude and increase in symmetry of the OHC receptor potential is qualitatively similar to that recorded for the IHC but the OHC also demonstrates a prolonged depolarization of the membrane potential that recovers in parallel with the sensitivity of the receptor potential. There is no obvious phase change in the waveform as recorded in the IHC. In contrast to the IHC, the OHC shows a greater loss of amplitude for the hyperpolarizing phase of the receptor potential. All records are single traces with no averaging

is a reduction in the driving voltage for the transducer current as a result of the OHC depolarization. However, measurements of the membrane impedance of the OHCs after the loud tone showed that the depolarization was not associated with an obvious conductance increase. It is possible that the decrease in the OHC receptor potential amplitude is the result of an increase in the intracellular levels of cations, where cation influx exceeds cation efflux during the intense tone There is a corresponding, but smaller, reduction in the amplitude of the IHC phasic response after the loud tone (Fig. 2*d*), but in contrast to the OHCs this was not associated with a persistent depolarization of the IHC membrane potential. Furthermore, it was clear that the voltage responses of IHCs were more vulnerable to acoustic overstimulation at their best frequency.

For both groups of cells the reduction in amplitude of their voltage responses to low frequency tones is greatest for one particular phase, depolarizing for the IHC (Fig. 2*e*) and hyperpolarizing for the OHC (Fig. 2*f*), which resulted in the increased symmetry of their waveforms. A phase delay of the IHC receptor potential of about 60° when compared with control responses was also recorded (Fig. 2*e*).

The primary source of mechanical input to cochlear hair cells is the relative shear between the tectorial membrane and their apical surfaces caused by basilar membrane motion during rarefactions (excitatory) and compressions (inhibitory) of the acoustic stimuli[7,8]. This causes displacement of their stereocilia and modulation of their transducer conductances[8-10]. Current theories of cochlear transduction propose that the OHCs, by virtue of the firm attachment of their stereocilia to the tectorial membrane[11], are in a position to regulate this displacement through a bidirectional mechano-electrical feedback loop[4-6]. That is, OHCs are both detectors and effectors[12,13] and can modify the system that drives them by feedback. In contrast, the IHCs are considered as primarily sensory cells responding to the shear of their free-standing stereocilia[11] through a fluid coupling to basilar membrane motion[7,9].

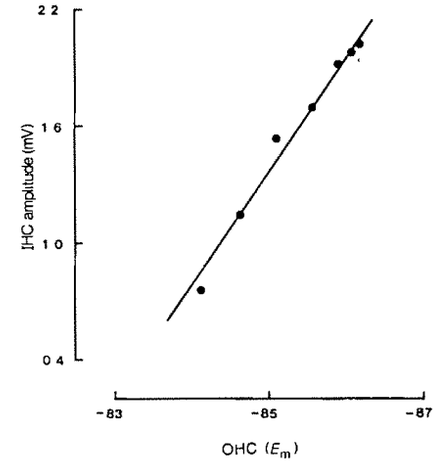


Fig. 3 OHC membrane potential (E_m) and the d.c. component of the IHC receptor potential (16 kHz) plotted over a 800-ms recovery period following exposure to a single 225-ms loud tone ($r^2 = 0.97$)

Our most important new finding is that OHCs are depolarized during and following acoustic overstimulation and that their repolarization parallels the recovery of the sensitivity of the IHCs and their afferent innervation. However, if OHCs are the protagonists in a mechano-electrical feedback loop, we do not know if the depolarization is the primary cause of the changes we have observed in the receptor potentials of the IHCs and OHCs during noise-induced hearing loss, or if it reflects more fundamental changes. In light of recent *in vitro* experiments on cochlear hair cells, there are two possibilities worth considering. One is that the primary site of such hearing losses are mechanical structures associated with the OHCs (stereocilia[14,15] and the tectorial membrane[6]), and that the depolarization of the OHCs is a response to the way these mechanical structures function

after overstimulation. The other possibility is that the OHC depolarization is a result of a massive influx of cations during the loud tone, and that one of these (Ca^{2+}) has interacted with contractile and structural proteins in the OHC[16]. This may lead to an alteration in the coupling of the stereocilia to the tectorial membrane, thereby resulting in a decrease in the mechanical input to the IHC. In this case the animal has suffered a noise-induced hearing loss.

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Human T-cell lymphotropic virus type-I antibodies in Falashas and other ethnic groups in Israel

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Epidemiological studies of the human T-cell leukaemia/lymphoma virus type I (HTLV-I), a type-C retrovirus of the human T-lymphotropic virus family, have used serological surveys to identify population subgroups possessing a high prevalence of naturally occurring HTLV-I-specific antibodies. Studies carried out to delineate the global distribution of the virus have demonstrated natural antibodies to HTLV-I in the serum of healthy donors from specific geographical areas, and have defined viral endemic areas in Japan[1–4], the Caribbean basin[5–7], Africa[8–10] and the southeastern United States[11]. Such studies have suggested that the prevalence of HTLV-I antibodies is directly correlated with age[3,12–14], is associated with the clinical syndrome of adult T-cell lymphoma[1–3,13,15–19], and is associated with transmission from mother to child[11,12,20]. A separate subtype of the human retrovirus, HTLV-II (refs 21, 22), has also been identified. The population of Israel in part comprises groups of immigrants of various ethnic and geographical origins. Because of this, and the fact that Israel has a highly developed public health system, we surmised that the ethnic groups in Israel could be used in a seroepidemiological survey of HTLV infection. The serological survey reported here demonstrates a high prevalence of HTLV-I antibodies in new immigrants from Ethiopia. This previously ethnically and geographically isolated group, the 'Black Jews' or 'Falashas', from the Gondar region in the northern rural highlands of Ethiopia, has the highest endemic rate of HTLV-I yet reported outside Japan.

Analysis of 1,048 serum samples from Israelis of different ethnic/geographical origins revealed major differences in the prevalence of antibodies to HTLV-I (Table 1). Of the 1,008 sera that were divided into ethnic groups I to VI in Table 1, 104 sera were positive for HTLV-I antibodies and 86 of these sera (83%) were donated by immigrants of Jewish Ethiopian (Falasha) descent. Thus, 37% of the sera from Ethiopian donors were positive for HTLV-I antibodies. Groups II and III, consisting of donors originating in North Africa and the Middle East, showed a 10-fold lower prevalence of antibodies to HTLV-I (4.2% and 3.7%, respectively). The remaining groups exhibited levels of prevalence (1–2%) similar to those found in non-endemic areas of Europe, the United States and the higher socioeconomic groups in urban Venezuela[11–12,23].

Serum samples that were positive for antibodies against HTLV-I in an enzyme-linked immunosorbent assay (ELISA) were further assayed in an absorption test in which the sera were preabsorbed with titrated, saturating concentrations of purified HTLV-I virion antigen, followed by assay of any non-complexed antibody in an HTLV-I ELISA. Whereas the

Table 1 Prevalence of antibodies to HTLV-I in various immigrant groups in Israel

Ethnic or geographical origin of serum donors	No. positive/ no. tested	% Positive
I, Ethiopia	86/233	36.9
II, North Africa (Morocco, Tunisia, Algeria, Libya)	7/168	4.2
III, Middle East Yemen	2/30)	
Iraq	1/61) 4/109	3.7
Iran	1/18)	
IV, Israeli-born (Ashkenazi)	3/150	2.0
V, Western Europe (Ashkenazi)	3/265	1.1
VI, Eastern Europe (Ashkenazi)	1/83	1.2
Other	3/40	NS*

We collected 1,048 serum samples randomly from apparently healthy donors of ages 19–65 yr, male and female, in Magen David Adom (Israeli blood bank) stations, mobile stations and army camps. Blood was drawn for the purpose of routine donation of blood. Sera were obtained from clotted samples remaining after routine clinical phlebotomy. Sera were stored frozen at −70 °C and shipped on dry ice to San Diego for assay. The information collected from the blood donors included age, country or region of birth, origin (ethnic and national) of the donors and their parents, and year of immigration to Israel. For the purpose of this survey, the donors were divided into six groups of ethnic and national origin. I, Ethiopia, II, North Africa (Morocco, Tunisia, Algeria, Libya), III, Middle East (Yemen, Iraq, Iran), IV, Israeli-born (predominantly of Ashkenazi descent), V, Ashkenazis born of Western European descent, VI, Eastern European-born Ashkenazis. All serum samples were collected in the spring of 1984, before the entry of thousands of additional new immigrants from Ethiopia in October–December 1984. Antibodies against HTLV-I antigens were detected by ELISA as developed by Cytotech Inc. HTLV-I was collected from the supernatant fluid of C10/MJ2 cells (provided by Dr R. C. Gallo). Virus-producing cells were pelleted by centrifugation at 3,500 r.p.m. in a RC 3 B centrifuge and the cell-free supernatant was filtered through a 0.45-μm Millipore filter. The extracellular virus was concentrated 50-fold by Pellicon Ultrafiltration (Millipore) and the concentrated virus pelleted at 25,000 r.p.m. on a cushion of 50% Renografin (Squibb). Virus was resuspended in phosphate-buffered saline (PBS) and layered onto a 15–50% Renografin gradient. Centrifugation was for 18 h at 25,000 r.p.m. in a SW28 rotor in a OTD 50 B Sorvall Ultracentrifuge. After gradient fractionation, the virus was resuspended in PBS and pelleted for 1 h at 25,000 r.p.m. Virus purified by this procedure is exceptionally pure and contains <2% non-viral protein (by weight). Solid-phase enzyme immunoassays were used for the detection of HTLV-I antibodies. Each donor serum sample was diluted 1:100 and assayed in duplicate on three separate days. A goat anti-human IgG (heavy+light chains) conjugated to glucose oxidase was used as a second antibody. To each microtitre well was added 200 μl of an enzyme substrate consisting of 200 μl 2,2'-azino-*bis*-3-ethylbenzthiazolinesulphonic acid, 200 μl horseradish peroxidase at 1 mg ml^{-1}, 3 ml of 18% β-D-glucose, in 25 ml 0.1 M phosphate buffer *p*H 6.0, and the development of a green colour was determined at 405 nm after exactly 30 min incubation in a Multiscan (Flow Laboratories) automated spectrophotometer. A reading of twice the background reading obtained with HTLV-negative sera in the same test was considered positive. In an attempt to preclude any false-positive serum antibody determinations, all positive sera were titrated and tested in a competition assay in which the serum samples were preabsorbed with purified HTLV-I virion antigen before being retested in the HTLV-I ELISA. Aliquots (250 μl) of a 1:100 dilution of donor serum samples were mixed with 1 μg of purified HTLV-I virus in 5 μl and incubated for 60 min at room temperature on a rotary shaker. After absorption with purified virus, the virus–antibody complexes were assayed for unabsorbed antibody by solid-phase immunoassay in duplicate. As a positive control serum in these assays, we used the HTLV-I antibody-positive serum SRL-SL11, a serum from a confirmed case of adult T-cell leukaemia from Japan. Preabsorption of confirmed HTLV-I-positive sera with our purified HTLV-I preparations completely removed all reactivity from these sera.

* Serum samples from a variety of sources, data not significant.

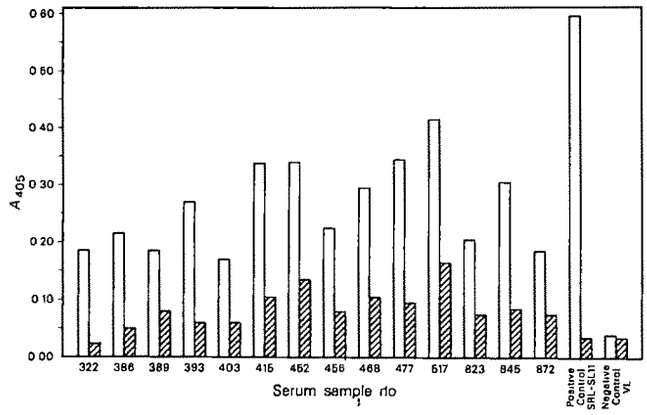


Fig. 1 Absorption of HTLV-I-positive Falasha serum samples with purified HTLV-I virions Open bars, unabsorbed serum samples, cross-hatched bars, HTLV-I-absorbed samples. See text for a discussion of the methods. All serum samples were assayed at a dilution of 1.100

antibodies that were present in most of the positive serum samples could thus be shown to be specific for HTLV-I, some 30% of the serum samples donated by Falasha individuals could not be absorbed completely with purified HTLV-I antigen. Figure 1 shows the results of absorption experiments with 14 positive Falasha serum samples; a control serum having a high titre of antibodies against HTLV-I (SRL-SL11) and an HTLV-I-negative control serum (VL) were included in the tests We found that antibodies from 4 of the 14 Falasha serum samples shown (samples 415, 452, 468 and 517) were incompletely absorbed although preabsorption with HTLV-I virions completely absorbed the antibodies from the high-titre control serum. For other serum samples (for example, samples 322, 386, 393 and 403), antibodies to HTLV-I were absorbed efficiently.

It is striking that in this serological survey of HTLV-I infection among immigrant groups in Israel, individuals of one ethnic group should display such a high prevalence of antibodies to HTLV-I and, by inference, have experienced a high frequency of virus infection. Our results show that 37% of the Black Jews of Ethiopian descent, most of them newcomers to Israel of 1 to 2 years at the time of sample collection, had been exposed to HTLV-I and had antibodies to the virus. Healthy Ethiopian-born immigrants to Israel thus have the highest frequency of exposure to HTLV-I encountered or reported to date outside Japan[4]. We are uncertain whether a similar prevalence occurs among non-Jewish Ethiopians in the highlands of that country or in other geographical rural and urban Ethiopian areas. Additional studies in other East African countries may reveal the origin of HTLV and may give rise to the isolation of variant subtypes of HTLV.

In competition immunoassays with purified HTLV-I virions as well as with purified HTLV-II virions (not shown), some HTLV-positive Falasha serum samples could be only partially absorbed. There are two possible explanations for this lack of absorption; either most antibodies in these serum samples are directed against viral core proteins p24, p19 and p15 which are not as readily available in the absorbing whole virions as are the viral envelope antigens, or the unabsorbed sera contain antibodies directed against a new subtype of HTLV which has cross-reacting antigenicities with HTLV-I, but also contains a unique antigenicity. We will investigate these possibilities using alternative methods.

HTLV-I has been shown to be associated with the development of certain T-cell leukaemias and lymphomas[2,5,13,18,19,24]. However, no direct causal relationship has been found between infection with HTLV-I and subsequent development of leukaemia or lymphoma. Unequivocal evidence linking HTLV-I infection with the development of human malignant disease has been hampered by the relatively low incidence of most cancers as well as by the low incidence of infection of most human populations with HTLV. Together with the long incubation period between exposure to HTLV and the possible development of disease, these difficulties have made prospective studies difficult because of the large number of persons who must be kept under observation. However, the Ethiopian immigrants to Israel, 37% of whom are positive for HTLV, may provide a practical population laboratory for prospective studies linking HTLV infection with subsequent disease. Studies concerning the modes of transmission of HTLV in a human population should also be feasible now that some 12,000 Ethiopian Jewish immigrants have moved to Israel and are living under improved hygienic conditions, with a modern public health system. These directions are being explored.

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Thymocyte subpopulation enriched for progenitors with an unrearranged T-cell receptor β-chain gene

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The development of T cells within the thymus is not well understood. It is known that thymocytes are derived from a progenitor cell in the bone marrow, the prothymocyte[1–5], and that cells in the subcapsular area of the thymus can give rise to progeny in both the cortex and the medulla[6]. However, it is not clear whether all medullary thymocytes are necessarily derived from cortical cells[7,8]. In particular, it has been difficult to distinguish intrathymic progenitor cells. Recently, however, Lesley *et al.*[9] have defined a thymocyte subpopulation which can be isolated by treatment of the thymus with cytotoxic anti-Thy-1 antibodies and that seems to be enriched for thymocyte progenitors as measured first by its ability to repopulate transiently the thymus of an irradiated host, and second, by its high content of cells bearing Pgp-1 (refs 10, 11), a cell-surface glycoprotein of relative molecular mass 95,000 that is present on most or all prothymocytes of the bone marrow and on fetal thymocytes[29], but on only a few per cent of cells in the adult thymus[10]. We show here that the gene encoding the β-chain of the T-cell receptor for antigen, which is rearranged during T-cell ontogeny[12–15], is predominantly in the germline configuration in these cells.

In the initial series of experiments, mouse thymocytes were treated in the presence of complement with a mouse anti-Thy-1.2

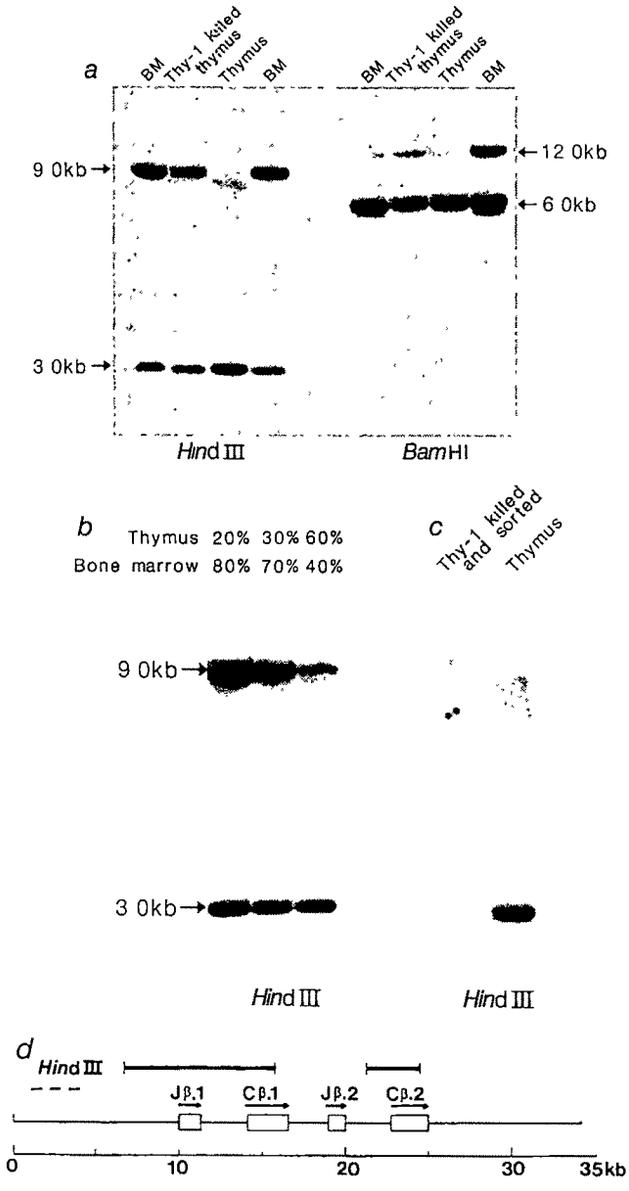


Fig. 1 *a*, Southern blots of Thy-1-killed thymus compared with bone marrow (BM) and total thymus. All lanes contained 10 μg digested DNA except the second BM sample which was 20 μg. *b*, Reconstruction experiment in which bone marrow DNA and thymus DNA were mixed in the proportions indicated. A total of 10 μg DNA was applied to each slot. *c*, Comparison of the Southern blots obtained with total thymus and Thy-1-killed thymus from which RB6-8C5-positive cells had been removed by fluorescence-activated cell sorting. Approximately 2 μg DNA from the Thy-1-killed and sorted population and 10 μg DNA from total thymus were loaded. *d*, Map of mouse germline T-cell receptor C_β-chain genes and their associated J_β segments indicating the origin of the *Hin*dIII restriction fragments observed in *a*–*c*. The map is derived from data presented in ref. 17.

Methods. Thymocytes from 50–80 C57BL/6 mice were treated with the mouse monoclonal anti-Thy-1.2 antibody HO 13.4.9[25] and complement as described in ref. 9. Briefly, $8–13 \times 10^9$ thymocytes were washed, treated with Tris-buffered ammonium chloride, and samples of 1×10^9 cells were spun down. Each cell pellet was resuspended in a total volume of 10 ml of HEPES-buffered Dulbecco's modified Eagle's medium with 2% newborn calf serum and containing tissue culture supernatant of HO 13.4.9 antibody (1:4 final dilution) and rabbit complement (1·20 final dilution). After incubation at 37 °C for 45 min, the cells were overlayed on 15 ml of Ficoll–Hypaque (Pharmacia) and centrifuged at room temperature for 25 min at 1,700*g*. The interface layer was recovered and washed. In some experiments, in which significant numbers of dead cells remained, the Ficoll–Hypaque treatment was repeated. After killing and Ficoll–Hypaque treatment, 0.2–0.4% of the input cells were recovered. A portion of the recovered cells was taken for flow cytometry (see Fig. 2 legend). High relative molecular mass DNA was prepared from the remaining cells by standard procedures and digested completely with either *Bam*HI or *Hin*dIII[26]. The digested DNA was fractionated on a 0.7% agarose gel and transferred to nitrocellulose filters. Filters were hybridized at 42 °C overnight with the ^{32}P-labelled *Eco*RI fragment of the 86T5 murine β-chain plasmid (0.25 μg; 2×10^8 c.p.m. per μg) in 40% v/v formamide, 4×S.S.C., 10% w/v dextran sulphate, 0.8×Denhart's solution, 500 μg ml^{-1} salmon sperm DNA in 7 mM Tris-HCl *p*H 7.6. Filters were washed twice for 30 min at room temperature, with 2×SSC/0.1% SDS and then washes were repeated with 0.1 SSC/0.1% SDS at 55 °C. Autoradiography was for 2–5 days.

monoclonal antibody (see Fig. 1 legend). After incubation, dead cells were removed by centrifugation over Ficoll–Hypaque and a portion of the viable cells was used to prepare high relative molecular mass DNA. The remaining cells were then stained with rat monoclonal antibodies against cell-surface antigens and analysed by flow cytometry using as a second-stage reagent a fluoresceinated goat anti-rat IgG which does not crossreact with mouse IgG. This made it possible to determine the cell-surface phenotype of the cell population in the presence of the mouse anti-Thy-1 antibody used for cytotoxic ablation. Concomitantly, by hybridizing DNA from these cells by the method of Southern[16] with a ^{32}P-labelled mouse T-cell receptor β-chain probe, it was possible to determine whether the T-cell receptor β-chain gene had undergone rearrangement in these cells. Figure 1*a* shows a typical experiment in which DNA from bone marrow, unfractionated thymus, and thymocytes surviving treatment with the cytotoxic anti-Thy-1 antibody had been digested with either *Hin*dIII or *Bam*HI restriction enzymes. In both cases, two bands hybridized to the β-chain probe, 86T5, which is an *Eco*RI cDNA fragment spanning the *V–J* (variable-joining) and *C* (constant) regions of the gene[14]. Comparison of the pattern obtained with total thymus and bone marrow shows the clear-cut distinction between the non-rearranged and rearranged genes. Whereas the higher relative molecular mass band obtained with both restriction enzymes is sharp when bone marrow DNA is probed, the total thymus gives a heterogeneous smear of bands. Most strikingly, the autoradiograph also clearly shows that the T-cell receptor β-chain gene in the 0.5% of thymocytes surviving treatment with anti-Thy-1 antibody and complement is predominantly non-rearranged. From reconstruction experiments using bone marrow and total thymus DNA in different proportions (Fig. 1*b*), it can be estimated that >80% of the T-cell β-chain genes in this thymocyte subpopulation are non-rearranged. The pattern of bands obtained from the *Hin*dIII digests are particularly informative. Previous work has shown that the 9-kilobase (kb) fragment extends upstream of the $J_\beta 1$ segment to the 3′-end of the $C_\beta 1$ gene (Fig. 1*d* and refs 17–19). Thus, this restriction fragment is variably reduced in size during diversity-joining rearrangements in the T-cell receptor β1-chain cluster. In contrast, the 3-kb fragment is derived from the *C* region of the β2-chain gene and is unaffected by rearrangements within the β2-chain gene cluster. Consequently, deletions in the 9-kb fragment serve as an indicator of all rearranged β1-chain genes, whereas the 3-kb fragment derived from the $C_\beta 2$ region serves as an internal control.

The antigenic phenotype of the thymus subpopulation was determined with two objectives in mind: first, to determine whether the phenotype of these cells differed from that of the bulk population of thymocytes, and second, to estimate the extent to which the cells surviving treatment with anti-Thy-1 antibody may be derived either from non-lymphoid thymic tissue or from mature haematopoietic cells derived from either blood

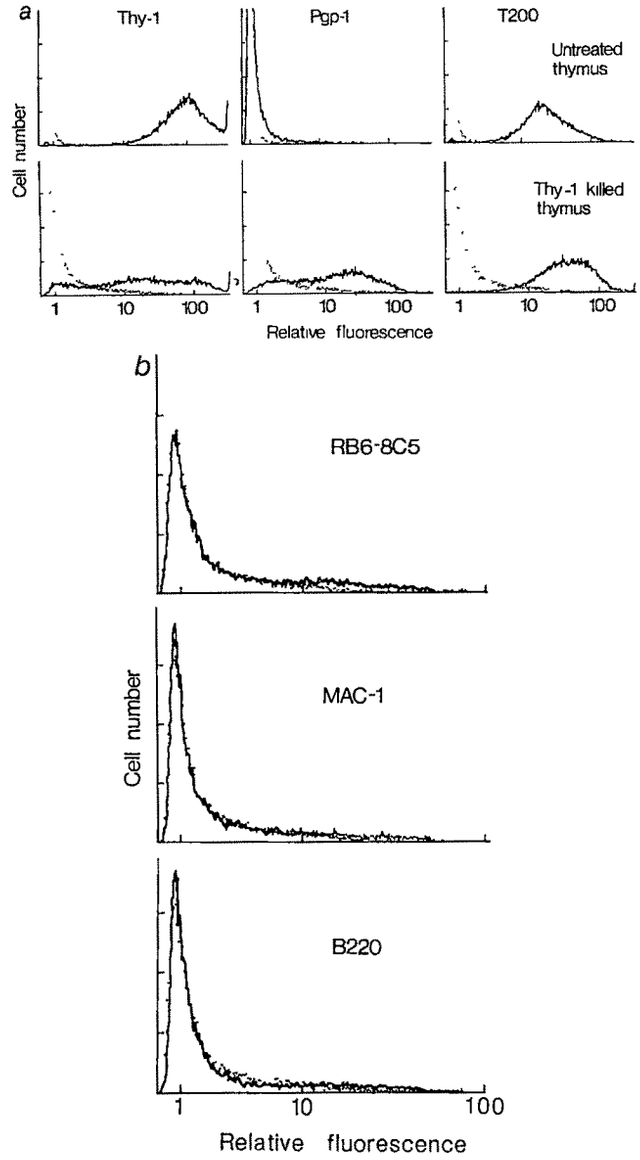


Fig. 2 Surface antigens of the anti-Thy-1-killed thymocyte population analysed by flow cytometry. Specific fluorescence (solid curves) is expressed relative to the peak of the background population in which normal tissue culture medium was substituted for monoclonal antibody containing tissue culture supernatant (dotted curves). *a*, Relative to untreated thymus, Thy-1-killed thymocyte populations show heterogeneous staining for Thy-1 with a subpopulation at or near background levels. Thy-1-killed thymocytes are enriched in highly fluorescent Pgp-1^+ cells and are essentially all $T200^+$. *b*, Most cells in Thy-1-killed populations do not stain with antibodies recognizing mature granulocytes (RB6-8C5), macrophages and monocytes (Mac-1), or B cells (B220) Ordinate: cell number

Methods. Aliquots of 1×10^6 untreated thymocytes and $2\text{–}5\times10^5$ thymocytes recovered after cytotoxic treatment with the mouse monoclonal anti-Thy 1.2 antibody, HO 13.4.9, and centrifugation on Ficoll-Hypaque as described in Fig. 1 legend ('Thy-1-killed thymocytes') were stained with tissue culture supernatants of the rat anti-mouse hybridomas C22/22.7.1.1 (ref. 27) (anti-Thy-1), IM7.8.1 (ref. 10) (anti-Pgp-1), I3/2.3[20] (anti-T200), RB6-8C5, which recognizes mature granulocytes (R. Coffman *et al.*, in preparation), M1/70.15[22] (anti-Mac-1), and RA3-3A1[21] (anti-B220), followed by staining with a fluorescein-labelled goat anti-rat immunoglobulin which had been absorbed to remove antibodies reacting with mouse immunoglobulin (Hyclone). Flow cytometry was carried out on the Salk Institute flow microfluorimeter. Data were stored as list mode data files which were gated to remove dead cells stained with propidium iodide as detected in the red channel. Fluorescein fluorescence in the green channel was log-compressed with a three-decade logarithmic amplifier before analog to digital conversion as previously described[28].

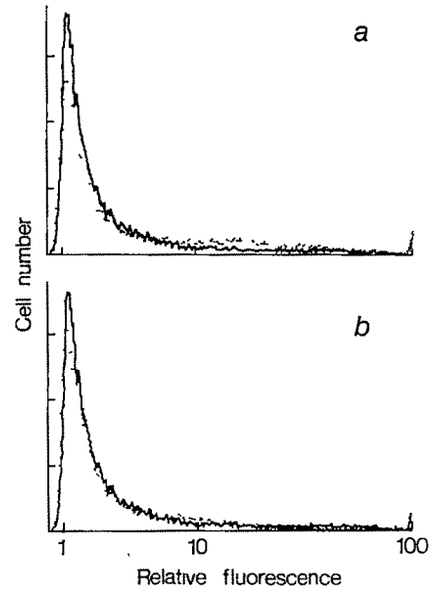


Fig. 3 Thy-1 population sorted for RB6-8C5-negative cells. *a*, Sorted (solid curve) and not sorted (dotted curve) Thy-1-killed thymocytes stained for RB6-8C5. The sorted population is depleted in cells staining with the RB6-8C5 antibody *b*, Sorted cells stained with RB6-8C5 (solid curve) compared with the background staining of the Thy-1-killed population which was not sorted (dotted curve). Specific staining approaches background levels.

Methods. Thymocytes were treated as described in the legend to Fig. 1. Thy-1-killed cells (1.4×10^7) from the same experiment as that illustrated in Fig. 2 were stained with RB6-8C5 antibody and fluorescent goat anti-mouse immunoglobulin. These stained cells were sorted on the Salk Institute BCS-176 cell sorter with the instrument optimized for the rapid selection of RB6-8C5-negative cells using anti-coincidence logic in the green fluorescence channel. The cells were sorted at a rate of 2,500 cells s^{-1}, deflecting three drops per event. The cells recovered from the sort were restained with various monoclonal antibodies and fluorescent goat anti-rat immunoglobulin as described in Fig. 2 legend.

or parathymic lymph nodes that may be present in the thymocyte preparations. The antigenic profile which has been consistently seen in four such replicate experiments is shown in Fig. 2. The data show that essentially all the cells express T200 glycoprotein[20] on their cell surface, suggesting that there are few, if any, non-lymphoid cells in the subpopulation of cells surviving treatment with anti-Thy-1 antibody. Moreover, the surviving cell population is enriched for Pgp-1^+ cells, consistent with the hypothesis that Pgp-1 glycoprotein is expressed on thymocyte progenitor cells. Most cells express Thy-1 but at levels much lower than the bulk thymocyte population and further analysis of these data indicates that a small subpopulation of cells expresses little or no Thy-1. With regard to the possible contribution of cells from either blood or parathymic lymph nodes, in this particular experiment ~5% of cells stained with a monoclonal antibody against B220, which is thought to be specific for B cells[21]; 7% stained with a monoclonal antibody against Mac-1, which is considered specific for myeloid cells[22]; and 13% stained with monoclonal antibody RB6-8C5, which is known to stain mature granulocytes (R. Coffman, J. Carty, L. Gemmell, J. Lesley, I. Weissman and D. Rennick, in preparation). On the basis of these results, it can be estimated that in this experiment the maximum contribution of non-T cells in the subpopulation of the thymus surviving treatment with anti-Thy-1 antibody is 25%. This is a maximum estimate since it is not known whether the RB6-8C5 monoclonal antibody reacts with thymocyte progenitor cells. It should also be noted that any contaminating mature T cells from blood or lymph nodes would yield DNA in which the T-cell receptor genes are rearranged.

These results make it unlikely that most of the non-rearranged T-cell receptor β-chain genes found in the subpopulation of

cells surviving treatment with anti-Thy-1 antibody were derived from cells other than thymocytes. However, to address this question more rigorously, an additional set of experiments were carried out. Thymocytes were first treated with anti-Thy-1 antibody and complement and then the viable cells remaining were isolated and stained with monoclonal antibody RB6-8C5. The cells were then fractionated by fluorescent cell sorting to remove cells staining with monoclonal antibody RB6-8C5. As shown in Fig. 3, this procedure reduced the number of cells staining with this antibody approximately 10-fold. The Thy-1 and Pgp-1 profiles of the sorted and nonsorted populations were essentially identical. When DNA from the sorted cells was digested with *Hin*dIII and blotted, it was clear that most cells had unrearranged β-chain genes although the limited amount of DNA available for analysis precluded precise quantitation (Fig. 1*c*).

In conclusion, we have shown that the subpopulation of thymocytes surviving treatment with anti-Thy-1 antibody and complement consists largely of cells in which the gene for the β1-chain of the T-cell receptor has not yet undergone rearrangement. This direct evidence that in the mouse the rearrangement of the β-chain gene is an intrathymic event is consistent with previous data from studies in man[23,24]. The cell-surface phenotype of these surviving cells indicates that the great majority of them are bona fide thymocytes that express Pgp-1 glycoprotein. We believe that the previous data showing that thymocyte subpopulations enriched in Pgp-1$^+$ cells can transiently repopulate the thymus and that Pgp-1$^+$ cells are found in abundance in early fetal thymus, together with the observations in the present paper, indicate that, although this population is still heterogeneous, thymocyte progenitor cells are enriched in this subpopulation of thymocytes. It is interesting that recently a thymic subpopulation representing 2–4% of unfractionated cells has been isolated in which the the β-chain genes have undergone rearrangement but the α-chain genes have not (M. Davis *et al.*, personal communication). Although other explanations are possible, these cells may represent the next stage of development of the progenitor cells identified in the present studies.

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Similarities between interleukin-2 receptor number and affinity on activated B and T lymphocytes

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Interleukin-2 (IL-2) is a T-cell-derived polypeptide hormone of 133 amino acids which exerts its growth-promoting activity via a surface receptor[1]. Originally, IL-2 was believed to be a unique growth factor for activated T cells[2]; more recent studies, however, have demonstrated that certain B-cell tumours[3] as well as normal activated B lymphocytes[4–6] express a surface molecule which is recognized by monoclonal antibodies directed against the IL-2 receptor. Furthermore, we[7] and others[6] have shown recently that activated B cells proliferate in response to either immunoaffinity-purified[8] or recombinant[9] IL-2. These controversial findings prompted us to undertake a detailed quantitative comparison of IL-2 receptor expression on activated B and T cells. We show here, using biosynthetically labelled IL-2 (^{3}H-IL-2) and anti-IL-2 receptor antibody (^{3}H-PC61) that activated B and T cells express both high-affinity (apparent dissociation constant, $K_d \sim 20$ pM) and low-affinity ($K_d \sim 1{,}000$ pM) IL-2 receptors. Binding of IL-2 to both classes of receptor is inhibited by the monoclonal anti-IL-2 receptor antibody PC61. B blasts express half as many total IL-2 binding sites or PC61 binding sites as T blasts, and the ratio of the number of low- to high-affinity receptors for each cell type is ~10:1. Immunoprecipitation analysis of surface-labelled blasts indicates that B and T cells have IL-2 receptors of similar relative molecular mass. Taken together, these data suggest strongly that IL-2 can act as a growth hormone for both B and T lymphocytes.

To quantitate and compare the number of IL-2 receptors expressed on activated B and T cells, we used ^{3}H-IL-2 in equilibrium binding assays[2]. Previous studies have shown that murine B cells activated with lipopolysaccharide (LPS) alone do not respond to[7], nor do they express detectable receptors for, IL-2[2,7]; however, B cells activated with LPS and anti-immunoglobulin antibodies (anti-Ig)[7] bind ^{3}H-IL-2 in a specific manner (Fig. 1*A*). Scatchard plot analysis of the specific binding data (using the Scatfit computer program) demonstrates that the activated B cells, like T cells, express two classes of IL-2 receptor. Figure 1*B* shows that the B blasts express 2,000 high-affinity IL-2 receptors ($K_d = 22$ pM) and ~10 times as many receptors of relatively lower affinity ($K_d = 1{,}330$ pM). Although the numbers of both classes of receptors expressed by concanavalin A (Con A)-activated T cells are about twice those on LPS/anti-Ig-activated B cells, the K_d values are very similar (17 and 1,270 pM for high- and low-affinity receptors, respectively). Binding of ^{3}H-IL-2 to low-affinity receptors is saturable at an IL-2 concentration of 3–5 nM (not shown); furthermore, it can be inhibited either with unlabelled IL-2 or with monoclonal antibody PC61 (ref. 10; Fig. 1*A*) but not with monoclonal antibodies to other cell-surface antigens (not shown). Although previous studies[2] reported that activated T cells express a single class of high-affinity receptors ($K_d = 5$–20 pM), a more recent report by Robb *et al.*[11] demonstrated that a second class of receptors of lower affinity is also detectable on murine and human T cells when much higher concentrations of IL-2 than those previously tested are used. Our results with various cell types (Table 1) including human phytohaemagglutinin (PHA)-induced blasts (not shown) show a 50–100-fold difference in K_d between the two classes of IL-2 receptor and an 8–14-fold excess of low- over high-affinity receptors, which is in close quantitative agreement with studies involving other hormone systems such as epidermal growth factor[12,13] and insulin[14,15]. Furthermore, both

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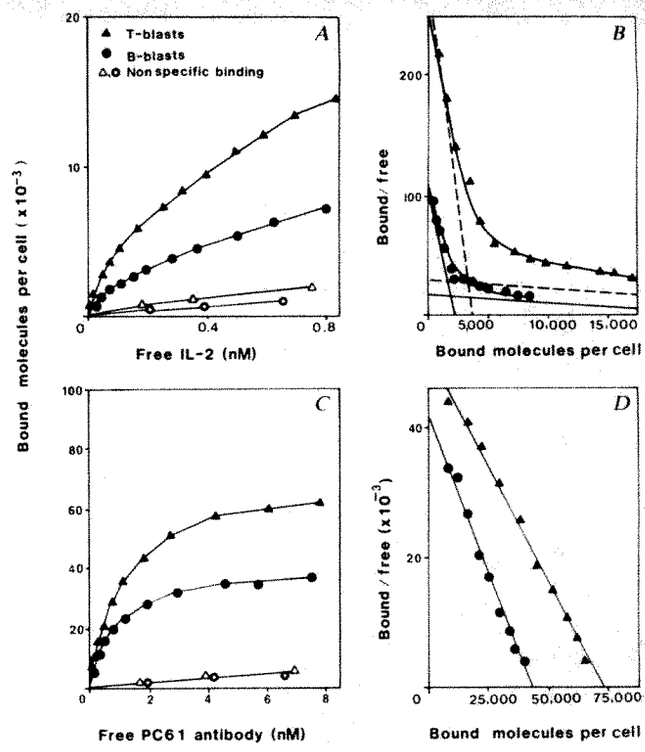


Fig. 1 Comparison between equilibrium binding of IL-2 and PC61 on B (●) and T(▲) blasts. In the presence of either excess unlabelled IL-2 or PC61 antibody, the binding of ^{3}H-IL-2 was reduced by 90–95% at all IL-2 concentrations tested (*A*, open symbols). The binding of ^{3}H-PC61 was inhibited by excess unlabelled PC61 (*C*, open symbols) but not by unlabelled IL-2 (not shown). Specific equilibrium binding (solid symbols) of IL-2 and PC61 is shown in *A* and *C*, respectively, after subtraction of nonspecific binding (open symbols). Scatchard plot analyses of these data were performed using a weighted least-squares curve-fitting computer program[22] (*B* and *D*). For IL-2 binding (*B*), the computer chose a two-binding-site model over a one-site model as the best fit to the experimental data. Broken lines (T blasts) and solid lines (B blasts) represent the computer-generated high- and low-affinity binding components. Similar results were obtained in three independent experiments.

Methods. B blasts were prepared as described elsewhere[7] by culturing purified C57BL/6 splenic B cells in the presence of LPS, anti-Ig beads and 10% EL4 supernatant (SN). T blasts were prepared by culturing spleen cells from the same mouse strain at a concentration of 2×10^6 ml^{-1} in the presence of 1.5 μg ml^{-1} Con A and 10% EL4 SN. Both cell types were collected after 4 days, centrifuged on a Ficoll/Hypaque gradient and washed extensively. The preparation of affinity-purified ^{3}H-IL-2 and the IL-2 binding assay have been described in detail[2,8]. In the experiments described here, all incubations were carried out at 4 °C in the presence of azide. For PC61 binding, aliquots of 10^6 cells were incubated for 20 min (equilibrium binding occurs within 15 min) in the presence of various amounts of protein A–Sepharose-purified ^{3}H-PC61 (ref. 10) in a volume of 200 μl. Before the antibody binding assay, cells were pre-incubated in the presence of 10% normal rat serum in order to block nonspecific binding of PC61 via Fc receptors. Free and cell-bound PC61 were measured by liquid scintillation counting after centrifugation through a paraffin–silicon oil gradient[2]. The number of bound IL-2 and PC61 molecules per cell was calculated given the specific activities of 2,500 c.p.m. ng^{-1} and 210 c.p.m. ng^{-1}, respectively. The M_r of IL-2 and PC61, as judged by SDS-PAGE, is 15K and 170K, respectively.

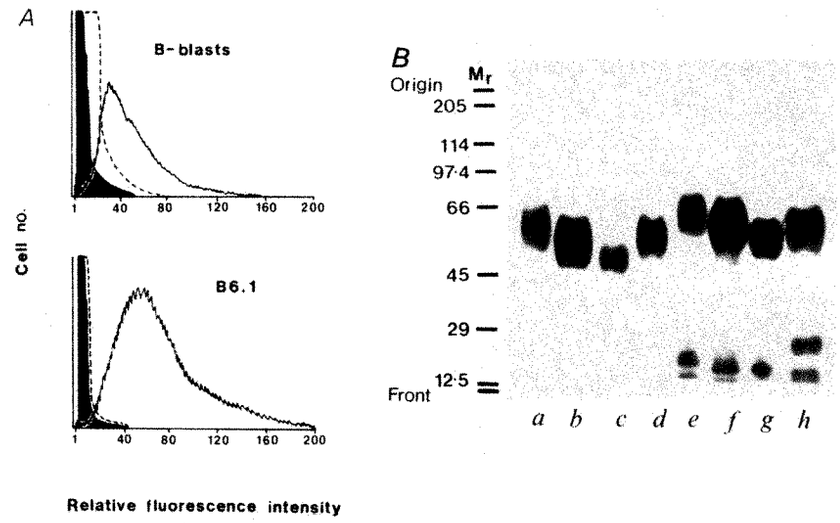


Fig. 2 *A*, Most activated B and T cells express the PC61 antigen. Solid lines represent the fluorescence profile of the PC61 antigen distribution (B blasts on gain 16; B6.1, gain 4); broken lines show the level of nonspecific staining after incubation in the presence of excess unlabelled PC61; shaded areas show background staining with avidin-fluorescein isothiocyanate (FITC) alone. In B-blast preparations, Thy-1-positive cells were not detected above background fluorescence. In addition, we have shown previously[7] by immunoperoxidase staining that cultures of B blasts contain >97% immunoglobulin-positive cells and <0.3% Thy-1-positive cells. The mean fluorescence intensity of B6.1 cells was about five times higher than for B blasts when normalized to the same gain, which is consistent with the data shown in Table 1. *B*, PC61 immunoprecipitates membrane components of similar M_r from different types of IL-2-responsive cells. Electrophoretic patterns (lanes *a–d*, non-reduced; *e–h*, reduced conditions) were obtained after immunoprecipitation with PC61 of lysates obtained from various types of IL-2-dependent cells: B1.8 (*a*, *e*), Con A blasts (*b*, *f*), B blasts (*c*, *g*) and BD2.10 (*d*, *h*). Lane *g* was exposed for 7 days, and all other lanes for 4 days.

Methods. *A*, B blasts were prepared as described in Fig. 1 legend, centrifuged on a Ficoll/Hypaque gradient, washed and pre-incubated in 10% normal rat serum. 5×10^5 B blasts or B6.1 cells were then incubated (20 min at 4 °C) in 100 μl of phosphate-buffered saline (PBS) containing 2 μg of biotinylated PC61 antibody in the presence or absence of 100 μg unlabelled PC61. The cells were washed, incubated for 20 min with avidin-FITC and analysed on a fluorescence-activated cell sorter (FACS II; Becton and Dickinson) gated to exclude non-viable cells[23]. *B*, Cells ($1–2\times10^7$) were surface-labelled with ^{125}I by the glucose oxidase–lactoperoxidase method of Hubbard and Cohn[24] as modified by Luescher *et al.*[25]. The labelled cells were lysed in 0.5 ml of cold 1% recondensed Triton X-114 in 50 nM Tris-HCl (*p*H 7.7) containing 0.3 M NaCl and 5 mM EDTA. The aqueous and detergent phases were separated according to Bordier[26]. Triton-insoluble material was removed by centrifugation of the lysates for 3 min at 4 °C. The lysates were heated to 30 °C for 1 min, then spun in an Eppendorf centrifuge at room temperature for 1 min. The aqueous phase was removed, the detergent phase re-dissolved in 0.5 ml of cold buffer and the separation repeated. The lysates were pre-cleared for immunoprecipitation by sequential addition of 5 μl normal rat serum, 20 μl rabbit anti-rat immunoglobulin and 12.5 μl protein-A–Sepharose 4B (twice), each step followed by incubation for 20 min on a rotating mixer. The pre-cleared lysates were then incubated for 60 min with purified PC61 antibody coupled to Sepharose 4B, followed by extensive washing of the lysates with two buffers: (1) 10 mM phosphate buffer (*p*H 8.2) containing 0.05% SDS, 0.05% deoxycholate, 0.5% Nonidet P-40 (NP40) and 10 mM EDTA; and (2) 0.12 M Tris-HCl (*p*H 8.2) containing 0.5 M NaCl, 0.5% NP40 and 10 mM EDTA. The immunoprecipitates were analysed by SDS-PAGE according to Laemmli[27] on 7.5–15% gradient gels. Samples were reduced with 0.1 M dithiothreitol and denatured by boiling in 4% SDS. After electrophoresis, the gels were autoradiographed at −70 °C on Kodak XAR film. M_r standards were: myosin (205K), β-galactosidase (114K), phosphorylase B (97.4K), bovine serum albumin (66K), ovalbumin (45K), carbonic anhydrase (29K) and cytochrome *c* (12.5K).

Table 1 Comparison of the number of binding sites for PC61 antibody and IL-2 on activated B and T cells

Cell type (no. of experiments)	IL-2 binding sites			PC61 binding sites	Ratio PC61/IL-2 binding sites
	Total ($\times 10^{-3}$)	Ratio low/high	K_d (pM)	($\times 10^{-3}$)	
B blasts (3)	18 ± 3	10	23/1,150	40	2.2
T blasts (3)	37 ± 5	12	19/1,360	73	2.0
B1.8 (2)	29 ± 4	8	17/1,420	48	1.7
B6.1 (2)	79 ± 9	9	16/690	165	2.1
BD210 (3)	166 ± 14	14	25/1,800	285	1.7

Equilibrium binding of IL-2 and PC61 antibody was measured as described in Fig. 1 legend and the data processed by computer analysis. For each cell type we show the total number of IL-2 binding sites per cell, the ratio of the number of low- to high-affinity IL-2 binding sites per cell and the K_d of both classes of receptor (high/low affinity). The number of PC61 binding sites was also calculated by Scatchard analysis. The final column shows the ratio of the number of PC61 antibody binding sites to the total number of IL-2 binding sites per cell. The following IL-2-independent cell lines were found to be negative for specific binding of both IL-2 and PC61; Jurkat, EL4, BW5174, LSTRA, and mouse spleen and thymus cells. B and T blasts were prepared as described in Fig. 1 legend. B1.8 (ref. 20) is an antigen-dependent cytotoxic T lymphocyte (CTL) clone derived from a (Cremona × CD)F_1 mouse. B6.1 and BD2.10 are two antigen-independent CTL clones derived from C57BL/6 and (C57BL/6 × DBA)F_1 mice, respectively[21].

the K_d values that we find for the high-affinity IL-2 receptors and the absolute number of receptors of both classes (Table 1) agree closely with those of Robb *et al.*[11]; however, they report a 10–20-fold lower K_d (20–40 nM) for the low-affinity IL-2 receptors. Because we have found that the dissociation rate of IL-2 from the low-affinity receptors is very fast ($t_{1/2} \sim 45$ s at 4 °C in medium; our unpublished data), we believe that this discrepancy in the estimates of the K_d of the low-affinity receptors results from the additional washing steps in the protocol used in the previous study. Regardless of these technical considerations, it still remains to be established whether the low-affinity receptors are biologically functional or whether they are a recycled or degraded form of the high-affinity receptor.

Recently, we have characterized a rat monoclonal antibody, PC61 (ref. 10), which specifically recognizes the murine IL-2 receptor as shown by the fact that it (1) inhibits by 95% the IL-2-dependent proliferation of cloned cytolytic T cells; (2) binds specifically to IL-2-responsive cells; (3) inhibits by >90% (at either 4 °C or 37 °C) the binding of ^{3}H-IL-2 to both high- and low-affinity receptors; and (4) immunoprecipitates from surface-labelled activated T cells a band of similar relative molecular mass (M_r) to those reported for other anti-IL-2 receptor antibodies[4,5,16]. Figure 1*c* compares the equilibrium binding of ^{3}H-PC61 by LPS/anti-Ig-activated B blasts and Con A-activated T blasts; the binding is saturable and can be competed for by excess unlabelled PC61, and Scatchard analysis (Fig. 1*d*) reveals that both types of cell bind PC61 with equal affinity (K_d ~1 nM). At saturation, T blasts bind 73,000 molecules per cell, almost twice the number bound by B blasts, which is consistent with the IL-2 binding data. Our results are also in good agreement with the recent finding that rat and human T blasts bind, respectively, 75,000 and 100,000 anti-IL-2 receptor antibody molecules per cell[17,18].

To date, it has been difficult to reconcile estimates of the number of IL-2 receptors expressed per cell as measured by IL-2 binding, with those derived from binding of anti-receptor antibody. Table 1 compares quantitative IL-2 and PC61 binding to a variety of IL-2-dependent cells derived from different mouse strains. Even over an almost 10-fold range in total number of receptors per cell, the number of PC61 binding sites is about twice (1.7–2.2) the number of IL-2 binding sites. A similar finding for human cells has been published recently[11]; PHA-blasts and HUT-102 cells were found to have 2.0–2.6 times as many anti-

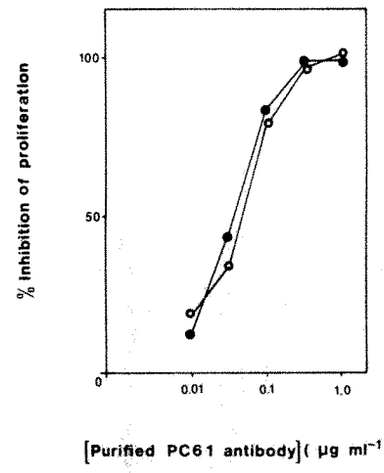


Fig. 3 PC61 antibody inhibits the IL-2-dependent proliferation of activated B and T cells. B blasts were prepared as described in Fig. 1 legend, collected on day 3, centrifuged on a Ficoll/Hypaque gradient and washed. 10^4 cells were cultured in 200 μl of RPMI-1640 medium containing 10% fetal calf serum (FCS). Cloned murine IL-2-dependent T cells (CTLL[28]) were washed three times and seeded at 4×10^3 cells per 200 μl of Dulbecco's modified Eagle's medium (DMEM) containing 5% FCS. To each well was added a suboptimal amount of IL-2 (giving 75% of maximum response in each assay) and various amounts of purified PC61 antibody. Cell proliferation was measured at 72 h (B blasts, ○) or 24 h (CTLL, ●) after a 6-h pulse with 1 μCi of ^{3}H-thymidine per well. A typical experiment is shown here, where symbols represent mean values of triplicate wells (s.e.m. = 10% of mean). Maximum c.p.m. in the absence of PC61 was 3.3×10^4 and 1.6×10^4 for B blasts and CTLLs, respectively. Thymidine incorporation, in the absence of added IL-2, was 500 c.p.m. for both populations. Similar results were obtained from 48-h cultures of CTLLs. From several experiments, the concentration of antibody giving 50% inhibition of proliferation was in the range 25–50 ng ml^{-1} for both cell types.

Tac[18] antibody binding sites as IL-2 binding sites. The reason for this difference is not clear, but a possible explanation is that two antibodies bind to a single IL-2 receptor. Alternatively, the difference could be explained by the binding of PC61 to molecules which do not bind IL-2 with a detectable affinity.

Flow microfluorometric analysis (Fig. 2*A*) shows that the distribution of PC61 antigen on LPS/anti-Ig-activated B blasts is similar to that seen on an IL-2-dependent cytolytic T-cell clone. Over 95% of the cells express the PC61 antigen. Analysis by SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 2*B*) reveals that the PC61 antigen immunoprecipitated from LPS/anti-Ig-activated B cells, T blasts or cloned T cells migrates as a broad band with an apparent M_r of 50,000–70,000 (50–70K) (reduced form) and 45–65K (non-reduced). This major band is similar to those reported by others using independently derived anti-IL-2 receptor antibodies[4,5,18,19]. In addition, three minor bands of lower M_r are consistently observed under reducing conditions; one at ~25K and two at 14–17K. The presence of low-M_r bands (20–25 K) has been reported previously for murine T cells[16]. The broad, high-M_r band precipitated from B blasts migrates slightly faster (reduced and non-reduced) than that of T cells and the low-M_r bands are less pronounced (Fig. 2*B*, lane *g*). We have also observed bands of similar M_r following precipitation with the 7D4 anti-IL-2 receptor antibody[4] (provided by Dr T. R. Malek). The heterogeneity in immunoprecipitation patterns observed between the different cell types studied (also reported for a variety of human T cells[19]), necessitates further biochemical analysis, such as peptide mapping, before the fine structure of the IL-2 receptors expressed by activated B and T cells can be compared.

Figure 3 shows that PC61 inhibits the IL-2-dependent proliferation of activated B and T cells at antibody concentrations very similar to those which inhibit the binding of IL-2 to its receptor (unpublished results).

Thus, by several independent criteria, the IL-2 receptors expressed by LPS/anti-Ig-activated B cells and activated T cells are similar. Quantitative ^{3}H-IL-2 binding reveals that B blasts, like a variety of independently derived activated T cells, express two classes of IL-2 binding sites; one has a high affinity for IL-2 and the other a 50–100-fold lower affinity. Binding to both classes can be inhibited by the same monoclonal antibody. The ratio of the number of low- to high-affinity sites per cell for B blasts (10) is within the range found for activated T cells (8–14). In addition, studies using antibody PC61 show that: (1) >95% of activated B and T cells express IL-2 receptors; (2) IL-2-dependent proliferation of both cell types is inhibited at similar antibody concentrations; and (3) bands of similar M_r are immunoprecipitated from activated T and B cells. On the basis of these results, we propose that IL-2 promotes the growth of appropriately stimulated B and T cells via a common mechanism.

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γ-Interferon transcriptionally regulates an early-response gene containing homology to platelet proteins

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Interferons are a family of proteins first identified by their ability to induce cellular resistance to infection by many viruses. In addition to the antiviral properties it shares with the α- and β-interferons, γ-interferon (IFN-γ), a lymphokine secreted by activated T cells, activates macrophages, stimulates B cells, increases fibroblast and endothelial cell resistance to many non-viral intracellular parasites and modulates cell-surface proteins central to immune cell regulation[1–13]. To identify molecules involved in the IFN-γ response and characterize their modulation, we have isolated genes that are induced following recombinant IFN-γ treatment of U937 cells, a histiocytic lymphoma cell line with monocytic characteristics[14,15]. We report here the molecular cloning and characterization of a gene regulated by rIFN-γ in U937 cells as well as in human mononuclear cells, fibroblasts and endothelial cells. Messenger RNA from this gene is induced within 30 min of rIFN-γ treatment and demonstrates maximal (>30-fold) accumulation within 5 h. Increased transcription is partly responsible for this accumulation. This gene encodes a protein of relative molecular mass (M_r) 12,378 which has significant amino-acid homology to platelet factor-4 and β-thromboglobulin, two chemotatic proteins released by platelets on degranulation. This IFN-γ-inducible protein may be a member of a family of proteins involved in the inflammatory process.

A complementary DNA library was constructed from poly(A)$^+$ RNA isolated from U937 cells treated with recombinant γ-interferon (18 h induction, 100 U ml^{-1}) and cloned into the *Pst*I site of pBR322 (refs 16, 17). Approximately 10,000 recombinants were screened for rIFN-γ-inducible sequences by differential colony hybridization using cDNAs prepared from rIFN-γ-induced and control poly(A)$^+$ RNAs. One cDNA clone, pIFNγ-31, showed a dramatic induction by Northern blot analysis and was chosen for further characterization.

rIFN-γ induces the accumulation of pIFNγ-31 mRNA in a dose-dependent manner. Figure 1*a* shows the accumulation of pIFNγ-31 mRNA in U937 cells in response to an 18-h incubation with various concentrations of rIFN-γ and rIFN-β. A detectable increase in pIFNγ-31 mRNA levels was seen at 10 U ml^{-1} rIFN-γ, with maximal accumulation at 100 U ml^{-1} rIFN-γ. pIFNγ-31 mRNA seems to be preferentially modulated by rIFN-γ; 100 times more antiviral units of rIFN-β than rIFN-γ were needed to produce a comparable effect (Fig. 1*a*, compare 10 U ml^{-1} rIFN-γ and 1,000 U ml^{-1} rIFN-β). Even at very high doses of rIFN-β (5,000 U ml^{-1}), the accumulation of pIFNγ-31 mRNA was only 40% of the maximal accumulation seen with 100 U ml^{-1} rIFN-γ. The submaximal stimulation of pIFNγ-31 mRNA by rIFN-β may result from cross-reactivity of this molecule with the IFN-γ receptor at very high IFN-β levels[18].

Figure 1*b* shows the kinetics of pIFNγ-31 mRNA accumulation at optimal rIFN-γ concentration (100 U ml^{-1}, see Fig. 1*a*). mRNA levels doubled within 30 min of induction and reached a maximal 34-fold increase within 5 h. By 18 h, half-maximal levels were observed, decreasing to a two-fold level by 34 h. In addition, 50 μg ml^{-1} of cycloheximide, which inhibited protein synthesis by >97%, did not inhibit the accumulation of pIFNγ-31 mRNA (data not shown). The rapidity with which pIFNγ-31 mRNA accumulates after rIFN-γ induction and its insensitivity to protein synthesis inhibitors suggest that this is a primary response gene in IFN-γ induction[19].

To determine whether IFN-γ affects transcription, the nuclear run-on transcription assay on isolated nuclei was used to measure the level of transcription of pIFNγ-31 mRNA in resting and rIFN-γ-treated cells. Figure 1*c* demonstrates that rIFN-γ increases the rate of transcription of pIFNγ-31 mRNA, thus establishing a role for γ-interferon in transcriptional activation. Recent studies[20,21] have demonstrated that the α/β-interferons can affect the transcription of several genes within minutes after the binding of interferon to its receptor. The analysis of an IFN-α-responsive gene from neuroblastoma cells suggests that IFN-α is also regulating post-transcriptional events[20].

To elucidate the function of pIFNγ-31, we determined the inducibility of this mRNA in different human cells. Figure 2 shows a panel of paired RNAs using various human cell lines, human peripheral blood mononuclear cells (PBM) and normal human spleen. To date, we have observed no primary cells which express this gene in the absence of rIFN-γ induction, in contrast

to DR-α and HLA-B7, two molecules shown to be induced by IFN-γ, which are constitutively expressed in many cells. pIFNγ-31 mRNA was preferentially induced in a primary human foreskin fibroblast after an 18-h incubation with 100 U ml^{-1} rIFN-γ. Human umbilical cord vein endothelial cells expressed pIFNγ-31 mRNA after incubation with rIFN-γ (100 U ml^{-1}, 12 h), but not rIFN-β (100 U ml^{-1}, 12 h) (data not shown). Figure 2 also demonstrates that pIFNγ-31 mRNA is induced in PBM and U937 cells within 5 h of treatment with 100 U ml^{-1} rIFN-γ, again in contrast to the regulation of DR-α and HLA-B7 mRNAs in

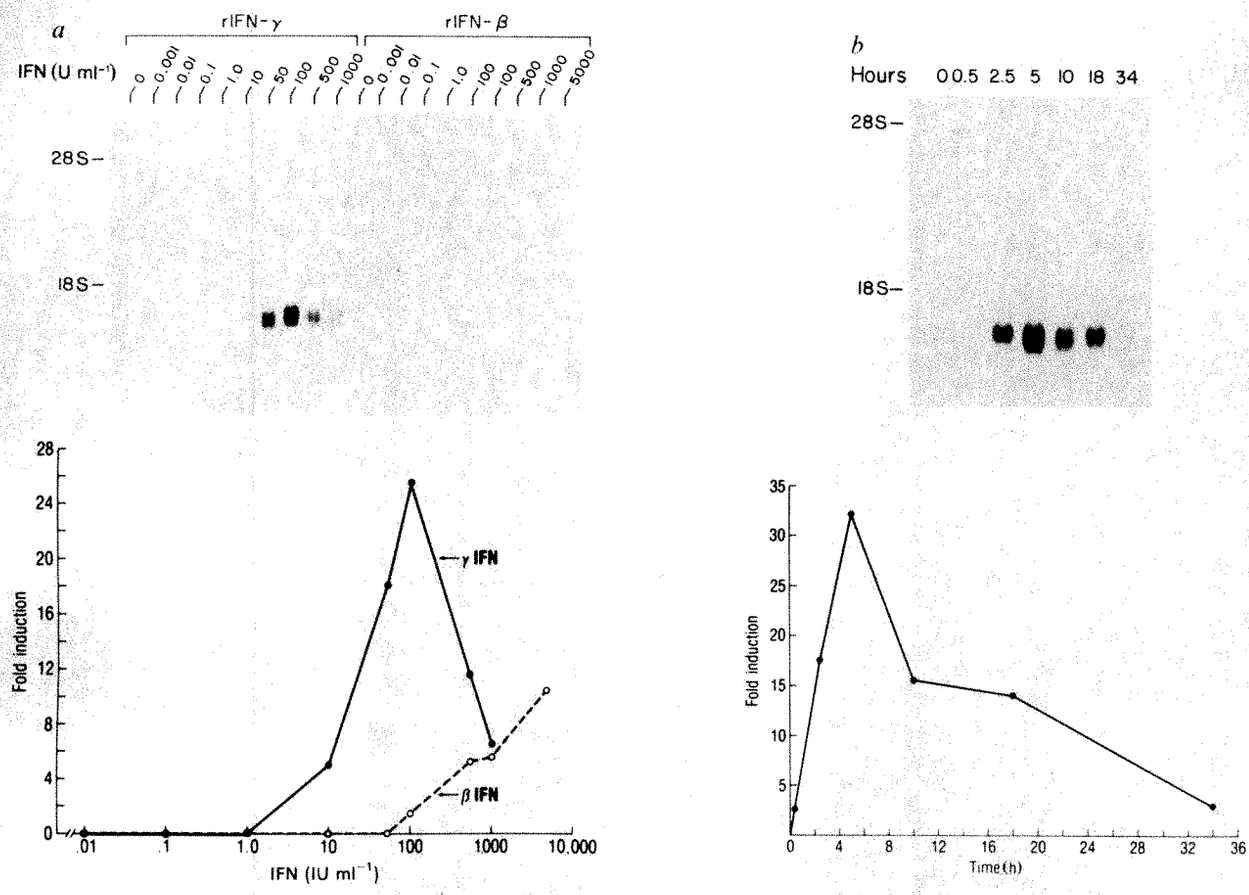


Fig. 1 Dose response, kinetics and transcription of pIFNγ-31 mRNA in response to IFN. *a*, Dose response of pIFNγ-31 mRNA accumulation in response to interferons; *b*, kinetics of accumulation; *c*, nuclear run-on transcriptional analysis. *a*, Top, autoradiogram of RNA blot analysis of the accumulation of pIFNγ-31 mRNA in U937 cells in response to 18-h incubations with various concentrations of rIFN-γ and rIFN-β. Bottom, quantitation of the RNA blot shown in the top panel by densitometer scanning (fold induction = densitometer units induced/densitometer units uninduced). *b*, Top, autoradiogram of the time course of pIFNγ-31 mRNA accumulation in U937 cells in response to 100 U ml^{-1} rIFN-γ, with quantitation shown in the bottom panel. 28S and 18S ribosomal RNA size markers are indicated in both upper panels. *c*, Plasmids containing the indicated genes were bound to nitrocellulose filters and hybridized with ^{32}P-labelled run-on transcripts from nuclei isolated at 3.5 h after addition of 100 U ml^{-1} rIFN-γ to U937 cells. To quantify the transcriptional induction, the hybridizing signals were excised and counted by liquid scintillation. The relative rates of transcription are expressed as parts per million (p.p.m.). p.p.m. = (c.p.m. sample − c.p.m. control)/total input c.p.m. C, Control (uninduced); γ, rIFN-γ induced.

Methods. *a*, *b*, The human histiocytic lymphoma cell line U937[14] was grown in RPMI-1640 supplemented with 10% fetal bovine serum. The recombinant interferons produced in *Escherichia coli* were generously provided by Hoffmann-La Roche. All the interferons were standardized by Hoffmann-La Roche using a cytopathic effect inhibition assay with vesicular stomatitis virus in WISH (HeLa) cells; activity was expressed in international antiviral units (U) per ml. The rIFN-γ lot used had a specific activity of 6.3×10^6 U mg^{-1} and the rIFN-β was clinical-grade material with a specific activity of 8×10^7 U mg^{-1}. The cells were induced with the interferons for this and all subsequent experiments at $2\text{–}5\times10^5$ cells ml^{-1}. RNA was isolated by the guanidine thiocyanate method[38] and 10 μg per line was fractionated on a 1% agarose/2.2 M formaldehyde gel and transferred to nitrocellulose as described elsewhere[39]. The filters were hybridized with ^{32}P-nick-translated pIFNγ-31.0 cDNA insert at a specific activity of $2\text{–}5\times10^8$ c.p.m. per μg, washed in stringent conditions (0.1 × SSC, 0.1% SDS, 52 °C), then subjected to autoradiography with Kodak XAR film using a Dupont Cronex intensifying screen at −70 °C for 2.5 h. *c*, RNA chain elongation in isolated nuclei was performed as described elsewhere[40], except that unincorporated nucleotide was removed by gel filtration of the purified nuclear RNA[41]. 10^8 nuclei were resuspended in 200 μl of incubation buffer containing 5 mM dithiothreitol, 5 mM $MgCl_2$, 90 mM KCl, 50% glycerol, 20 mM HEPES *p*H 7.6, 1 mM GTP, 1 mM CTP, 3 mM ATP, 250 μCi of ^{32}P-UTP, and incubated for 10 min at 37 °C. RNA purification was performed as described previously[40]; DNase I digestion was followed by deproteination with phenol, chloroform/isoamyl alcohol extractions, and concentration by ethanol precipitation. Approximately 2 μg of recombinant plasmid were linearized with *Bam*HI, boiled for 10 min, base denatured, neutralized and applied to 0.45-μm nitrocellulose filters[42] using a Minifold dot apparatus (Schleicher & Schuell). 6.9×10^6 c.p.m. ml^{-1} and 5.6×10^6 c.p.m. ml^{-1} of trichloroacetic acid-precipitable counts were used in rIFN-γ-induced and control hybridization reactions, respectively. Hybridization, RNase A and T_1 digestion, and washing of filters were performed as described previously[40]. The X-ray film was exposed at −70 °C with an intensifying screen for 4 days. The HLA-B7 cDNA plasmid was generously provided by S. Weissman[43].

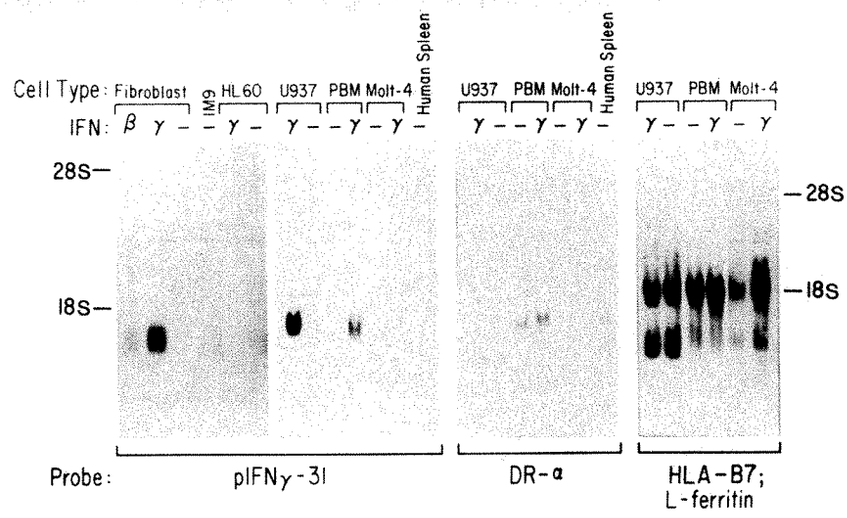


Fig. 2 Cell distribution and IFN-γ dependence of pIFNγ-31 mRNA accumulation. RNA blots were performed on the RNAs extracted from the indicated cells induced with rIFN-γ (γ) or induced with rIFN-β (β), or which received no treatment (−). Filters were hybridized with the probe indicated below the blots. **Methods.** RNA transfers contained U937, PBM, MOLT-4 and normal human spleen RNA. RNA (10 μg per lane) was fractionated; the 18S and 28S ribosomal RNAs are indicated on the far right. In the blot probed with HLA-B7 and L-ferritin, the HLA-B7 mRNA is the larger of the two transcripts. The far-left blot contains fibroblast, HL60 and IM9 RNA. In this blot, 2 μg of poly(A)$^+$ RNA was fractionated per lane; the 18S and 28S rRNA for this filter are indicated on the far left. U937 cells, PBM and MOLT-4 cells were induced with 100 U ml^{-1} rIFN-γ for 5 h. The fibroblasts and HL-60 cells were induced with 100 U ml^{-1} of both rIFN-β and rIFN-γ for 18 h. RNA isolation, RNA blot analysis and interferon induction were performed as indicated in Fig. 1 legend, except that the fibroblasts were confluent monolayers. The primary human foreskin fibroblast FS4 was obtained from S. Gupta and grown in minimum essential medium supplemented with 10% fetal calf serum (FCS). The cell lines HL60, IM9 and MOLT-4 were grown in RPMI-1640 supplemented with 10% FCS. Normal human peripheral blood was fractionated on a Ficoll–Hypaque gradient and the PBM were cultured in RPMI-1640 + 10% FCS. Normal human spleen was obtained from an incidental splenectomy and RNA was isolated by the guanidine thiocyanate method[38]. The HLA-DR α-chain cDNA plasmid was generously provided by J. Lee[44] and the human L-ferritin cDNA plasmid by M. H. Dorner[45].

PBM and U937 cells where, 5 h after rIFN-γ stimulation, no significant modulation in mRNA levels was detected. When cells from the human T-cell leukaemia cell line MOLT-4 were incubated for 18 h with 100 U ml^{-1} rIFN-γ, an increased accumulation of HLA-B7 mRNA was observed, but no expression of either DR-α mRNA or pIFNγ-31 mRNA. HL-60 cells, a promyelocytic leukaemia cell line, showed no induction of pIFNγ-31 mRNA after an 18-h incubation with 100 U ml^{-1} rIFN-γ. Induction of pIFNγ-31 mRNA by rIFN-γ in mononuclear cells, fibroblasts and endothelial cells indicates that this gene is induced in a diverse population of cells in response to IFN-γ.

The DNA sequence of pIFNγ-31 was obtained from two overlapping cDNA clones (Fig. 3*a*). Each base was sequenced from both strands using the dideoxy method of Sanger and Coulson[22] and the chemical cleavage method of Maxam and Gilbert[23]. The longest open reading frame begins at nucleotide 70, extending for 294 nucleotides before terminating at nucleotides 364–366 with TAA. pIFNγ-31 mRNA contains the classical polyadenylation signal AAUAAA, 12 bases from the start of the poly(A) tail (underlined in Fig. 3*a*). There is a long 811-base 3′-untranslated region, a 294-nucleotide coding region and a ~220-nucleotide 5′-untranslated region.

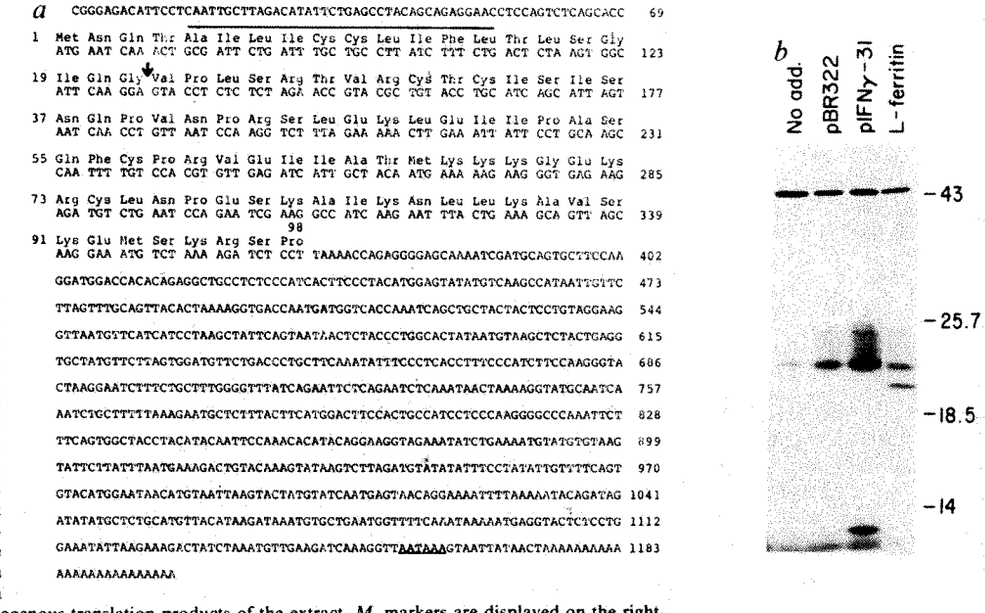


Fig. 3 Primary structural analysis of pIFNγ-31 cDNA clones. *a*, Determined nucleotide sequence and predicted amino-acid sequence of pIFNγ-31; *b*, hybridization selection and translation of pIFNγ-31 mRNA. The coding strand is displayed in *a*; the number of the nucleotide residue at the right end of each line is given. The polyadenylation signal is underlined. The deduced amino-acid sequence is shown above the nucleotide sequence, numbered beginning with the putative initiating methionine. The hydrophobic core of the predicted signal sequence is overlined and the predicted signal peptidase cleavage site is indicated by an arrow. Hybridization selection and translation of pIFNγ-31 mRNA (*b*) were performed to confirm the open reading frame predicted above. Poly(A)$^+$ mRNA from U937 cells induced by 5 h treatment with rIFN-γ (100 U ml^{-1}) was annealed to nitrocellulose filters containing pIFNγ-31 and control clones pBR322 and L-ferritin. The mRNA was eluted and translated *in vitro* using a rabbit reticulocyte lysate extract and ^{35}S-methionine label. An autoradiogram of the translation products fractionated on a 12.5% SDS-polyacrylamide gel is shown in *b*. The 'no add.' lane shows only the endogenous translation products of the extract. M_r markers are displayed on the right. **Methods.** *a*, DNA sequence analysis was performed using the dideoxy method of Sanger and Coulson[22] and the chemical cleavage method of Maxam and Gilbert[23]. The entire coding region of pIFNγ-31 consisted of two overlapping cDNAs. pIFNγ-31.0 was isolated in the differential colony hybridization screening, extended from nucleotides 451 to 1,198, and used to rescreen a size-fractionated cDNA library. 10,000 recombinants were screened with an *Eco*RI/*Pvu*III fragment of pIFNγ-31.0. Seven colonies hybridizing to this probe were picked, rescreened, then analysed by restriction mapping. One clone, pIFNγ-31.7, contained the most 5′-nucleotide sequences and extended from nucleotides 1 to 875. *b*, Hybridization selection of specific mRNAs was performed essentially as described previously[46]. Plasmid DNA (10 μg) were linearized and bound to 0.45-μm nitrocellulose filters as indicated in Fig. 1 legend. Hybridization was carried out at 50 °C for 3 h in a 250-μl volume containing 65% formamide, 20 mM PIPES (*p*H 6.4), 0.1% SDS, 0.4 M NaCl, 50 μg yeast transfer RNA and 45 μg poly(A)$^+$ RNA. Filters were then washed six times with 50 ml of 0.15 M NaCl, 0.01 M Tris *p*H 7.6, 1 mM EDTA, 0.5% SDS at 65 °C. The filters were washed twice more with 50 ml 0.15 M NaCl, 0.01 M Tris *p*H 7.6, 1 mM EDTA at 65 °C. Selected RNAs were eluted by boiling in 300 μl of H_2O for 60 s and then quickly freezing in a dry-ice ethanol bath. The eluted RNAs were extracted with $CHCl_3$/phenol and then ethanol precipitated in 0.25 M sodium acetate with 20 μg yeast tRNA as a carrier. The pellets were washed once with 70% ethanol and dissolved in 5 μl H_2O. Each selected RNA (1 μl) was used to program an Amersham RRL *in vitro* translation system using ^{35}S-methionine as label.

a

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MNQTAILICCLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRSLEKLEIIPASQFCPRVEIIATMKKKGEKRCLNPESKAIKNLLKAVSKEMSKRSP   γIP-10
                 ..: :.....: :.:: ...:.:. :.. :: ...:::. :.: : ::. ..   :...: .
          EAEEDGDLQCLCVKTTSQ-VRPRHITSLEVIKAGPHCPTAQLIATL-KNGRKICLDLQAPLYKKIIKKLLES                    PF4-Hu
      :..:.. .  . ...:.:.....  .::: ...:.:. :.  ::. ...:: ::.: : :::...   :..:: .
   ESSFPATFVPLPADSEGGEDEDLQCVCLKTTS-GINPRHISSLEVIGAGTHCPSPQLLAT-KKTGRKICLDQQRPLYKKILKKLLDGDES          PF4-Bo
             :  ..:: :.....  ..:.....:.:  .  : .::.:::. :.: : :::.:.  :::...
      GKEESLDSDLYAELRCMCIKTTS-GIHPKNIQSLEVIGKGTHCNQVEVIATL-KDGRKICLDPDAPRIKKIVQKKLAGDESAD              βTG-Hu
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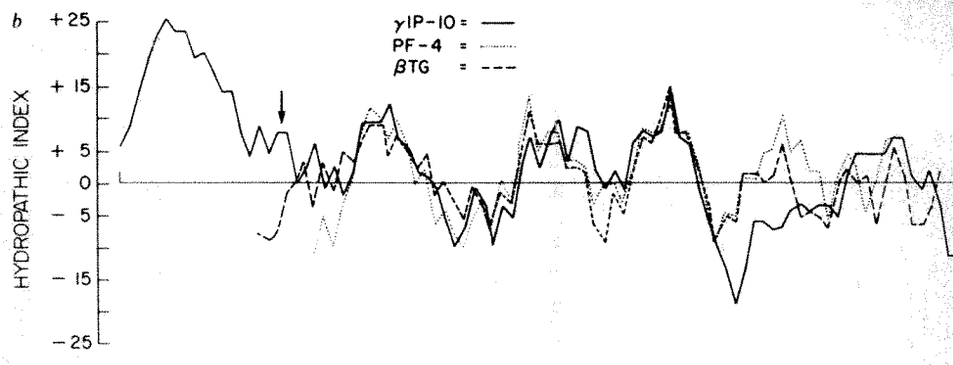


Fig. 4 *a*, Amino-acid homology between γIP-10, PF-4-human (PF-4), PF-4-bovine (PF4 30) and β-TG-human (βTG-Hu). The optimized amino-acid alignment, which includes gaps, was done by implementing a Needleman–Wunsch algorithm[47] over the initial dfastp alignment. The one-letter amino-acid notation is used. A colon indicates an exact match, a period a 'conservative mutation' (can be obtained with only one DNA base change) and a blank a 'non-conservative mutation' (at least two DNA bases would have to be changed). *b*, Hydropathy profile of γIP-10 (——), PF4-Hu (···) and βTG-Hu (---). The hydropathic value of each amino acid was determined by using the scoring system of Kyte and Doolittle[48]. The average hydropathic value at residue i is calculated across seven residues from $i-3$ up to and including $i+3$. Positive values indicate hydrophobic regions and negative values hydrophilic segments. Each protein was plotted independently and overlaid to obtain the composite seen above. The hydropathic value of each amino-acid residue is plotted from the amino terminus to the carboxyl terminus (left to right along the abscissa). The entire coding region of γIP-10 is plotted, but only the mature PF-4 and β-TG proteins are plotted. The arrow indicates the predicted signal peptide cleavage site of γIP-10.

The deduced amino-acid sequence (Fig. 3*a*) beginning with the initiation codon AUG at nucleotides 70–72 encodes a protein of 98 amino acids and predicts a protein of M_r 12,378, termed γIP-10. The highly hydrophobic stretch of 10 amino acids (residues 5–14 overlined in Fig. 3*a*) is typical of the hydrophobic core of a signal peptide[24]. Application of the empirical rules proposed by Von Heijne[25] predicts the signal peptidase cleavage site to be after residue 21 (Gly, arrow Fig. 3*a*). Analysis of the amino-acid sequence reveals no other hydrophobic regions which could serve as a transmembrane region and anchor the protein in the membrane. This suggests that the protein is secreted and that the unmodified mature protein has a M_r of 9,979. Consistent with γIP-10 containing a signal peptide, its mRNA is enriched in the microsomal fraction (data not shown). The predicted amino-acid sequence for γIP-10 contains no *N*-linked glycosylation sites (Asn-X-Thr/Ser).

Hybridization selection and translation of pIFNγ-31 were performed to confirm the predicted open reading frame. Poly(A)$^+$ mRNA from U937 cells treated for 5 h with rIFN-γ was annealed to nitrocellulose filters containing pIFNγ-31 and control clones (L-ferritin and pBR322) and then eluted. The selected mRNA was translated using a rabbit reticulocyte lysate with ^{35}S-methionine label and fractionated on a 12.5% SDS-polyacrylamide gel (Fig. 3*b*). mRNA from clone pIFNγ-31 directs the synthesis of a ~12,000-M_r protein which is not present in the control lanes, confirming the existence of an mRNA which can direct the synthesis of the γIP-10 protein in rIFN-γ-induced U937 cells.

Using the program 'dfastp' (ref. 26), we searched the National Biomedical Research Foundation Library for any protein containing homology to the predicted amino-acid sequence for γIP-10. Two proteins, platelet factor-4 (PF-4) and β-thromboglobulin (β-TG), have significant homology to the predicted amino-acid sequence of γIP-10. Alignment of these three sequences is shown in Fig. 4*a*. γIP-10, PF-4 and β-TG demonstrate 35% identity in a 62-amino-acid overlap. β-TG has been reported to contain two disulphide bonds linking residues 16–42 and 18–58 (ref. 27). When PF-4, β-TG and γIP-10 were aligned for maximum homology (Fig. 4*a*), the cysteine residues of the mature proteins were perfectly aligned, suggesting preservation of tertiary structure among these molecules. Consistent with this notion is the similarity in the hydropathy profile of the proteins (Fig. 4*b*); there is a striking conservation of hydrophobic (peaks) and hydrophilic (troughs) domains between the three proteins. The hydrophobic core of the predicted leader peptide of γIP-10 is clearly evident with the predicted signal peptidase cleavage site indicated by an arrow in Fig. 4*b*.

PF-4 and β-TG proteins are stored in the α granule of platelets and secreted concomitantly during the platelet-release reaction[28]. Although the *in vivo* functions of PF-4 and β-TG remain unclear, *in vitro* studies suggest that these proteins are important mediators of inflammation and wound healing[29–37]. The homology between γIP-10, PF-4 and β-TG suggests that γIP-10 may also be involved in the inflammatory response. The rapid and specific induction of pIFNγ-31 mRNA by IFN-γ in a diverse population of cells suggests that γIP-10 may be central to the IFN-γ response. The role for this family of related polypeptides in inflammation and immune cell regulation as well as the molecular characterization of transcriptional activation by IFN-γ are under investigation.

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Insulin receptor tyrosine kinase is defective in skeletal muscle of insulin-resistant obese mice

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Obese syndromes of genetic origin or experimentally induced are characterized by resistance to insulin both *in vivo* (association of hyperglycaemia and hyperinsulinaemia) and *in vitro*[1]. Thus, skeletal muscle of obese mice, which is the most important target organ for the action of insulin, displays a reduced response to insulin[2–4]. This hormonal resistance cannot be explained by the moderate decrease in the number of insulin receptors found in obese animals[3–5]. In fact, it is generally believed that a biochemical event occurring very early after binding of insulin to its receptor, which is the first step in insulin action, is defective in obesity. One of the earliest post-binding events so far recognized, and which is thought to have a key role in cellular signalling by the insulin receptor, is the insulin-stimulated phosphorylation of its receptor[6–8]. In an effort to localize the defect responsible for the insulin resistance in obesity, we have studied the insulin receptor protein kinase activity and we show here that insulin receptors from skeletal muscles of insulin-resistant obese mice have an altered kinase activity for phosphorylation of both the receptor itself and of exogeneous substrates.

The animals used in the present study were male Swiss albino mice. Mice were rendered obese with a double injection of gold thioglucose at 3 weeks of age and they were used at 25–30 weeks when obesity was fully expressed[3,4]. Lean controls were age- and sex-matched animals. All animals were fed *ad libitum* on a standard diet until the time of killing. Following gold thioglucose injection, which induces hyperphagia, mice became markedly obese (Table 1). They were hyperglycaemic and insulin-resistant since insulin induced *in vivo* a poor hypoglycaemic effect. Using wheat-germ lectin chromatography, partially purified insulin receptors were prepared from skeletal muscle of lean and obese mice, and they were then used in a cell-free phosphorylation assay as described previously[7–9]. For all tissues studied so far, insulin enhances several-fold the phosphorylation of its receptor β-subunit (relative molecular mass, M_r, 95,000); the other receptor subunit, termed α-subunit (M_r 130,000) and containing the insulin binding site, is generally not phosphorylated[10]. Similar results were found with receptors derived from rat skeletal muscle[11]. With insulin receptors obtained from skeletal muscle of lean mice, insulin markedly stimulated the phosphorylation of a polypeptide of M_r 95,000 (Fig. 1); this phosphoprotein corresponds to the β-subunit of the insulin receptor, as it was immunoprecipitated by a highly specific antiserum[12] to insulin receptor (lanes *d*, *h*), but not by normal serum (lanes *c*, *g*). More importantly, the effect of insulin on phosphorylation of its receptor was less pronounced in the preparation from insulin-resistant obese mice (lane *h*) compared with lean controls (lane *d*). The decreased insulin effect observed in obese mice is unlikely to result from a residual occupation of insulin receptors due to the high levels of circulating insulin in obese mice (Table 1), as basal phosphorylation was negligible in both groups of mice (lanes *b*, *f*).

Table 1 Characteristics of mice used (*a*) and binding capacity of partially purified insulin receptor preparations (*b*)

a	Lean	Obese
Body weight (g)	43.4 ± 1.2	66.5 ± 3.5
Blood glucose (mg per 100 ml)		
Before insulin	162 ± 8	334 ± 27
30 min after insulin	61 ± 4	294 ± 37
Insulinaemia (ng ml^{-1})	3.2 ± 0.7	50.8 ± 11.1
	Specific binding of ^{125}I-insulin	
b	Lean	Obese
Soleus muscles (per cent per mg protein)	0.52 ± 0.04	0.37 ± 0.03
Partially purified receptors (per cent per μg protein)	3.81 ± 0.21	2.94 ± 0.22

Mice were injected with gold thioglucose at 3 weeks of age and used at 25–30 weeks[3,4]. Insulin resistance was tested *in vivo* by measuring plasma glucose before and after an intravenous insulin injection (0.5 U per kg body weight). Insulin binding was measured in soleus muscles as described previously[3] or in partially purified insulin receptors: 10 μl of partially purified receptor preparations was incubated in HEPES (50 mM), NaCl (150 mM) buffer *p*H 7.6 containing ^{125}I-labelled insulin (10^{-11} M) in the absence or presence of unlabelled insulin (10^{-6} M) in a final volume of 200 μl. After 2 h at 20 °C, bound insulin was separated from unbound hormone using polyethylene glycol. Nonspecific binding represented 10–15% of total binding. Values are the mean ±s.e.m. of five measurements for insulin test, eight for soleus muscles or six for partially purified receptor preparations.

Insulin binding was decreased only to a moderate extent (~30%) in intact soleus muscles[3] from obese mice compared with controls, and a similar moderate defect (22%) in insulin binding was observed using partially purified insulin receptors from obese animals (Table 1). Scatchard analysis of the insulin binding data revealed that this was due only to a decrease in receptor number without any change of binding affinity (data not shown). The altered autophosphorylation of insulin receptors from skeletal muscle of obese mice could be due to a decreased number of receptors or, alternatively, due to an intrinsic defect of the insulin receptor kinase in obese mice. To approach this question, we next studied insulin receptors from skeletal muscle of lean and obese mice for self-phosphorylation of the receptor β-subunits present in identical amounts of receptors, as measured by Scatchard analysis and affinity labelling of α-subunits with ^{125}I-insulin. Partially purified insulin receptors were incubated with ^{125}I-photoreactive insulin followed by ultraviolet irradiation for covalent affinity labelling of insulin receptors. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of a major labelled polypeptide with M_r 130,000, which we recognize as the insulin receptor

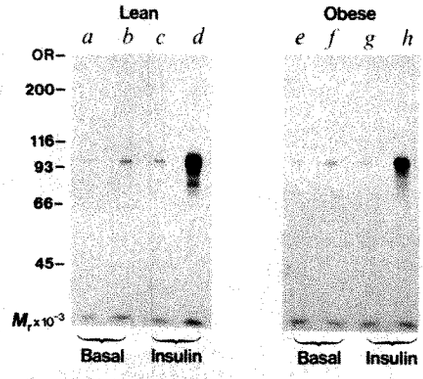


Fig. 1 Phosphorylation of skeletal muscle insulin receptors from lean and insulin-resistant obese mice. Skeletal muscles were obtained from the hindlegs of 30-week-old normal Swiss mice (lean) or mice injected with gold thioglucose (obese). Muscles (3 g) were homogenized (15 s, Polytron homogenizer, 2,000 r.p.m.) in 3 vol. HEPES (50 mM, *p*H 7.6), NaCl (150 mM), bacitracin (1 mM), aprotinin (1,000 trypsin-inhibitor units per ml), phenylmethylsulphonyl fluoride (1 mM) and Triton X-100 (1%). The homogenates were solubilized for 90 min at 4 °C by continuous stirring, and centrifuged at 150,000*g* at 4 °C for 90 min. The supernatants were applied to a wheat-germ-agglutinin-agarose column; after washing, bound glycoproteins were desorbed with 0.45 M *N*-acetylglucosamine[9]. Preparations from lean and obese mice were always performed in parallel. Partially purified insulin receptors were incubated in a reaction mixture containing HEPES (50 mM, *p*H 7.6), NaCl (150 mM) without (basal) or with 0.1 μM insulin at 15 °C for 90 min. Thereafter, the phosphorylation reaction was initiated adding [γ-^{32}P]ATP (15 μM), $MnCl_2$ (4 mM) and $MgCl_2$ (8 mM). After 30 min at 20 °C, the reaction was stopped by adding ice-cold stopping solution containing NaF (80 mM) and EDTA (30 mM). Insulin receptors were specifically immunoprecipitated with antiserum containing autoantibodies to the insulin receptor[12] (serum from patient B2 at a 1:300 dilution) (lanes *b, d, f, h*) or normal serum (lanes *a, c, e, g*) after a preclearing step with normal serum. The immunoprecipitates were boiled for 5 min in a solution containing SDS (3%, w/v), glycerol (10%, v/v), sodium phosphate (10 mM), 2-mercaptoethanol (5%) and bromophenol blue (0.05%) and subjected to one-dimensional SDS-PAGE with a 7.5% acrylamide resolving gel[21]. An autoradiograph of the gel is shown.

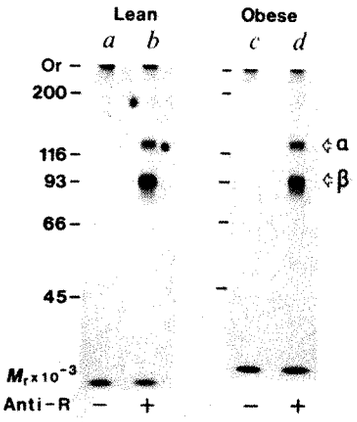


Fig. 2 Double labelling of α- and β-subunits of insulin receptors in lean and insulin-resistant obese mice. Partially purified insulin receptors were prepared from lean and obese mice as described in Fig. 1 legend. The amount of insulin receptors in the two preparations was determined by Scatchard analysis of insulin binding data; obese mice showed a reduction of 20% compared with lean mice (there was no change in insulin binding affinity). Identical amounts of receptors from lean and obese mice were first incubated with 5 nM ^{125}I-labelled photoreactive insulin (B29 (2-nitro,4-azido)phenylacetyl insulin) for 2 h at 15 °C in the dark; samples were then irradiated for 5 min at 4 °C using a high-pressure mercury lamp (Philips HPK; 125 W h^{-1}) as previously described[22]. Using this procedure, 20% of photoreactive ^{125}I-insulin was covalently linked to solubilized receptors after ultraviolet irradiation. At the end of photoactivation, phosphorylation was carried out as detailed in Fig. 1 legend. The labelled insulin receptors were then exposed to normal serum (lanes *a* and *c*) or anti-receptor serum B2 (lanes *b* and *d*), and the immunoprecipitates were subjected to SDS-PAGE under reducing conditions. An autoradiogram of the gel is shown.

α-subunit for the following reasons. First, the radioactive band with M_r 130,000 was not found when unlabelled insulin (10^{-7} M) was present together with ^{125}I-photoreactive insulin during the binding step. Second, the 130,000-M_r polypeptide was immunoprecipitable by anti-insulin antiserum and anti-receptor antiserum, but not by normal serum (data not shown). Based on estimates derived from Scatchard analysis of insulin binding, we took identical amounts of insulin receptors partially purified from skeletal muscle of lean mice and from insulin-resistant obese mice, respectively. Then, ^{125}I-photoreactive insulin was first covalently attached to the two receptor preparations, and the receptors were used in a cell-free phosphorylation assay as described previously. Finally, insulin receptors were specifically immunoprecipitated with antibodies to insulin receptor. The autoradiogram in Fig. 2 clearly shows that for an equal amount of insulin binding, as reflected by the identical labelling intensity of the receptor α-subunit, a much lower degree of phosphorylation of the insulin receptor β-subunit is found in partially purified receptors obtained from obese mice compared with lean mice. Thus, when the radioactivity present in the insulin receptor α- and β-subunits was measured by scanning densitometry in three different experiments, and the ratio of labelling of the β-subunit over the α-subunit was calculated, we found 2.9 ± 0.3 in lean mice, and 1.7 ± 0.2 in obese mice (mean ± s.e.m. of three experiments, each performed with a different insulin receptor preparation). This result indicates that in obese mice there is a marked alteration in the insulin-induced phosphorylation of the insulin receptor.

To evaluate further the possible role of a defect in insulin receptor kinase activity in the resistance to insulin seen in obesity, the ability of insulin receptors to catalyse the phosphorylation of exogenous substrates was investigated. The substrate used here was a copolymer of glutamate and tyrosine residues in a 4:1 ratio, which has been shown to be an excellent substrate for the insulin receptor protein kinase[13]. Insulin binding to the partially purified insulin receptors obtained from skeletal muscle of obese mice and used in these experiments was only decreased by 15% compared with lean mice. In contrast, phosphorylation of the substrate by partially purified insulin receptors from obese mice was decreased by 40% at all insulin concentrations compared with lean mice (Fig. 3). For both preparations, a half-maximal effect was obtained with 8×10^{-8} M insulin.

In our attempts to test the putative causal association between insulin resistance and loss of receptor kinase activity, we have investigated whether both defects evolve concomitantly in mice rendered obese. The insulin receptor tyrosine kinase activity of skeletal muscle was thus evaluated in young obese mice, which are not insulin-resistant, as they are hyperinsulinaemic and slightly hypoglycaemic, and they normally respond to exogenous insulin[4]. These 10-week-old non-insulin-resistant obese mice were compared with 30-week-old obese animals, which have developed an overt resistance to insulin. For both groups of obese mice, insulin binding to partially purified skeletal muscle insulin receptors was ~80% of the binding in the corresponding age-matched lean animals. In skeletal muscle from 10-week-old obese mice, the insulin-stimulated receptor tyrosine kinase activity towards an exogenous substrate was reduced in direct proportion to the decrease in insulin binding, that is, a reduction of approximately 20%. This is in striking contrast to the situation observed in 30-week-old insulin-resistant obese mice, where the diminution in receptor kinase activity (45%) is much more pronounced than would have been expected from the decrease in insulin binding (20%). Thus, in obesity with the development of insulin resistance and without any further change in insulin receptor number, a defect intrinsic to the insulin receptor tyrosine kinase activity appears.

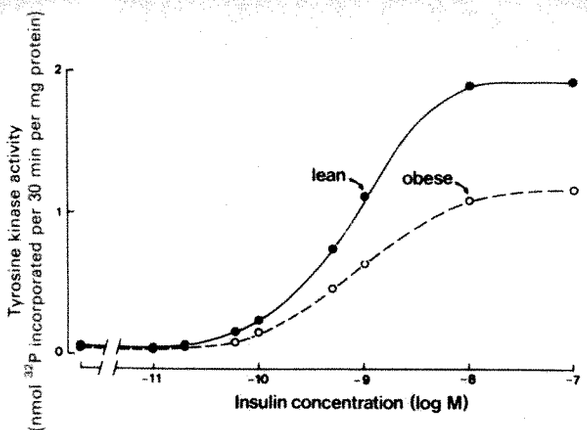


Fig. 3 Kinase activity of insulin receptors from lean and insulin-resistant obese mice towards an exogeneous substrate. Partially purified insulin receptors (25 μl), prepared from lean and obese mice as described in Fig. 1 legend, were preincubated in HEPES (50 mM), NaCl (150 mM), bovine serum albumin (0.2 mg ml^{-1}) buffer, *p*H 7.6 (final volume 140 μl) without or with various concentrations of insulin for 60 min at 24 °C. The substrate[13] (copolymer of glutamate/tyrosine, 4 : 1, 0.25 mg ml^{-1}) was then added for an additional 30 min. The phosphorylation reaction was initiated by adding 20 μl of a solution containing [γ-^{32}P]ATP, $MnCl_2$ and $MgCl_2$ at final concentrations of 20 μM, 3,000 c.p.m. pmol^{-1}, 4 mM and 8 mM, respectively. After 30 min the reaction was stopped by applying 75-μl samples to filter papers (Whatman ET31) and soaking the papers in a bath of 10% trichloroacetic acid (TCA), 10 mM sodium phosphate. After extensive washing in 5% TCA, radioactivity of the TCA-precipitable material was measured using Cerenkov radiation. Results are expressed as nmol of phosphate incorporated per mg receptor protein per 30 min.

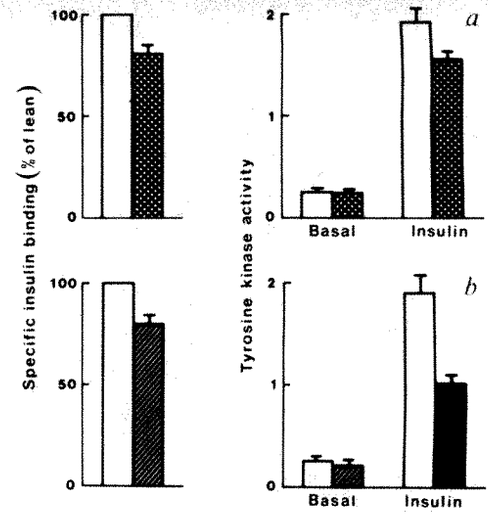


Fig. 4 A defect in insulin receptor tyrosine kinase activity appears with insulin resistance in skeletal muscle from obese mice. Insulin receptors were partially purified from skeletal muscle obtained from the following two groups of lean (open bars) and obese (shaded bars) mice: *a*, mice were aged 10 weeks, and the obese animals were not insulin-resistant (body weight (g) 35.9 ± 0.3 and 45.1 ± 1.0; blood glucose (mg per 100 ml) 217 ± 6 and 182 ± 6; insulinaemia (ng ml^{-1}) 1.9 ± 0.4 and 7.7 ± 1.8 for lean and obese mice, respectively; values are mean ± s.e.m. of 8–12 values; all differences were statistically significant with $P < 0.01$. *b*, Mice were 30 weeks old and the obese animals were insulin-resistant as described in Table 1. Insulin binding was measured in partially purified insulin receptors as described in Table 1, and the results are expressed as a percentage of the binding obtained in preparations from lean mice. Insulin receptor tyrosine kinase activity towards an exogenous substrate was measured in the absence (basal) or presence of insulin (10^{-7} M) as described in Fig. 3 legend and is expressed in nmol ^{32}P incorporated per mg receptor protein per 30 min. Values are the mean of five experiments, each performed with a different insulin receptor preparation.

Here we have presented evidence that the insulin receptor tyrosine kinase activity is markedly impaired in skeletal muscle of insulin-resistant obese mice. This defect in the enzyme function of the receptor was observed for both receptor autophosphorylation and the ability of the receptor to catalyse the phosphorylation of a synthetic substrate. This result indicates that the insulin-resistant state accompanying obesity can be related to an impaired activity of the insulin receptor kinase. Similarly, it has also been shown recently that the insulin resistance, which occurs in streptozotocin-diabetic rats, is accompanied by a decreased kinase activity of liver insulin receptors[14]. Our finding represents the earliest post-receptor binding alteration recognized so far in animal obesity, in which the insulin resistance is generally accepted to result mainly from a post-receptor defect. This observation may have important implications for our understanding of the insulin resistance commonly found in human obesity, which is the most frequent cause of hormone resistance in humans. Our model of animal obesity has a major feature in common with human obesity in that it is acquired. It thus excludes the possibility that the insulin receptor kinase activity is deficient due to the result of a genetic abnormality of the enzyme as it appears to occur in two recently reported cases of insulin resistance in extremely rare patients[15,16]. The alteration of intrinsic insulin receptor kinase activity in our obese animals could result from the chronic hyperinsulinaemia present in obese mice which induces a desensitized state of the receptor-associated enzyme function. Along this line, it has been suggested that diet-induced alterations of insulin receptor kinase activity could be related to different nutritional states or ambient plasma insulin levels in fasted and fasted, re-fed animals[17]. Our observations are similar to those of Carlin *et al.*[18] who show that senescent fibroblasts, which are resistant to epidermal growth factor (EGF) action, have a defect in their EGF receptor kinase despite an unchanged number of EGF receptors. Alternatively, the defective insulin receptor kinase activity found in obese mice could be due to inadequate coupling between the two insulin receptor subunits. The mechanism of signal transfer from the insulin binding subunit (α) to the β-subunit, responsible for the kinase activity, is unknown. It could be envisaged that this signal transfer is hampered in insulin-resistant obese mice. In contrast, a quantitative defect of solely the insulin receptor β-subunit in obese mice is very unlikely. Indeed, insulin-induced receptor loss (a phenomenon known as down-regulation[19]) leads to an identical decrease in both insulin receptor subunits[20]. Along the same line, the insulin receptor of obese mice could have a greater proportion of nicked β-subunits than lean mice. However, a careful analysis of our gels does not favour this contention.

It is generally believed that protein phosphorylation is an important mechanism by which a variety of extracellular stimuli regulate cellular functions. For the insulin receptor, the hormone-stimulated phosphorylation of its receptor is a very early post-binding event, and a fundamental property of the receptor as it occurs in all tissues studied thus far. The demonstration of an altered insulin-stimulated kinase associated with the insulin receptor activity in muscles of obese mice, which display insulin resistance for a variety of parameters (glucose transport and metabolism, glycogen synthase activation and amino-acid transport), argues strongly for a key role of receptor phosphorylation in insulin action.

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Novel preparation for studying excitation–secretion coupling in the isolated single cell

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Cellular mechanisms of secretion in exocrine and endocrine glands are technically difficult to study. We present here a model which offers fundamental advantages for studying excitation–secretion coupling at the level of the isolated single cell. The salivary gland of the leech *Haementeria ghilianii* possesses a unique combination of unusual properties which greatly facilitate research in this area. Its cells are exceptionally large (up to 1 mm in diameter), clearly visible and easy to penetrate with microelectrodes. They do not form a homogeneous population but consist of five distinct histochemical types, secreting a number of identified products such as the fibrinolytic enzyme hementin (for which there is a sensitive assay). The cells generate overshooting calcium-dependent action potentials up to 90 mV in amplitude and with a duration of 200–1,000 ms. One of their most useful and unusual features is a lack of electrical coupling which means that individual cells can be studied in an intact gland without interference from neighbouring cells.

H. ghilianii is the world's largest leech (up to 0.5 m long) and was known only from two nineteenth-century reports until it was rediscovered by one of us in swamps in French Guyana[1]. Unlike most other leeches (for example, *Hirudo medicinalis*) in which the salivary cells are relatively small and scattered throughout the head musculature[2], those of *H. ghilianii* are very large and form a discrete gland containing ~240 cells (Fig. 1). Intracellular injection of Lucifer yellow indicates that the cells are not coupled because the dye does not pass into neighbouring cells[3].

Leech salivary glands contain a number of medically interesting peptides, including an anti-elastase, a vasodilator, hyaluronidase, an anti-metastasis factor, a possible local anaesthetic and a species-specific range of anticoagulants[4,5]. For example, the anticoagulant of *H. ghilianii* is hementin, an enzyme that specifically cleaves fibrinogen/fibrin[6].

These properties, together with the electrophysiological experiments reported here, suggest that the gland is a valuable model for glandular secretion, particularly because much is known about the biochemistry and mechanisms of action of some of the biologically active secretions mentioned above[5]. Features of the electrophysiology of this gland have recently been examined by Marshall and Lent[7]. Here we present the results of parallel studies and summarize the evidence which has led us to recognize the potential of this system as a gland model.

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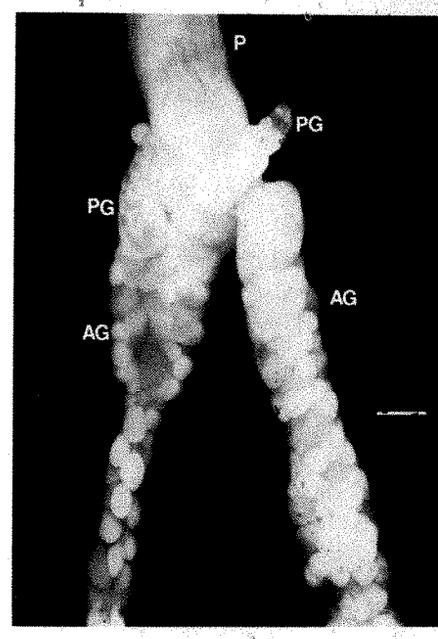


Fig. 1 Dissected salivary gland complex of *H. ghilianii*. P, proboscis; AG, anterior gland; PG, posterior gland. Cells, ranging up to 1 mm or more in diameter, are clearly visible for microelectrode penetration. Scale bar, 1 mm.

Dissected salivary glands were pinned to the base of a small Perspex bath and immersed in physiological saline[8]. Individual cells were impaled with two independent KCl-filled microelectrodes of 10–20 MΩ resistance; one electrode was used for recording potential and the other for passing current. Experiments were performed at room temperature (18–22 °C).

Resting potentials of between −35 and −80 mV were recorded, with no sign of spontaneous impulse activity or subthreshold potentials. Depolarizing pulses elicited overshooting action potentials which exceeded 90 mV in some cells. Action potentials were characterized by a shoulder on the repolarizing phase, pronounced after hyperpolarization (Fig. 2*a*, *b*), and a duration of 200–400 ms which increased during repetitive firing up to 1 s. In most cells only one or two action potentials could be elicited by maintained depolarization because of rapid adaptation; recovery could take many seconds or even minutes. Action potentials could, however, be elicited by termination of a hyperpolarizing pulse (Fig. 2*b*, *c*) even when depolarization produced no response. Addition of 5 mM $CoCl_2$ to the bathing medium reversibly suppressed action potential generation (Fig. 2*d*–*f*), indicating that Ca^{2+} is the major current carrier for the action potential.

To test for electrical coupling, pairs of cells were recorded simultaneously. We never detected responses in one cell to action potentials or applied current pulses (depolarizing or hyperpolarizing) in another (Fig. 2*c*), even when applied pulses were increased to cause eventual damage to the cell. Both neighbouring and more distant pairs of cells were tested in this way. This lack of coupling is unusual (the only other case of electrical isolation of gland cells is in the pedal gland of molluscs[9]). This property should eliminate many of the experimental problems associated with coupling in other systems[10]. For example, it is difficult to ascertain clearly whether any electrical activity observed in a given cell originates from that or from neighbouring cells, and the effective shunt resistance of the cells may vary (for example, by the actions of transmitters on cell membrane or junctional membrane permeability).

The electrical isolation of the *H. ghilianii* salivary cells is a fundamental advantage for secretion studies at the cellular level. It should be possible, for example, to study release of identified secretions from each of the five cell types, as has been done with neurotransmitters in identified invertebrate neurones[11].

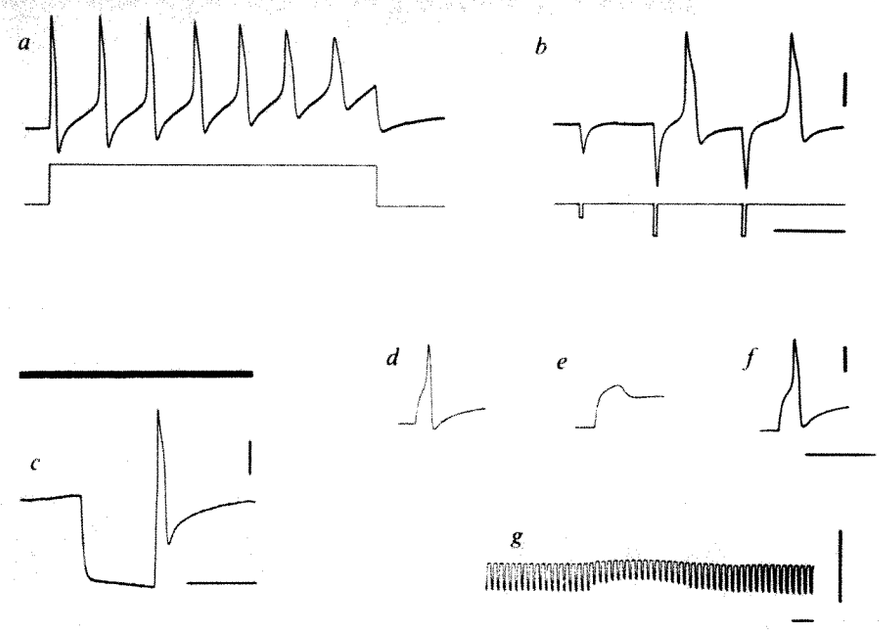


Fig. 2 Intracellular recordings from salivary gland cells of *H. ghilianii*. *a*, *b*, Action potentials, which may exceed 90 mV, are initiated by depolarizing current (*a*) and also following hyperpolarization by inward current (*b*; 100-ms pulses were applied). *c*, A hyperpolarizing current pulse in one salivary cell (lower trace) produces rebound excitation but there is no sign of electrical responses in an adjacent cell, recorded simultaneously at high gain on the upper trace. Experiments of this type indicate that the gland cells are not electrically coupled. *d*–*f*, Effect of 5 mM Co^{2+} added to the bathing solution. *d*, Control impulse in response to depolarizing current; *e*, 5 min after addition of $CoCl_2$ the action potential is abolished (a delayed rectification is apparent); *f*, recovery. This indicates that Ca^{2+} is the major current carrier for generation of action potentials. *g*, Brief application of 10^{-5} M serotonin produces a depolarization and increase in membrane conductance (indicated by reduction in amplitude of constant-current hyperpolarizing pulses). Voltage scales (vertical bars), 25 mV (1 mV in *c*, upper trace); time scales (horizontal bars), 2 s.

The natural stimulus for action potential generation, whether neural and/or hormonal, is unknown. Several putative neurotransmitters, however, (dopamine, serotonin and acetylcholine) were found to depolarize the gland cells, with an accompanying decrease in membrane resistance (Fig. 2*g*) and occasionally the production of no more than four impulses. Interestingly, in the presence of dopamine, applied depolarizing current was sometimes found to produce repetitive firing which was not simply a consequence of the depolarization produced by the drug. If the impulse provides a trigger for secretion, it seems unusual that the cells are normally so difficult to activate. Feeding, however, occurs very infrequently (every few months) and an action such as that of dopamine may mimic a natural process of bringing the gland into secreting condition.

In mammals, salivary and other exocrine gland cells are electrically inexcitable, producing graded potential changes (often hyperpolarizations) which may or may not be related to secretory function[10]. The *H. ghilianii* salivary cells are similar in their electrical excitability to mammalian endocrine cells such as those in the pancreas[12], adenohypophysis[13] and adrenal gland[14] (some molluscan exocrine glands produce action potentials[15]). This similarity extends to the anode-break excitation[8] shown by chromaffin[14] and anterior pituitary cells[13].

We have also found the *H. ghilianii* salivary gland to be suitable for molecular genetics because the cells have a very large ramifying nucleus that displays gene amplification of $\sim 10^6$ times; this should allow precise questions to be asked about the relationship between secretion and transcription/translation of identified genes. Thus, the *H. ghilianii* salivary gland, with its unusual combination of properties, represents a simple, accessible preparation with distinct experimental advantages for cellular studies of glandular secretion.

This specialized leech has been generally unavailable because in its natural habitat it is restricted to Amazonia. We have developed techniques for breeding this species and a facility has been set up by Biopharm to supply hementin to interested researchers.

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Production of transgenic rabbits, sheep and pigs by microinjection

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Direct microinjection has been used to introduce foreign DNA into a number of terminally differentiated cell types as well as embryos of several species including sea urchin[1], *Candida elegans*[2], *Xenopus*[3], *Drosophila*[4,5] and mice[6–11]. Various genes have been successfully introduced into mice including constructs consisting of the mouse metallothionein-I (MT) promoter/regulator region fused to either the rat or human growth hormone (hGH) structural genes. Transgenic mice harbouring such genes commonly exhibit high, metal-inducible levels of the fusion messenger RNA in several organs, substantial quantities of the foreign growth hormone in serum and enhanced growth[12,13]. In addition, the gene is stably incorporated into the germ line, making the phenotype heritable. Because of the scientific importance and potential economic value of transgenic livestock containing foreign genes, we initiated studies on large animals by microinjecting the fusion gene, *MT–hGH*[13], into the pronuclei or nuclei of eggs from superovulated rabbits, sheep and pigs. We report here integration of the gene in all three species and expression of the gene in transgenic rabbits and pigs.

Studies with mouse ova indicated that integration of a gene into host chromosomes is much more efficient with nuclear than with cytoplasmic injection[14]. On this basis, we reasoned that nuclear injection would be an appropriate first approach with other species. The first problem encountered was visualization

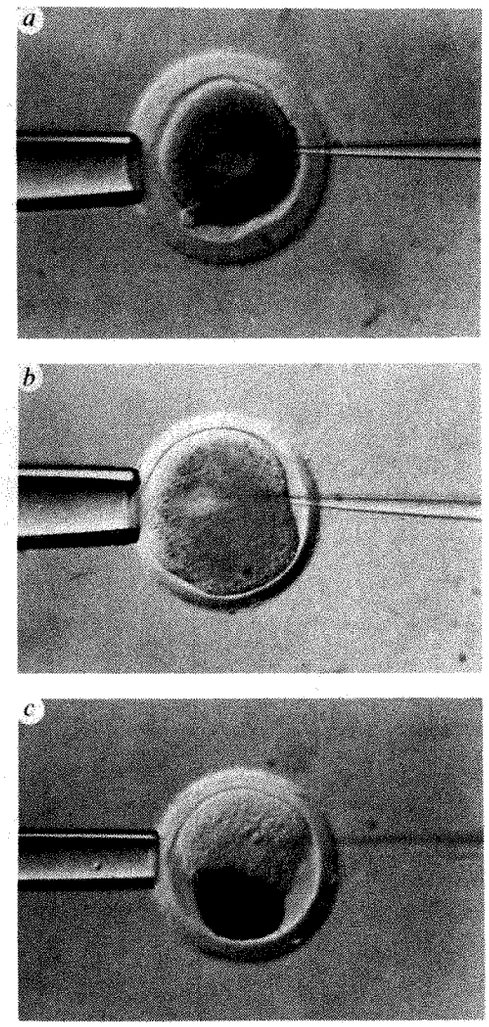


Fig. 1 Interference-contrast photomicrographs of one-cell fertilized ova from rabbit (*a*), sheep (*b*) and pig (*c*) following microinjection. Ova are held by a blunt holding pipette (diameter ~50 μm); an injecting pipette (diameter ~1.5 μm) has penetrated the zona pellucida, plasma membrane and pronuclear envelope. The tip is seen within the nucleoplasm immediately following injection of buffer containing DNA. The porcine ova (*c*) has been centrifuged at 15,000*g* for 3 min to reveal the normally obscure pronuclei[15]. Visualization of nuclear structures is aided by the use of interference-contrast optics, and microinjection is carried out under × 250 magnification using a Leitz microinjecting apparatus[10,14]. Injection was monitored by observing the diameter of the pronuclus or nucleus, which was expanded ~50%.

of the pronuclei or nuclei in the ova. Rabbit nuclear structures are readily seen (Fig. 1*a*). However, pronuclei and nuclei in sheep ova are difficult to locate and can only be seen by fluorescent microscopy using DNA specific fluorochromes (Hoechst, 33258) or by interference contrast (IC) microscopy (Fig. 1*b*). The combination of stain and ultraviolet light is damaging to the ovum (data not shown), so we used IC microscopy for microinjection. Fluorescent analysis indicated that IC microscopy is an effective method for pronuclear localization in approximately 80% of fertilized sheep eggs. Pig ova are opaque and no nuclear structures can be seen even with IC microscopy, but we found that centrifugation of pig ova at 15,000*g* for 3 min stratifies the cytoplasm (Fig. 1*c*), leaving the pronuclei or nuclei visible[15].

Once the nuclei could be visualized, microinjection was performed as described previously[10,14]. A few hundred copies of a 2.6 kilobase (kb) linear fragment containing the *MT-hGH* gene (see Fig. 2) were injected. Approximately 5,000 ova were injected and subsequently transferred to foster animals; about 500 of these resulted in fetuses or neonates (Table 1). The frequency of *MT-GH* integration was similar in the rabbit (12.8%) and the pig (11.0%) and low in sheep (1.3%). These integration efficiencies are probably accurate for the techniques being used because they are based on a large number of animals. The reasons for the lower integration frequency in these species compared to the mouse where it is ~27% are unknown but could be related to factors such as the concentration of DNA, buffer composition, age of the ovum and the structure of the chromosomes[14].

The number of copies of the *MT-hGH* gene that integrated was estimated by quantitative dot hybridization. Figure 2*d* shows the quantitation method as applied to transgenic pigs. Gene copy numbers ranged from 1 to 490 copies per cell (Table 2). The DNA from some of the transgenic animals was also analysed by restriction enzyme digestion of the chromosomal DNA followed by agarose gel electrophoresis and Southern blotting. Figure 2*a* shows the results obtained when the DNA was restricted with *Eco*RI, an enzyme that cuts once within the injected DNA. The probe detects two prominent bands in several rabbits and pigs. One band is close to the length of the injected DNA fragment (2.6 kb) and probably represents a tandem, head-to-tail array of the *MT-hGH* genes as is typically observed in transgenic mice[10,12]. The other band is approximately twice that length and might represent a head-to-head dimer, but further analysis will be required to test that possibility. When the DNA was restricted with *Sst*I (Fig. 2*b*), an enzyme that cuts twice within the injected DNA, two bands of the expected size were observed in all of the pigs and rabbits. Analysis of the sheep sample with *Eco*RI (not shown) and *Sst*I (Fig. 2*c*) revealed bands that were inconsistent with an intact *MT-hGH* gene, suggesting that the DNA had been trimmed or rearranged prior to integration.

Expression of the integrated genes was examined by quantitating *MT-hGH* mRNA by solution hybridization (Table 2). Only 4 of 16 rabbits analysed had any detectable *MT-hGH* mRNA in the liver, but the level was substantial in one of these. In mice, the frequency of expression of this gene is close to 70% (ref. 13). In pigs, mRNA levels were measured only in tail or ear samples because we did not want to risk adverse consequences of liver biopsy. Although tail and ear tissues are not primary sites of *MT* gene expression, we detected low levels of *MT-hGH* mRNA in several of the transgenic pigs (Table 2).

Plasma samples taken from pigs at birth and ~1 month later were analysed for hGH by radioimmunoassay. At birth, 11 of 18 pigs had detectable levels of hGH, ranging between 2 and 730 ng ml^{-1} (Table 2). One month later, hGH exceeded 300 ng ml^{-1} in three pigs. One rabbit also had a high level of hGH. Serum hGH as high as 64,000 ng ml^{-1} has been detected in transgenic mice, but accelerated growth rate was observed at levels of 20 to 80 ng ml^{-1} (ref. 13). None of these animals were exposed to high levels of zinc, a treatment that has been shown to activate *MT-hGH* gene expression ~10-fold in mice[13].

The effects of hGH on the growth of rabbits cannot be evaluated at present because only one live rabbit had detectable serum hGH and unfortunately it had malocclusion that impaired normal food consumption. Early indications are that the levels of hGH found in these transgenic pigs do not increase body weight dramatically. This may not be surprising considering that daily injections of bacterially synthesized hGH had no effect[16], and exogenous, highly purified porcine GH only stimulated growth by 10% when delivered during the major growth phase of the pig[17]. Transgenic offspring and littermate controls will need to be raised on normal and zinc-supplemented diets to determine precisely the effects of hGH on growth rate and other nutritional as well as endocrine parameters.

These experiments demonstrate that foreign genes can be introduced into several large animal species by microinjection of ova. Furthermore, expression of MT-hGH was obtained in rabbits and pigs. We used a fusion gene that has worked well in mice to demonstrate the feasibility of such techniques, and we are now trying several modifications in an effort to improve the level of expression and physiological response.

Table 1 Efficiency of producing *MT-hGH* transgenic rabbits, sheep and pigs by microinjection

Species	Transferred injected ova	Recipients	Integration frequency (%)	Expression frequency *MT-hGH* mRNA	Expression frequency Serum or plasma hGH
Rabbit*	1,907	73	28/218 (12.8)	4/16	1/1
Sheep†	1,032	192	1/73 (1.3)	ND	ND
Pig‡	2,035	64	20/192 (10.4)	11/20	11/18

Integration frequency is the number of animals (fetuses, stillborns and neonates) that retained the injected DNA/total number of animals resulting from injected ova. Six gilts bearing only injected eggs farrowed, producing 52 neonates, 5 of which retained DNA. In 31 gilts that farrowed, 204 fertilized control ova were transferred along with 859 injected ova to ensure sufficient embryos at implantation to maintain pregnancy. If survival of injected eggs to fetuses (16.4%) was similar for both groups, then injected eggs resulted in 140 of 252 fetuses and piglets produced, 15 of which retained injected DNA. We combined the data from the two groups to estimate integration efficiency. Expression frequency is the number of fetuses or neonates containing *MT-hGH* mRNA or plasma hGH per total number of animals examined. ND, not determined.

* Fertilized one-cell rabbit ova were flushed from the oviducts of superovulated New Zealand White (NZW) females 19 h after mating[20]. For microinjection the ova were placed in the well of a depression slide containing ~100 μl modified BMOC culture medium[21] with the $NaHCO_3$ replaced by 25 mM HEPES[22] and covered by silicone oil. Microinjection of embryos (1,857 one-cell and 50 two-cell) was performed as described for mouse ova[10,14]. Following injection, the ova were washed in fresh modified BMOC and surgically transferred to the oviducts of synchronized pseudopregnant rabbits[23].

† Rambouillet ewes were superovulated after exhibiting at least one prior oestrus period. On about day 10 of the oestrous cycle, progestagen-impregnated vaginal sponges (6α-methyl-17α-acetoxy progesterone, 60 mg; from Dr J. Lauderdale, Upjohn) were inserted and left for 12 days. Gonadotropin treatment (porcine follicle stimulating hormone, Burns) began three days before sponge removal and was continued twice daily (2.5 mg per injection, intramuscular) until the day following sponge removal[24]. At the onset of oestrus, ewes were either hand mated to fertile rams or inseminated *in utero* with 0.2 ml per horn of washed ram semen; 72 h after sponge removal, one-cell fertilized ova and cleaved ova were surgically collected from the reproductive tracts of anaesthetized ewes by flushing 6 ml Ham's F-10 medium containing 10% heat-inactivated fetal calf serum (FCS) from the utero-tubal junction through the cannulated infundibular end of each oviduct. The flushings were collected in sterile Petri dishes, and ova were removed under a dissecting scope. Ova were transferred to fresh Ham's F-10 containing 10% FCS and transported (~2.5 h) to Philadelphia in temperature-controlled containers. Microinjection of embryos (641 one-cell, 375 two-cell and 16 four-cell) was performed as previously described[10,14]. After embryos were injected, they were washed and transported to Beltsville. Embryos were aspirated into a glass micropipet tip with 10 μl Ham's F-10 and expelled 1-3 cm into the fimbriated end of the oviduct in synchronized recipient ewes. To assess the effects of transport and microinjection of DNA on egg development, a number of recipients bearing control and injected eggs were flushed 8 days following transfer. In recipients in which eggs were recovered, 26% of transported, uninjected and 10% of injected sheep eggs developed to blastocysts. Because of the high mortality of transported, injected eggs and the concern of multiple births, 5 or 6 embryos were transferred per recipient.

‡ Mature gilts were superovulated and bred as previously described[15]. At 18 to 27 h after the expected time of ovulation, gilts were anaesthetized and one cell fertilized the oviduct with 20 ml modified BMOC[21]. Ova were transferred to fresh BMOC and transported to Philadelphia. Microinjection of embryos (316 one-cell, and 1,719 two-cell) was performed as previously described[10,14]. The obscured pronuclei or nuclei or one- and two-cell pig ova were visible after centrifugation for 3 min at 15,000 g. Centrifugation of pig ova at this force and length of time has no detectable effect on development[15]. After embryos were injected, they were transported to Beltsville and transferred to the oviducts of recipient gilts as previously described[15]. To assess the effects of transport and microinjection of DNA on egg development, recipients bearing control and injected eggs were flushed 5 days following transfer. Approximately 52% of transported, uninjected and 23% of injected pig eggs developed to blastocysts. The pregnancy rate in recipients bearing only injected eggs was 50% while in recipients bearing both injected and control eggs 58% farrowed.

Table 2 Characteristics of transgenic rabbits, sheep and pigs

Species	Animal and sex	Gene copy (no. per cell)	*hGH* mRNA (molecules $cell^{-1}$)	Immuno-assayable hGH (ng ml^{-1})	Species	Animal and sex	Gene copy (no. per cell)	*hGH* mRNA (molecules $cell^{-1}$)	Immuno-assayable hGH (ng ml^{-1})
Rabbit	59-3*	20	0		Pig	100-3*	4	0	ND
	64-2♀	18	0			163-4*	140	0	ND
	68-3†	28	39	ND		3-2♂	330	26	Neg.
	68-4♀	24	0			3-6♂	490	53	17
	122-9♀	11	0			7-3♀	90	12	80
	131-8†	88	0			10-4♀	23	0	Neg.
	157-1♀	3	0			11-2♂	1	0	40
	163-3♂	3	15	250		16-3♀	3	0	Neg.
	167-5♀	10	0			16-8♀	10	18	53
	179-1♀	16	0			16-9♂	1	5	65
	179-2♂	36	0			17-4♀	3	0	Neg.
	179-5♀	6	0			18-3♀	3	6	60
	200-3*	8	920	ND		20-2♂	2	4	40
	221-1*	40	140	ND		20-8♂	110	1	2
	223-4*	5	0			21-4♂	1	2	Neg.
	223-5*	40	0			21-5♀	1	0	Neg.
Sheep	693-1♀	1	ND	ND		22-1♀	50	24	56
						23-8♀	7	41	730
						25-2♀	17	0	108
						25-4♀	2	0	Neg

A 2.6-kb linear fragment of the fusion gene *MT-hGH*[13] containing the mouse MT-I regulator/promoter fused to hGH[19] was injected into fertilized one-cell and two-cell rabbit, sheep and pig eggs as described for mouse ova[10,14]. The male or female pronuclei of one-cell ova and both nuclei of two-cell ova were microinjected with a 3 ng μl^{-1} solution of DNA in Tris-EDTA buffer[14]. The ova were transferred into the oviducts of recipients at the same stage post oestrus as the donors (see Table 1). Animals without identified gender were either killed as fetuses(*) or were stillborn(†). The number of foreign fusion genes per cell was estimated by extracting total nucleic acids from a piece of fetal liver, neonatal ear or tail samples and performing quantitative dot hybridization with a 1.0-kb *Pvu*II probe spanning most of the *hGH* structural gene[19] (see Fig. 2). *MT-hGH* mRNA was measured by solution hybridization with a ^{32}P-labelled oligonucleotide (21-mer)[25]. For rabbits, either a partial hepatectomy was performed or fetal liver was used. For pigs, the *MT-hGH* mRNA content of ear or tail samples was quantitated. The concentration of *hGH* was measured in pig plasma obtained shortly after birth and serum from a 9-month-old rabbit. Samples were assayed in duplicate at 2.5 and 10 μl by radioimmunoassay using a hGH kit provided by Dr Raiti (National Hormone and Pituitary Program). The assay did not cross-react with porcine GH but required extra normal rabbit serum and anti-rabbit gammaglobulin to quantify hGH in rabbit samples. Pigs with hGH values less than 2 ng ml^{-1} at birth were designated negative for hGH. At about one month of age, these pigs were also negative for hGH. ND, not determined; Neg, negative for hGH.

The key element in our success was the ability to visualize pronuclei and nuclei. Microinjection of the *MT-hGH* gene into the cytoplasm of 485 pig ova failed to produce DNA integration in 42 fetuses. Separate techniques for sheep and pigs were necessary because the opacity of the eggs differed. Although both contain dense cytoplasm, centrifugation did not help visualize pronuclei of sheep eggs and IC microscopy did not allow nuclear localization in pig ova. Preliminary work indicates

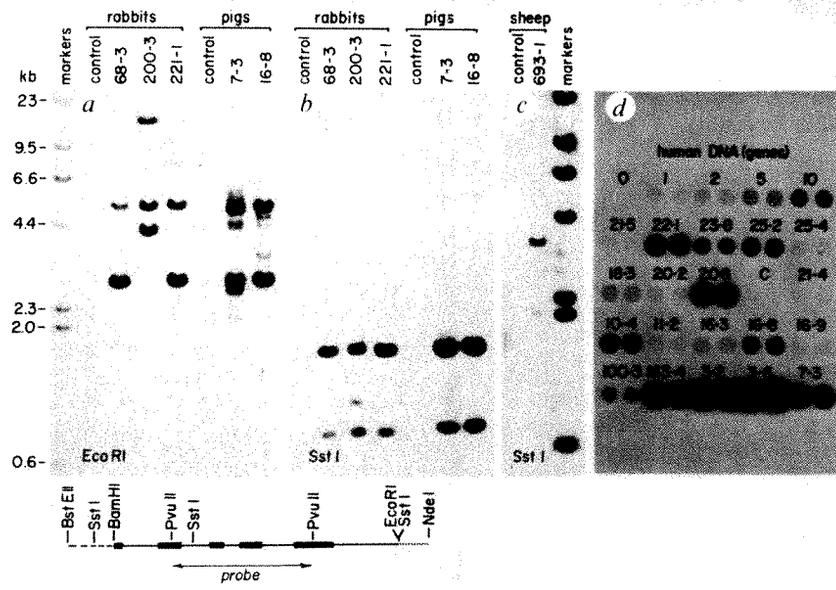


Fig. 2 Analysis of *MT-hGH* DNA introduced into rabbits, pigs and sheep. The diagram at the bottom shows the 2.6-kb *Bst*EII/*Eco*RI DNA fragment isolated from *MT-hGH* gene plasmid 111 that was microinjected[13]; the mouse MT-I promoter region is dashed, the *hGH* gene is solid, with the exons indicated as boxes, and the residual pBR322 sequences are dotted. The internal *Pvu*II fragment was isolated, nick-translated and used as a probe for quantitation of genes and Southern blots. Panels *a*, *b* and *c*: DNA (5 μg for controls and transgenic animals 200-3, 16-8 and 693-1; 1 μg mixed with 4 μg of control DNA for 68-3, 221-1 and 7-3) were digested with the indicated restriction enzymes (10 units, 6 h), electrophoresed on a 1% agarose gel, transferred to nitrocellulose and hybridized with the nick-translated probe, washed and autoradiographed as described previously[18]. *a* and *b*, Exposure was 4.5 h; *c*, 24 h. For quantitation of gene copy number, 5 μg of DNA was spotted in duplicate onto nitrocellulose along with standards of 0, 0.5, 1, 2 and 5 μg of human DNA mixed with control DNA to make a total of 5 μg. *d* Shows the visualization *MT-hGH* gene copy number in transgenic pigs. After exposure, the spots were cut out and the radioactivity determined in a scintillation counter. Gene copy numbers were calculated from the standards assuming that the genome size of pigs and humans are comparable and that diploid human cells contain 10 genes homologous to the *Pvu*II fragment used as probe[19]. The results are shown in Table 2.

that these two techniques can be used for ova of other species; for example, IC microscopy allowed visualization of pronuclei in goat ova (unpublished observations) and centrifugation allowed localization of pronuclei in cow ova[15]. Although improvements in integration efficiency should be possible, the techniques have immediate application for both scientific and practical purposes.

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Expression of active human clotting factor IX from recombinant DNA clones in mammalian cells

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Haemophilia B, or Christmas disease, is an inherited X-chromosome-linked bleeding disorder caused by a defect in clotting factor IX and occurs in about 1 in 30,000 males in the United Kingdom[1]. Injection of factor IX concentrate obtained from blood donors allows most patients to be successfully managed. However, because of impurities in the factor IX concentrate presently in use, this treatment involves some risk of infection by blood-borne viruses such as non-A, non-B hepatitis and the virus causing acquired immune deficiency syndrome (AIDS)[2]. Because of the recent concern about the increasing incidence of AIDS amongst haemophiliacs, a factor IX preparation derived from a source other than blood is desirable. Here, we report that after introduction of human factor IX DNA clones[3] into a rat hepatoma cell line using recombinant DNA methods, we were able to isolate small amounts of biologically active human factor IX.

Factor IX is a plasma glycoprotein which has an essential role in the middle phase of the intrinsic clotting pathway[4] where, in an activated form, IXa, it interacts with factor VIII, phospholipid and calcium ions to form a complex that converts factor X to Xa. Factor IX is synthesized in liver hepatocytes where it undergoes three distinct types of post-translational modification before secretion into the bloodstream as a 415-amino-acid-long, highly modified protein. These modifications are the vitamin K-dependent γ-carboxylation of 12 glutamic acid residues[5], the addition of several carbohydrate residues[6] and the β-hydroxylation of a single aspartic acid residue[7]. The first two modifications are known to be required for activity of factor IX[5,6]. Because of the complex and specialized nature of these modifications, it seemed probable that the expression of active factor IX, derived from factor IX DNA clones, would be most likely to succeed in a hepatic cell or a transformed cell line derived from a hepatocyte. None of the standard mammalian hepatoma cell

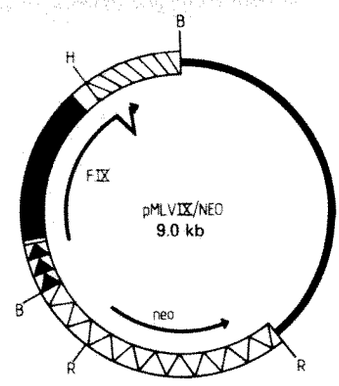


Fig. 1 Map of the expression plasmid pMLVIX/NEO. Restriction sites for *Eco*RI, *Bam*HI and *Hin*dIII are denoted R, B and H, respectively. The factor IX (abbreviated FIX) and *tk/neo* (abbreviated neo) sections are indicated by arrows which show the 5′→3′ direction of the mRNA transcribed from these genes. pAT153 sequences are indicated by the narrow solid line, the *tk/neo* gene by the zig-zag line. The factor IX transcription unit, from 5′ to 3′, consists of the M-MuLV LTR (▲), factor IX 5′-noncoding sequence (open wide line), factor IX coding sequence (solid wide line), factor IX 3′-noncoding sequence (open wide line) and SV40 sequences (diagonals).

Methods. Construction of the plasmid: The region from nucleotides 25 to 93 was removed from the factor IX cDNA cVI by digesting cVI[3] (held in the plasmid vector pAT153/PvuII/8) with *Bam*HI, followed by 'filling-in' the overhanging ends with deoxynucleoside triphosphates using the Klenow fragment of DNA polymerase I (*Escherichia coli*). After dephosphorylation with calf intestinal phosphatase, a partial digest with *Eco*RV was performed. *Eco*RV recognizes the sequence GATATC at nucleotides 91–96. A partial digest was necessary because of the presence of an additional *Eco*RV site at nucleotide 508. The resulting linear fragment of ~4.9 kb was purified by agarose gel electrophoresis. A short (0.1-kb) *Taq*I/*Eco*RV fragment was generated from the factor IX genomic DNA XI[3] by digesting XI (also held in the plasmid vector pAT153/PvuII/8) with *Taq*I, filling-in the overhanging ends with deoxynucleoside triphosphates as before in the presence of tracer amounts of [α^{32}P]dGTP and re-cutting with *Eco*RV. This labelled fragment, corresponding to nucleotides 288–386 of the genomic DNA (see Fig. 4 of ref. 3), was separated from other *Taq*I and *Eco*RV fragments derived from the plasmid vector by 6% acrylamide gel electrophoresis. The 4.9-kb and 0.1-kb fragments were ligated together and cloned in *E. coli* MC1061. One clone, designated p5′G3′cVI, contained the reconstructed factor IX gene corresponding to nucleotides 1–1,572 of the mRNA[3], effectively adding nucleotides 1–25 to, and correcting the rearrangement in, the first 15 residues of clone cVI. The clone also contained eight extra nucleotides, genomic DNA residues 288–295, derived from the presumed factor IX promoter region. The factor IX DNA insert present in p5′G/3′VI was removed by restriction with *Bam*HI and *Hin*dIII and isolated by agarose gel electrophoresis. Construction of the expression vector pMLV/NEO: pTKMoLTR1 (given by Dr J. C. Lang[20]) containing the M-MuLV LTR and the herpes simplex virus *tk* gene, was restricted with *Hin*dIII and *Bam*HI to remove the *tk* coding sequence. The remainder of the molecule (pAT153 and M-MuLV LTR) was purified by agarose gel electrophoresis, then used as a vector for cloning of an agarose gel-purified 0.9-kb *Bam* HI/*Hin*dIII fragment containing the SV40 small-t intron and early gene polyadenylation signal which was derived from pSV1[17] (given by Dr Colman). Recombinants containing the 0.9-kb fragment were identified by restriction mapping and one was chosen and designated pMLV. pMLV was restricted with *Pvu*I and *Eco*RI, and the *Eco*RI ends blunt-ended by filling in using the Klenow fragment of DNA polymerase I. The 4.4-kb *Pvu*I/*Eco*RI fragment containing the M-MuLV LTR, SV40 sequences and most of the pAT153 sequences, was purified by agarose gel electrophoresis. The *tk/neo* gene and part of the ampicillin resistance gene (*amp*^{r})[9] was purified as a *Pvu*I/*Bam*HI fragment from the cosmid vector pTM[18]. The *Bam*HI end was blunt-ended by filling in (see above). This fragment was then ligated to the pMLV-derived *Pvu*I/*Eco*RI fragment using T4 DNA ligase and recombinants selected on ampicillin. Note that the *Pvu*I site interrupts the *amp*^{r} gene so that in this ligation the *amp*^{r} gene was reconstructed in part from one fragment and in part from the other. The structure of recombinants was checked by restriction mapping and one clone, designated pMLV/NEO, was used in the next stage. The isolated factor IX DNA from stage 1 (above) was ligated into the *Hin*dIII site of the expression vector pMLV/NEO using T4 DNA ligase and the Klenow fragment of DNA polymerase I. Clones containing the factor IX DNA insert in the correct orientation were identified by restriction mapping, and one such clone, pMLVIX/NEO, was used for transfection.

lines secretes active endogenous factor IX. The rat hepatoma line H4-11-E-C3, however, is known to secrete a biologically active form of another vitamin K-dependent clotting factor, prothrombin[8], indicating the presence of an active γ-carboxylase system; therefore we chose this cell line for our studies.

We first constructed a factor IX expression plasmid (Fig. 1) from previously isolated human complementary DNA and genomic clones as follows. A full-length factor IX cDNA clone, p5′G/3′cVI, was assembled from the cDNA clone cVI[3] and a small fragment of genomic factor IX sequence encoding the 5′-noncoding sequence of the normal mRNA and the first domain of the protein. The resulting cDNA sequence was then placed under the transcriptional control of the promoter of the long terminal repeat (LTR) of Moloney murine leukaemia virus (M-MuLV). The transcription unit was completed by placing the simian virus 40 (SV40) small-t-antigen intron and early polyadenylation signal downstream of the factor IX coding sequence. The plasmid also contains the *tk/neo* gene[9] in order to provide a dominant selectable marker for expression in mammalian cells. In preliminary experiments, we tested the expression plasmid pMLVIX/NEO, by transfecting[10] mouse fibroblast Ltk$^-$ cells and selecting permanent cell lines for resistance to the antibiotic G418 (ref. 11). One cell line was characterized in detail and secreted detectable human factor IX in an enzyme-linked immunosorbent assay, but the secreted factor IX failed to adsorb to barium sulphate (see Fig. 2 legend and ref. 12 for assay details). Thus, the expression plasmid was proved functional in producing a protein. However, as expected, the mouse fibroblast cell line lacked the enzymes required for γ-carboxylation of the N-terminal glutamyl residues of factor IX, which are necessary for its biological activity.

The rat hepatoma line H4-11-E-C3 (see above) was transfected with the factor IX expression plasmid by standard calcium phosphate precipitation methodology[10]. Cells containing a functional *tk/neo* gene were selected for G418 resistance[11] and 24 of the resultant colonies were cloned by limiting dilution. These permanent cell lines were then grown up and the growth medium of each assayed for the presence of secreted human factor IX by ELISA (see Fig. 2 legend). Four of the cell lines secreted detectable levels of factor IX and the highest producer, line 4A, was chosen for further analysis. Southern blot analysis indicated that there was only one copy of the plasmid integrated into the chromosomal DNA per cell (data not shown). Northern blot analysis of total cellular RNA derived from line 4A revealed the presence of a factor IX mRNA species of ~1.6 kilobase (kb), which is the expected size of factor IX mRNA. On both Southern and Northern blot analysis, no cross-hybridizing species were detected in the control hepatic cell line H4-11-E-C3 (data not shown).

Figure 2 shows the time course of secretion of human factor IX by line 4A as measured by a specific, quantitative ELISA (see Fig. 2 legend); from this experiment, we estimate the rate of secretion of factor IX to be ~6 ng per 10^7 cells per 24 h. Furthermore (see Fig. 2), adsorption of the secreted factor IX onto barium sulphate[12], a procedure which depends on the γ-carboxylation of factor IX, suggests that the protein is γ-carboxylated. As the recovery of factor IX was not corrected for any losses during the experimental procedure, we cannot distinguish between the possibility that either about 70% of the material has γ-carboxyglutamic acid residues or, alternatively, that the material is all γ-carboxylated and there were losses of ~30% during recovery of the protein. No factor IX was detectable in lysates of the 4A cells, indicating that there was insignificant accumulation within the cell. No cross-reacting material was detected either in control H4-11-E-C3 conditioned media or in cell lysates.

Table 1 shows the results of a clotting and antigenic analysis of a sample of affinity-purified human factor IX from conditioned medium of line 4A cells, together with an analysis of a parallel control sample of H4-11-E-C3 cells. The purified human

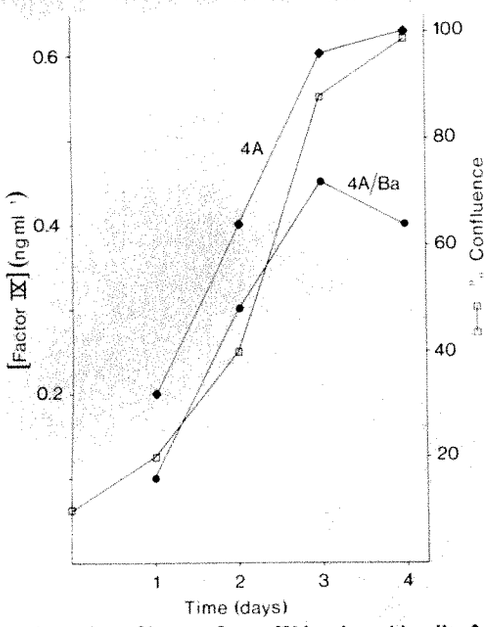


Fig. 2 Secretion of human factor IX by clone 4A cells. ◆, Factor IX levels; ●, factor IX recoverable by barium sulphate adsorption; □, degree of confluency.

Methods. Cells were seeded at 10% confluency in an 80-cm^2 flask containing 10 ml of minimal essential medium (MEM: 10% fetal calf serum containing penicillin, streptomycin, kanamycin and 100 ng ml^{-1} vitamin K). The medium was changed every 24 h and the conditioned medium assayed for human factor IX by ELISA. The ELISA was done by first binding 0.2 ml of a mouse anti-factor IX monoclonal antibody 3A6[14], diluted 1: 5,000 in 200 mM sodium carbonate *p*H 9.0, to microtitre wells at room temperature for 2 h. Samples (150 μl) of conditioned medium were then bound overnight at 4 °C. The second antibody, 150 μl of rabbit anti-human factor IX (Calbiochem-Behring), diluted 1:500 in PBS, 1% NP40 and 10% horse serum, was then bound at room temperature for 2 h. The third antibody, peroxidase-conjugated swine anti-rabbit IgG (Dako), was bound in the same manner. The assay was finally developed with 0.2 ml of 40 mM ABTS (azinodi-(3-ethylbenzthiazole sulphonic acid)-diammonium salt) in 0.008% H_2O_2 in 50 mM citrate buffer *p*H 4.0 for 30–45 min. Colour change of the solution was measured at 405 nm in a spectrophotometer. A standard curve was constructed using twofold dilutions of normal plasma in the range 10^{-3}–10^{-5} dilution, assuming a concentration of 5 μg ml^{-1} (ref. 1) for plasma factor IX. Undetectable quantities (<0.02 ng ml^{-1}) of factor IX were observed in conditioned medium from control H4-11-E-C3 cells using ELISA.

factor IX is active in a one-stage clotting assay[13], giving 7% of the activity of normal plasma. The control shows only 1% activity and this is presumably due to the trace amounts of bovine factor IX which were found in both line 4A and control samples, as indicated by an ELISA for bovine factor IX antigen (see Table 1). Further evidence (Table 1) that the cell line 4A is secreting active human factor IX is provided by the inhibitory effect of the specific anti-human factor IX monoclonal antibody 3A6[14] in the clotting assay. Control experiments showed that this antibody does not significantly inhibit bovine or rat factor IX activity at the concentration used here (data not shown). The fact that the activity level (7% of normal plasma) in the line 4A sample correlates well with the level of human factor IX antigen (6.8% of normal plasma) suggests that the protein is fully active. We conclude that line 4A cells secrete biologically active human factor IX, albeit at a low level.

We are testing alternative expression plasmids and attempting to isolate improved cell lines which might secrete more factor IX, thus facilitating purification of larger amounts of material. This should allow a detailed examination of the properties of the genetically engineered factor IX protein in comparison with the plasma protein, and should eventually allow us to start *in vivo* experiments of the kind necessary for the development and testing of a therapeutic product. For example, it is important to determine the half-life and efficacy of the genetically

Table 1 Analysis of clotting factor IX activity and of human and bovine factor IX antigen in purified samples from 4A and H4-11-E-C3 conditioned media

Assay	Cell line 4A	H4-11-E-C3
Clotting (%)	7	1
Clotting (%) + 3A6 antibody	<1	<1
Human IX antigen (%)	6.8	ND
Bovine IX antigen	0.8	1.2

Factor IX clotting activity (measured using the one-stage clotting assay[13] in which factor IX-deficient plasma is used as a substrate), and human factor IX antigen (measured by ELISA) are both given as percentages of average normal plasma levels. The inhibitory effect of the monoclonal antibody 3A6 was measured by preincubation of the sample to be tested with a final concentration of 2.5% 3A6 ascites fluid for 15 min at room temperature. Bovine antigen is given in arbitrary units and was assayed by quantitative ELISA as follows. Dilutions of pure bovine factor IX or samples to be assayed were diluted in 200 mM sodium bicarbonate, *p*H 9.0 buffer and dried onto microtitre plates. Bovine factor IX was then detected using a polyclonal rabbit anti-bovine factor IX antibody and a peroxidase-conjugated anti-rabbit IgG antibody as for the ELISA for human factor IX. The human factor IX sample from 1.5 l of conditioned media from line 4A cells, and the control sample from the same volume of H4-11-E-C3 cells were purified by barium citrate adsorption[16] and immunoaffinity chromatography. An anti-human factor IX antibody affinity column was prepared by binding the 3A6 monoclonal antibody (purified from ascites fluid by sodium sulphate precipitation) to the support Affigel 10 (Biorad) using conditions recommended by the manufacturer. The sample to be bound was passed twice over the column (vol. 0.3 ml) then washed with >100 vol. phosphate-buffered saline (PBS)), 1% Nonidet P-40 (NP40) and a similar volume of PBS, 1 M NaCl, 1% NP40. Finally, the column was rinsed with 5 vol. PBS, 1 M NaCl and eluted with 7 M urea, 1 M NaCl, and 0.4-ml fractions were collected. The first three fractions were pooled and immediately dialysed overnight against 2% PBS at 4 °C, followed by freeze-drying. The samples were finally dissolved in 200 μl H_2O. ND, not determined.

engineered product compared with plasma factor IX. A clinical product derived from the cell line described here is therefore not an immediate prospect, but the results reported here represent an important first step in demonstrating the feasiblity of this approach. However, we are optimistic that further studies in conjunction with work already done on clotting factor VIII(C)[15,19], will eventually allow the majority of haemophiliacs to be treated with improved virus-free products.

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Lattice mobility and anomalous temperature factor behaviour in cytochrome *c'*

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Atomic temperature factors (*B*-values) obtained from X-ray refinement experiments provide empirical estimates of protein mobility that have been correlated with both theoretical simulations of protein dynamics[1] and experimental studies of antibody reactivity[2,3]. The comparison of *B*-values with protein solution properties requires adjustment of the apparent atomic mobilities to compensate for the effects of the crystal environment[4–6]. Here we compare crystallographically independent subunits of the dimeric cytochrome *c'* from the bacterium *Rhodospirillum molischianum* to examine how lattice effects influence refined *B*-values. In addition to local effects on protein mobility at crystal contacts[4,5], we show that *B*-value differences up to 12 Å^2 between subunits result from lattice disordering effects that approximate to concerted rotations of the molecules about a crystal symmetry axis.

Ferricytochrome *c'* from *R. molischianum* is a dimeric haem protein composed of identical 128-residue polypeptide chains. The protein crystallizes in the orthorhombic space group $P2_12_12_1$ ($a = 56.6$, $b = 72.4$, $c = 75.4$ Å), with a dimer in the crystallographic asymmetrical unit[7]. The structure has been crystallographically refined to an *R*-factor of 0.19 for 30,533 reflections with $F > 2\sigma F$ to 1.67 Å resolution[8]. Independently refined subunits are related by a non-crystallographic dyad axis with an r.m.s. difference between corresponding α-carbon positions of 0.40 Å. Many detailed features of the subunits, including the equivalent situations of some 40 bound solvent molecules per monomer, are conserved despite differing environments in the crystal lattice.

Figure 1*a,b* plots mean residue *B*-value against sequence number for the two subunits of the native dimer. Comparison of the subunits yields a correlation coefficient of 0.82. This is similar to values reported for structures determined in different crystal forms[5,9] and is indicative of the extent to which *B*-value behaviour may reflect intrinsic protein dynamical properties[1,4]. Despite the similarities, monomer *B*-values differ by up to 30 Å^2 locally and by some 6 Å^2 overall (Fig. 1*c*). These variations between chemically identical subunits are attributable to their different environments in the crystal lattice.

Lattice interactions could potentially affect *B*-value behaviour in a variety of ways[6]. One result of the intermolecular interactions formed at crystal contacts is the localized immobilization of protein side chains or surface backbone loops. Consequently, the *B*-values of groups involved in such interactions may imply local mobilities that are substantially smaller than actually occur in solution. Previous studies have correlated the magnitude of such localized effects with solvent-accessible surface area in the crystal lattice[5,10]. Comparison of the mean residue *B*-values and fractional accessible surface areas[11] for the individual cytochrome *c'* subunits yields correlation coefficients (0.69 and 0.61, Fig. 1*a, b*) comparable to those observed previously[5,10]. A difference plot between monomers (Fig. 1*c*) shows that *B*-value and accessible area variations frequently correspond to alternative lattice contacts made by the two monomers in the asymmetrical unit. Nevertheless, differences between monomers correlate relatively poorly (0.35), in part resulting from an average difference of 6 Å^2 in subunit *B*-value that is uncorrelated with any overall difference in accessible surface area. As shown below, these variations reflect a second type of lattice effect that involves systematic disordering of molecules relative to crystal symmetry axes.

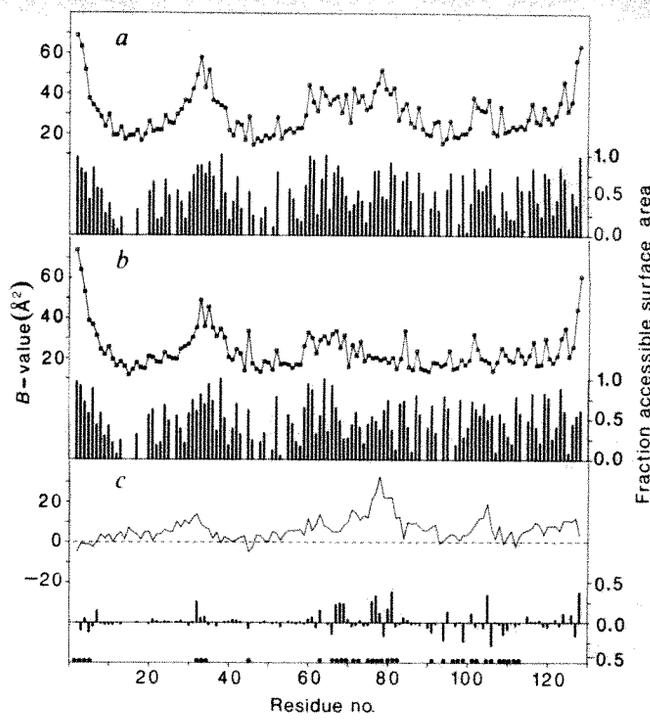


Fig. 1 *a, b*, Plots of residue *B*-value averaged over main-chain backbone atoms (connected curves), and fractional accessible surface areas[5,11] for entire residues in their respective crystal lattice environments (bars), against sequence number for crystallographically independent subunits of cytochrome *c'*. *c*, *B*-value (solid curve) and accessible surface area (bars) difference between subunits. Dots indicate sequence positions of residues that make different crystal contacts in the two subunits.

Figure 2 shows the crystal packing geometry and illustrates that subunits with sytematically low *B*-values (cool monomers) form continuous lattice interactions along alternate 2-fold screw axes parallel to the unit cell *c*-axis. Hot monomers make native dimer interactions with cool monomers aligned along each crystal screw, together with lattice contacts to cool monomers aligned along adjacent *c*-axis screws (Fig. 2*a*). Hot monomers interact only with cool monomers and are not in contact with each other in the crystal lattice. The crystal is thus organized as a parallel array of native dimer helices that are interconnected through hot monomer lattice interactions. This situation raises the possibility of systematic disordering of the hot monomers relative to the cool monomer arrays aligned along the *c*-axis crystallographic screws.

Figure 3 plots differences in mean *B*-value (ΔB) between corresponding residues of hot and cool monomers in the native dimer against their difference in radius (ΔR) from the *c*-axis crystallographic screw. This treatment compensates for variations in *B*-value that may arise from the local structural properties of the monomers (for example, extent of local secondary structure) and emphasizes differences that vary systematically relative to the crystallographic screw axis. The observed correlation (0.57) between ΔB and ΔR for dimer subunits indicates a continuous increase in average temperature factor for residues progressively more distant from the crystallographic screw. This behaviour is consistent with either static or dynamic lattice disordering phenomena involving rotational or wobbling displacements of the dimers relative to the crystal screw axis. The average behaviour (Fig. 3) suggests a maximum *B*-value contribution of 12 Å^2 at the hot monomer periphery resulting from extended lattice disordering effects. Assuming the simplest model involving rigid-body displacement of the dimer about the

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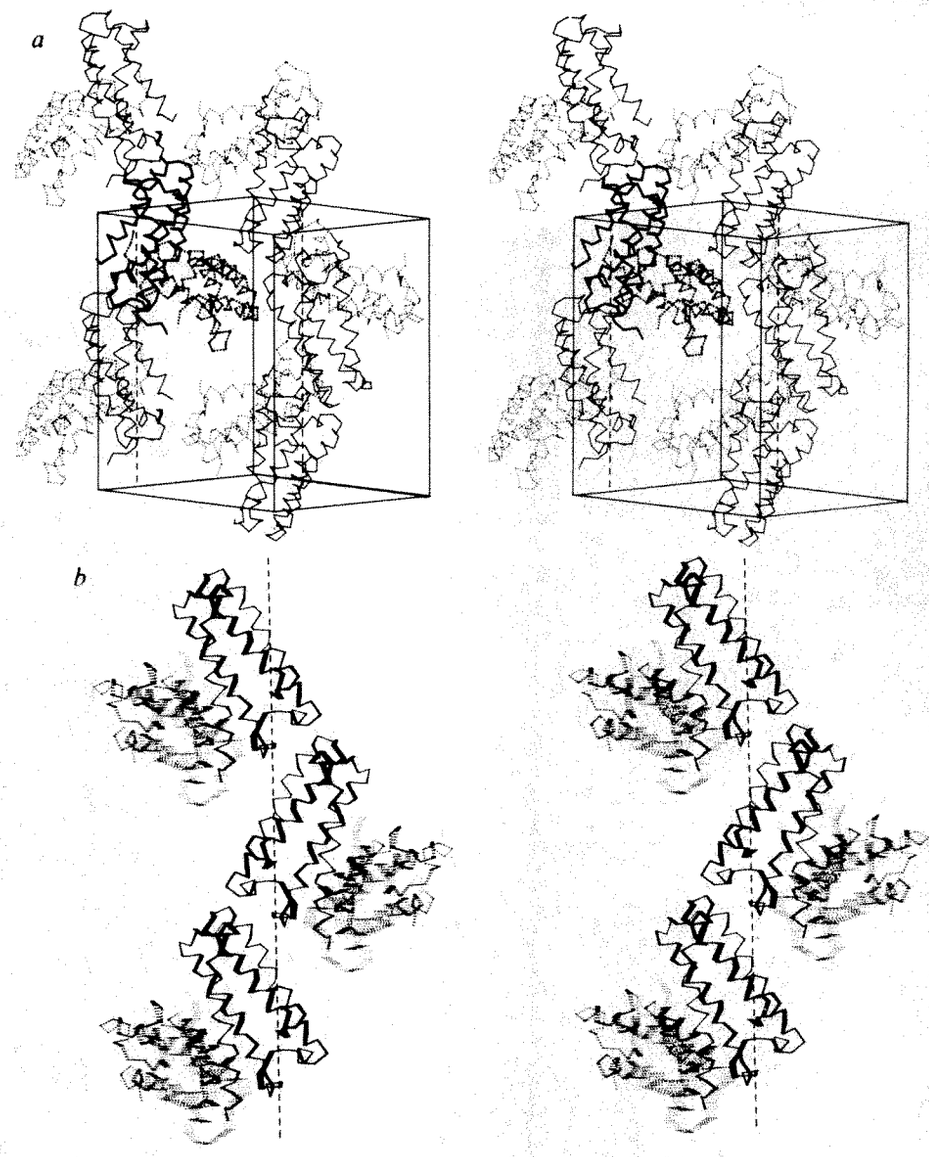


Fig. 2 *a*, Stereoview of the cytochrome *c'* crystal packing. Subunits (illustrated by α-carbon backbones) with lower average *B*-values (cool, solid) pack continuously along alternate 2-fold screws (long dashed) parallel to the unit cell (outlined) *c*-axis. Hot (dashed) monomers hold the lattice together by making native dimer (bold) interactions with each cool monomer array and lattice contacts with cool monomer arrays on adjacent screws. *b* Shows how the systematic *B*-value differences between monomers could reflect static or dynamic disordering involving rigid-body displacements of the molecular arrays about the *c*-axis screws. The observed *B*-value difference of 12 Å^2 at the hot monomer periphery (Fig. 3) suggests an axial rotation of 1.2° that produces a maximum displacement $U = 0.7$ Å, where $U^2 = 3B/8\pi^2$. The figure illustrates the superimposed effects of a rigid body rotation about the *c*-axis crystallographic screw that has been exaggerated by a factor of 3 for clarity.

crystallographic screw yields a corresponding r.m.s. amplitude of 0.7 Å (Fig. 2*b*).

The preceding experiment shows that observed differences in subunit *B*-factor behaviour in cytochrome *c'* result from a superposition of localized lattice packing effects that correlate with accessible surface area, and long-range disordering phenomena that reflect the extended pattern of molecular interactions in the crystal lattice. In the present case, the apparent motion may be related to a low-frequency vibrational mode of the V-shaped dimer, as suggested by the correlation (0.40) of monomer *B*-values with distance to the molecular dyad axis. Such concerted molecular flexing could produce the observed overall *B*-value behaviour, assuming that the cool monomers are relatively constrained in their aligned orientations along the *c*-axis crystallographic screws.

The comparison of protein structures determined in different crystal environments provides a basis for correcting *B*-values to account for the localized immobilizations that occur at crystal contacts[4,5], and thus allows estimates of protein dynamic flexibility in solution. Nevertheless, it is clear that extended lattice disordering effects, which may be unrelated to protein solution dynamics, can make comparable contributions to refined crystallographic *B*-values. It is anticipated that similar effects could occur in other high-resolution crystal structure determinations. However, such lattice effects could go unrecognized in the general case where the crystal asymmetrical unit contains only a monomer. In this case, the potentially non-uniform contributions to *B*-values from lattice disordering would be erroneously

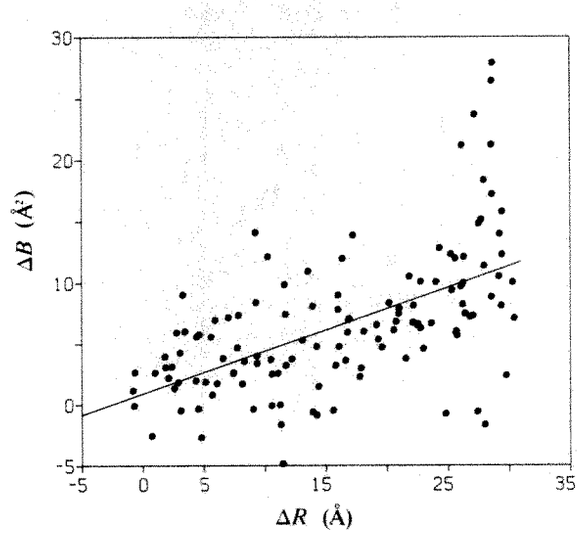


Fig. 3 Plot of the difference in mean backbone *B*-value (ΔB) between corresponding residues of the cytochrome *c'* subunits against their difference in radial distance (ΔR) from the crystal screw on which the cool monomers are aligned. The least-squares line suggests that the component varying systematically with ΔR contributes up to 12 Å^2 to the *B*-values of residues furthest from the crystal screw. Large deviations from the least-squares line correspond to residues forming different crystal contacts in the two monomer environments.

attributed to localized protein flexibility in solution. The significance attached to protein *B*-values, both in theoretical studies of protein dynamics[1] and practical applications of medical importance[2,3], justifies continued efforts at developing new theoretical and crystallographic refinement methods that can simulate crystal lattice effects.

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Yeast heat-shock protein of M_r 48,000 is an isoprotein of enolase

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Exposure of cells or tissues of various living organisms to elevated temperatures induces the synthesis of a family of specific proteins called heat-shock proteins (HSPs)[1–3]. This phenomenon has so far been investigated mostly with respect to the induction mechanism of these HSPs[4,5]. However, little is known about the function of such proteins, although it has been suggested that they are involved in the acquisition of thermal tolerance[3]. We have recently suggested that a specific class of HSPs may function as negatively regulatory molecules in the growth of eukaryotic cells[6–8], and that a HSP of relative molecular mass (M_r) 48,000 (HSP48) from the yeast *Saccharomyces cerevisiae* may be involved in both thermal tolerance and growth control in this organism[8]. We now present evidence that *S. cerevisiae* HSP48 is an isoprotein of a glycolytic enzyme, enolase (EC 4.2.1.11). This unexpected finding may provide new insight into the role of the protein in the acquisition of thermal tolerance and growth control.

In *S. cerevisiae*, 6 out of 13 HSPs, including HSP48, are durably induced when cells are induced to enter the resting state of the cell cycle, G_0 (ref. 7). These G_0 yeast cells are much more resistant to heat shock than are growing cells, probably because of the accumulation of some or all of the above six HSPs. Induction of certain HSPs was also found with cultured cells of higher eukaryotes when they were arrested in G_0 (ref. 7). Furthermore, we have isolated a heat-shock-resistant mutant of the yeast which is defective in the *HSR1* gene and which constitutively synthesizes six proteins, including two isoforms of HSP48 (ref. 8). The *hsr1* mutant reveals unusual properties involved in growth control, in addition to the selected property that it is ~1,000-fold more resistant to lethal heat shock than the parental *HSR1* strain[8]. Because HSP48 is the only protein that is commonly induced in both growing *hsr1* cells and pre-heated or G_0-arrested *HSR1* cells, all of which are highly heat-shock resistant, we believe that HSP48 is responsible for the heat-resistance associated with these cells[8]. This is compatible with previous findings indicating that neither the products of the amplified *HSP90* genes[9] nor those of the two cloned genes of the *HSP70* family[10] are directly involved in the acquisition of resistance to lethal heat shock.

Table 1 Physicochemical and biochemical properties of HSP48 and yeast enolase

	HSP48	Enolase
Sedimentation coefficient ($s^\circ_{20,w}$)	5.3S	6.0S*
Stokes radius (Å)	36.5	36.2†
M_r from $s^\circ_{20,w}$ and Stokes radius	90,000	96,000
M_r from sedimentation equilibrium	84,000	88,000*
M_r from SDS-PAGE	48,000	48,000*
Enolase specific activity	69 ± 1	68 ± 1

Enolase activity was determined by monitoring the increasing rate of absorption at 240 nm at 25 °C as a result of conversion of D(+)2-phosphoglyceric acid (PGA) to phosphoenolpyruvate (PEP)[20]. Standard assay buffer consisted of 50 mM Tris-HCl (*p*H 7.8 at 25 °C), 2.7 mM magnesium acetate, 0.01 mM EDTA and 1.2 mM PGA. Specific activity is defined as the conversion of 1 μmol PGA to PEP per min per mg protein. Yeast enolase was a product of Oriental Yeast Co. Ltd, Tokyo.

* Data from ref. 21; † data from ref. 22.

Table 2 Amino-acid composition of HSP48 and yeast enolase

	HSP48	Yeast enolase
Asx	11.7	11.6
Thr	4.8	4.7
Ser	6.5	7.2
Glx	8.2	7.9
Pro	3.5	3.5
Gly	8.8	8.6
Ala	13.3	12.8
Val	7.4	7.9
Met	1.1	1.2
Ile	5.3	5.1
Leu	9.7	9.3
Tyr	2.1	2.1
Phe	3.7	3.7
His	2.4	2.6
Lys	8.2	8.6
Arg	3.2	3.3

Values are represented in mol%. Purified HSP48 was resolved in 7.5% SDS-PAGE[23]. The gel was not fixed or stained. Gel slices containing HSP48 were incubated in 0.02% SDS solution (1.0 ml per cm^3 gel) to elute the protein, and the elution was repeated. The combined eluates were extensively dialysed against distilled water for 2 days and lyophilized. The protein was hydrolysed in 6 M HCl at 110 °C *in vacuo* for 22 h and 70 h and analysed in an automatic amino-acid analyser (Hitachi model 835, Hitachi). Amino-acid composition of yeast enolase (peptide 1) was calculated from the amino-acid sequence of this protein deduced from the nucleotide sequence of the complementary DNA[12]. Cys and Trp residues were omitted from the above calculation as we did not determine the content of Cys or Trp in HSP48.

To elucidate the function of HSP48, extracts of yeast cells in the stationary phase underwent four successive purification steps and HSP48 was purified to homogeneity (see Fig. 1 legend). Purified HSP48 is composed of two isoforms, HSP48A (acidic form) and HSP48B (basic form)(Fig. 1*A*), as we have shown previously[7,8] by two-dimensional non-equilibrium *p*H gradient electrophoresis/SDS-polyacrylamide gel electrophoresis (NEPHGE/SDS-PAGE) of ^{35}S-methionine-labelled cells. Peptide mapping confirmed that the purified protein is identical to ^{35}S-methionine-labelled HSP48 (Fig. 1*B*).

We determined the N-terminal 23 amino acids of the purified HSP48 on a gas phase sequencer (Fig. 2). Using the Dayhoff protein sequence bank[11] to compare the sequence with those of other proteins, we found that the N-terminal 23 amino-acid sequence of HSP48 is identical to the corresponding sequence of yeast enolase (Fig. 2). In addition, physicochemical properties (Table 1) and amino-acid composition (Table 2) of the purified HSP48 were essentially the same as those of yeast enolase. Furthermore, the specific activity of the enolase in the purified HSP48 protein was the same as that of the authentic yeast

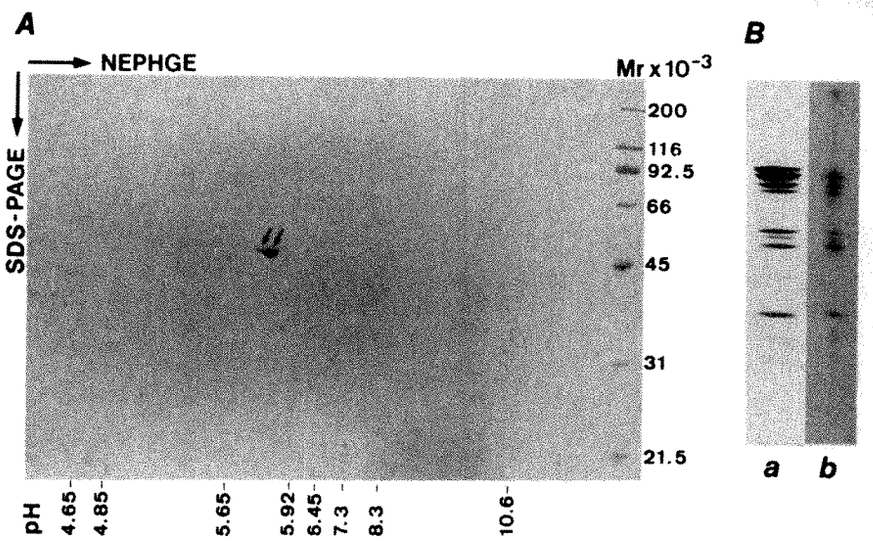


Fig. 1 *A*, Two-dimensional gel electrophoresis of purified yeast HSP48 (5 μg). *B*, peptide mapping of purified HSP48 (lane *a*) and ^{35}S-methionine-labelled HSP48B recovered from the corresponding spot in a two-dimensional gel (lane *b*).

Methods. *A*, Isolation of HSP48: Cells of strain X2180-1A (ref. 6) were grown to stationary phase at 23 °C with aeration in SD10 medium[6] in two 2.5-l batches (1×10^8 cells per ml), after which the cultures were incubated for 24 h in the same conditions. After chilling the cell suspensions, we collected the cells by centrifugation at 4 °C at 5,000 r.p.m., and washed them once with 0.05 M Tris-HCl (*p*H 7.9 at 4 °C). The pelleted cells were 35 g in wet weight, and were stored at −80 °C until use. Half of the frozen cells were thawed and suspended in 20 ml of buffer A (0.05 M Tris-HCl, *p*H 7.9 at 4 °C, 0.01 M $MgCl_2$, 0.01 M 2-mercaptoethanol, 0.1 mM EDTA, 0.25 M mannitol, 1 mM phenylmethylsulphonyl fluoride), after which they were homogenized at 4 °C with 120 g of 0.5-mm glass beads in a Universal Homogenizer (Nihon Seiki Seisakusho Co., Tokyo) for 3 × 2 min. The homogenate was centrifuged at 5,000 r.p.m. at 4 °C for 5 min, after which the resulting pellet was again homogenized and centrifuged. The supernatants were combined (58 ml) and centrifuged at 35,000 r.p.m. in a Beckman Type 35 rotor (Beckman Japan) for 2 h at 4 °C. The supernatant containing most of the HSP48 was dialysed twice against 2 l of buffer B (0.05 M Tris-HCl, *p*H 8.8 at 4 °C, 0.01 M 2-mercaptoethanol, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.02 M NaCl) for 24 h at 4 °C, and applied to a DEAE-cellulose (Watman DE52) column (3 × 24 cm) equilibrated in buffer B. HSP48 was recovered in the flow-through fractions in buffer B. The fractions were combined and dialysed twice against buffer C (0.01 M sodium phosphate, *p*H 5.5 at 4 °C, 0.01 M 2-mercaptoethanol, 1 mM $MgCl_2$, 0.1 mM EDTA) for 20 h, and applied to a CM-Sepharose CL-6B column (1.4 × 20 cm) equilibrated with buffer C. HSP48 was recovered in flow-through fractions. One-tenth of the combined fractions containing HSP48 was loaded onto a Sephacryl S-200 column (1.5 × 75 cm) equilibrated with buffer D (0.01 M sodium phosphate, *p*H 6.0 at 4 °C, 0.01 M 2-mercaptoethanol, 1 mM $MgCl_2$, 0.1 mM EDTA) and eluted at a flow rate of 10 ml h^{-1}. HSP48 was eluted in a single peak and found to be purified to 99% homogeneity as judged by SDS-PAGE. The HSP48 content of fractions obtained during the purification was determined by densitometry after one-dimensional and two-dimensional SDS-PAGE. About 15–19 mg of purified HSP48 was reproducibly obtained from 17.5 g (wet weight) of yeast cells in the stationary phase with the recovery of 65%. Two-dimensional gel electrophoresis: The purified HSP48 was lyophilized, dissolved in the O'Farrell lysis buffer, and resolved by NEPHGE[24], followed by second-dimension electrophoresis in 11% SDS-PAGE[25]. The gels were stained with Coomassie brilliant blue. M_r (Bio-Rad) and isoelectric point (BDH) markers were used[8]. *B*, Cells grown in the stationary phase in synthetic medium (6.7 g l^{-1} Bacto yeast nitrogen base without amino acids, 10 g l^{-1} succinic acid, 6 g l^{-1} NaOH, 2 g l^{-1} glucose) were pulse-labelled with 10 μCi ml^{-1} L-^{35}S-methionine (1,200 Ci/mmol, Amersham Japan) at 23 °C for 10 min, and chased for 3 min by the addition of non-radioactive methionine to 0.5 mg ml^{-1}. The whole-cell extracts were resolved by two-dimensional NEPHGE/SDS-PAGE. ^{35}S-Methionine-labelled HSP48B, cut out from the corresponding spot in the gel, and purified HSP48 (5 μg) were separately subjected to peptide mapping with *Staphylococcus aureus* V8 protease (5 ng for each) according to the method of Cleveland *et al.*[26]. Lane *a*, digested products of purified HSP48 stained with Coomassie brilliant blue; lane *b*, digested products of ^{35}S-methionine-labelled HSP48B revealed by fluorography. We have previously shown that HSP48A and HSP48B give similar peptide mapping results[7,8].

enolase (Table 1). Lastly, a rabbit antiserum directed against yeast HSP48 reacted to authentic yeast enolase (Fig. 3*A*). From these results, we conclude that yeast HSP48 is enolase.

Searching for further homology between the partial amino-acid sequence of HSP48 and other proteins, we found that the 23 amino-acid N-terminal sequence of HSP48 and the N-terminal 41 amino-acid sequence of yeast enolase[12] were similar in part to the most extensive homologous region[13] found in *Drosophila* HSP70, yeast HSP70 and in the *Escherichia coli* heat-shock *dnaK* protein (Fig. 2). This partial homology suggests an evolutionary relationship between enolase and the HSP70 family. The possibility that enolase and the HSP70 family share a common function (for example, an interaction with the substrate of enolase) remains to be investigated.

An immune blot analysis of total yeast-cell proteins showed that two proteins, HSP48 and another protein of M_r 47,000 (p47), reacted with anti-HSP48 serum (Fig. 3*A*). Peptide mapping indicated that HSP48 and p47 are quite similar (Fig. 3*B*), suggesting that the latter is another isoprotein of enolase. Unlike HSP48, the synthesis rate of p47 was not significantly altered by heat shock or growth arrest (Fig. 3*C*). *S. cerevisiae* contains two non-tandemly repeated enolase structural genes, designated *ENO1* and *ENO2* (ref. 12). Enolase 2 polypeptide, the product of *ENO2*, migrates faster than enolase 1 polypeptide, the product of *ENO1*, in SDS-PAGE and the contents of the two enolase polypeptides differ with the growth conditions[14]. We found that the levels of HSP48 and p47 present in cells grown in media containing various carbon sources were quantitatively different (data not shown). Because of this and because of similarities in relative molecular mass, we conclude that HSP48 and p47 are identical to enolase 1 and enolase 2, respectively.

As is the case for HSP48 (HSP48A and HSP48B), p47 is also composed of two isoforms (Fig. 3*C*). The relative amounts of the two isoforms in each of the two proteins also differ for cells grown in different conditions[7,8], and it is possible that the enolase activities associated with these isoforms and their intracellular distribution also differ. Thus, the differential expression of the two enolase genes and the differential processing of their products probably have biological significance. Indeed, differential expression of mammalian enolase isoproteins has been observed

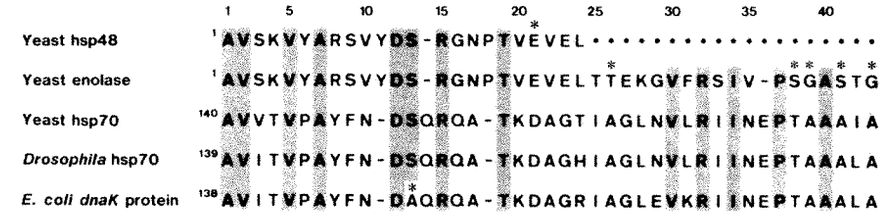


Fig. 2 Comparison of amino-acid sequences of HSP48, yeast enolase and possibly related heat-shock proteins. The sequences of yeast HSP48 (residues 1–23), yeast enolase (Residues 1–41)[12], yeast HSP70 (the *YG100* gene product, residues 140–180)[27], *Drosophila* HSP70 (residues 139–179)[28] and *E. coli dnaK* protein (residues 138–178)[13] are aligned to show the maximum homology among these proteins. Numbers on the left indicate the position of the residue in the molecule. Shading indicates homology. Asterisks indicate conservative amino-acid substitutions as defined by Schwartz and Dayhoff[29]. Dots indicate residues which have not been experimentally determined. − Indicates a space added to maximize the homology.

Methods. An aliquot of purified HSP48 was dialysed against 1 mM ammonium bicarbonate, lyophilized and dissolved in 1% trifluoroacetic acid. Amino-acid sequence results were obtained by using a protein sequencer (Model 470A; Applied Biosystems).

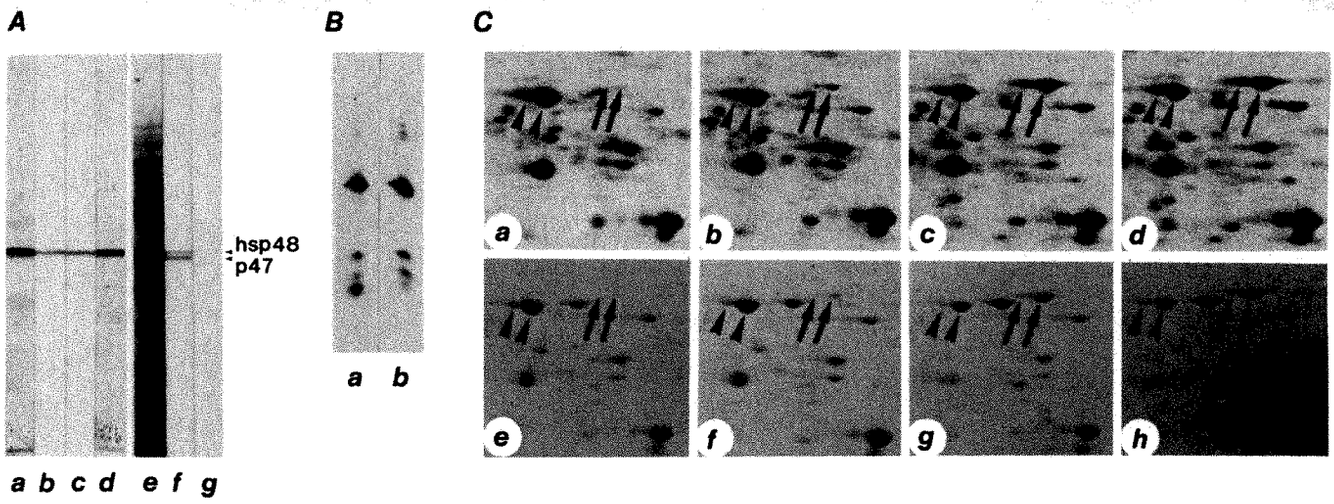


Fig. 3 *A*, Immune blotting of yeast whole-cell extracts and authentic yeast enolase with anti-yeast HSP48 antibody. Purified HSP48 (lanes *a*, *b*), yeast enolase (lanes *c*, *d*) and extracts of X2180-1A cells in the stationary phase (lanes *e*–*g*) were resolved in SDS-PAGE and electrophoretically transferred to a nitrocellulose sheet. The sheet was stripped and strips were separately incubated with 1:500 diluted rabbit yeast anti-HSP48 antiserum (lanes *b*, *c*, *f*) and preimmune rabbit serum (lane *g*), followed by incubation with peroxidase-conjugated goat anti-rabbit IgG (Cappel). A strip was stained with Auro Dye (Janssen) (lanes *a*, *d*, *e*). ***B***, Peptide mapping of HSP48 and p47. The spots of ^{35}S-methionine-labelled HSP48 (lane *a*) and p47 (lane *b*) were subjected to peptide mapping with *S. aureus* V8 protease as described in Fig. 1 legend. ***C***, synthesis rate and content of HSP48 and p47 in heat-shocked or G_0-arrested yeast cells. *a*, *e*, Exponentially growing X2180-1A cells at 23 °C in synthetic complete medium; *b*, *f*, heat-shocked cells at 36 °C for 1 h; *c*, *g*, cells kept in stationary phase for 24 h; *d*, *h*, cells starved of sulphur for 24 h by incubation in sulphur-free medium[8]. The cells in each of the above cultures were pulse-labelled with ^{35}S-methionine and chased as described in Fig. 1 legend. The whole proteins (25 μg each) extracted from these labelled cells were analysed in two-dimensional gel electrophoresis as described for Fig. 1. Protein spots were revealed by autoradiography (*a*–*d*) and staining (*e*–*h*). A pair of arrowheads indicate p47A (left; acidic form) and p47B (right; basic form). A pair of arrows indicate HSP48A (left) and HSP48B (right).

during differentiation and maturation of neurones and teratocarcinoma and neuroblastoma cell lines[15–18].

The expression of the enolase genes seems to be regulated by many systems. First, it depends on carbon sources in the media[14]. Second, we have shown previously that the *HSR1* gene negatively regulates the synthesis of six proteins—HSP48A, HSP48B, two G_0-induced proteins (p73 and p56) and two unidentified proteins (p63 and p60)[8]. Third, exposure of *HSR1* cells to mild heat shock increases the synthesis rates of the two HSP48s and of other HSPs[7]. Finally, *hsr1* cells starved of sulphur or arrested in the stationary phase synthesize HSP48A, HSP48B, p73 and p56 at higher rates than do exponentially growing *hsr1* cells[8]. These results suggest that the synthesis rate of HSP48 (enolase 1) is affected by at least three factors, the *HSR1* gene, growth conditions and heat shock, although these factors may partly overlap. In agreement with our results, McAlister and Holland have noted that the content of enolase 1, but not of enolase 2, is significantly increased in yeast cells grown to the stationary phase[14].

Finally, the question arises of whether HSP48 functions solely as a glycolytic enzyme when it is involved in growth control and in protecting yeast cells from lethal heat shock. The synthesis of other glycolytic enzymes linked to enolase, such as glyceraldehyde 3-phosphate dehydrogenase or pyruvate kinase, is not significantly altered in *hsr1* cells or when *HSR1* cells are preheated by 36 °C (ref. 19 and our unpublished observation). This result is consistent with the hypothesis that the heat-shock resistance acquired by the *hsr1* mutation and heat pretreatment may be ascribed to some function associated with HSP48 other than enolase activity. Analysis of the intracellular distribution and molecular interactions of HSP48 (enolase 1) with other molecules, especially in comparison with p47 (enolase 2), should provide more direct evidence.

We thank Drs Hiroshi Kawasaki and Koichi Suzuki for amino-acid sequencing, Ms Yoko Nadaoka and Dr Tsuguchika Kaminuma for analysis of the protein sequence bank, and Dr Kazuko Iida for immune blotting. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

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Erratum

Colour, albedo and nucleus size of Halley's comet

D. P. Cruikshank, W. K. Hartmann & D. J. Tholen
Nature **315**, 122–124 (1985).

ON page 122, column 1, line 3 of the first paragraph should contain the values J (1.25 μm) and V (0.55 μm).

Retroviral protease-like sequence in the yeast transposon Ty*1*

RECENTLY, Mellor *et al.*[1] determined the nucleotide sequence of the 5'-terminal portion of the yeast transposon Ty*1* and identified two open reading frames, ORF1 and ORF2, which are in the same relative positions as the *gag* and *pol* coding sequences in retroviruses. In addition, they showed that Ty*1* produces a fusion protein by a specific frameshifting process similar to that used by retroviruses. We have shown that retroviruses, cauliflower mosaic virus (CaMV), *Drosophila copia*-like element 17.6 and Syrian hamster intracisternal A-type particle (IAP) contain sequences homologous to the *gag*-specific protease p15 of Rous sarcoma virus (RSV) in the same relative positions[2,3]. Furthermore, these p15-related sequences contain a highly conserved stretch of amino acids which shows a close similarity with sequences around the active-site amino acids Asp-Thr-Gly of the acid protease family, suggesting that they possess a similar activity to that of acid proteases[2].

A computer-assisted search for homology has revealed the presence of the p15-related sequence in the ORF2 of Ty*1* (Fig. 1, Table 1). Thus, the N-terminal portion of the ORF2 product may have an activity similar to that of acid proteases. Such a widespread occurrence of the protease sequence suggests that it is vital for these viruses and transposons. Mellor *et al.* suggested that the fusion protein is processed proteolytically[1]; the ORF2-coded protease may be involved in this processing. The relative positions of the conserved segments are almost the same among Ty*1*, CaMV, 17.6 and Moloney murine leukaemia virus (M-MuLV) (Fig. 1), suggesting that the observed homologies are the result of divergence, not convergence. This also supports the notion that the ORF2 corresponds to *pol* in retroviruses[1], and thus the reverse transcriptase coding sequence, which is highly homologous among reverse transcriptase-containing viruses and transposons[2-5], probably lies in a region immediately downstream from the protease region.

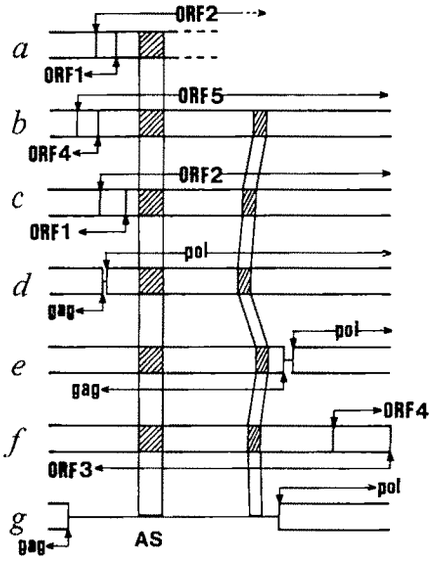


Fig. 1 Maps of homology between protease regions of reverse transcriptase-containing viruses and transposons: *a*, Ty*1*, *b*, CaMV; *c*, 17 6; *d*, M-MuLV, *e*, RSV; *f*, IAP; *g*, HTLV-I (human adult T-cell leukaemia virus). Homologous segments are linked by slanted lines. No open reading frames are assigned between *gag* and *pol* in HTLV-I. Data were taken from ref 2 for *b–e* and *g*, and from ref. 3 for *f*. AS, the region showing a close sequence similarity with active-site amino acids of the acid protease family

Table 1 Amino-acid sequence comparison between p15-related proteases and pepsin-related acid proteases

Viral protease	
Ty*1* ORF2	GHLLL-DSGASRTLIRS
CaMV ORF5	LHCFV-DTGASLCIA-S
17.6 ORF2	LKCLI-DTGSTVNMT-S
M-MuLV *pol*	VTFLV-DTGAQHSVL-T
RSV *gag* p15	ITALL-DSGADITII-S
IAP ORF3	FEGIM-DSGADKSII-S
HTLV (*gag-pol*)	IEALL-DTGADMTVL-P
	*
Acid protease (C-terminal)	
Pepsinogen (human)	CQAIV-DTGTSLLTG-P
(porcine)	CQAIV-DTGTSLLTG-P
Prochymosin (bovine)	CQAIL-DTGTSKLVG-P
Penicillopepsin	FSGIA-DTGTTLLLL-B
Renin (mouse)	CEVVV-DTGSSFISA-P
(human)	CLALV-DTGASYISG-S
	*
Acid protease (N-terminal)	
Pepsinogen (human)	DFTVVFDTGSSNLWV-P
(bovine)	DFTVIFDTGSSNLWV-P
(porcine)	DFTVIFDTGSSNLWV-P
Prochymosin (bovine)	EFTVLFDTGSSDFWV-P
Penicillopepsin	TLNLNFDTGSADLWV-F
Renin (mouse)	TFKVMFDTGSANLWV-P
(human)	TFKVVFDTGSSNVWV-P

As pepsin-related proteases consist of two topologically similar domains and two active-site aspartic acids, the amino-acid sequences around the two active sites were aligned between the N- and C-terminal halves. Bold type indicates amino acids that are identical or chemically similar among 18 sequences (90%) out of 20 at each position. Gaps(-) were inserted to increase sequence similarity. * Active sites of acid protease family For sequence data, see refs 2 and 3.

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MELLOR *ET AL* REPLY—WE thank Toh *et al.* for their interest in our paper[1] and for pointing out the homology with acid proteases at the beginning of ORF2 in Ty*1-15*. We would like to add that in pulse-chase experiments the protein, p1(Ty1-15), encoded by ORF1 in Ty*1-15* seems to be processed, presumably by proteolytic cleavage. Furthermore, we have recently identified a candidate for the 'natural' product of ORF1/ORF2 fusion. This protein, p3(Ty1-15), has a relative molecular mass of 190,000 and can be seen only in short pulse-labelling experiments. Like p1(Ty1-15), therefore, it also appears to be proteolytically cleaved. We have observed four possible cleavage products and their relationships with p1(Ty1-15) and p3(Ty1-15) are presently being established. The 'processing' of p1(Ty1-15) is dependent on the expression of ORF2, a result that is consistent with the processing protease being encoded within this region and therefore in agreement with the

observations of Toh *et al.* The interrelationships of Ty proteins will be reported elsewhere[2].

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Forest succession

MOST forest ecologists will agree with the conclusion of Finegan[1] that in real forests, succession commonly has characteristics of both the contrasting models he has described: a 'holistic' facilitation model where successive species replace each other and a 'reductionist' model where all species arrive at the outset.

Finegan is wrong, however, to say that no direct studies have been made of forest succession. Many studies do indeed rely on contemporaneous observations of forest patches of purportedly known age and history, but in the Eastern tropical rainforests there have been four studies of forest patches through time which clearly illustrate various departures from the two paradigms These studies, originally reported in the specialist forestry literature, were used in the analysis of dynamic processes in a general account of the Eastern rainforests[2].

At Kepong, Malaysia, forest on an abandoned field has been monitored since 1947[3]. Here, the flora changed little between 1949 and 1960 because of a fern blanket, which was eventually shaded out, so from 1960 to 1976 there was a marked change, though climax species were still scarce. At Sungai Kroh, Malaysia, a similar succession was monitored for 17 years from 1946 to 1963[4]. Here, some tree species, including the dominant species, were present throughout while others progressively arrived. No fern blanket developed. At Sungai Menyala, Malaysia, a secondary forest established in 1917 has been monitored since 1947[2,5]; here spasmodic floristic change is still occurring. After natural catastrophic destruction by repeated cyclones, succession at Kolombangara in the Solomon Islands has been under observation since 1964, and analysed for the first 6.6 years[2,6]. The latter two studies also include areas of 'climax' forest which are showing 'cyclic replacement'[7] (rather than 'directional change in time' as Finegan[1] defines succession) much more rapid than suggested by conventional wisdom about the stability of primary equatorial forests. Finegan excluded cyclic replacement from his discussion. It does not involve directional change in time and is the process whereby climax ecosystems are maintained in the absence of catastrophic destruction. (The latter is followed by 'secondary' succession (as it is usually termed) back to climax.) Cyclic replacement is occurring at Sungai Menyala.

At Kolombangara island, the north-coast forests are believed to be repeatedly destroyed by cyclones; they show succession as discussed by Finegan. The west-coast forests, by contrast, are outside the influence of cyclones and show cyclic replacement Sungai Menyala and Kolombangara demonstrate the value of long-term studies, and in fact these studies have continued longer than any others in climax tropical forest. The Kolombangara study includes the monitoring of recruitment, growth and mortality of seedlings of the 12 main large tree species and how these are influenced by different amounts of leafy canopy overlying them. For these species the total population is being monitored and the resultant understanding of their autecology has aided the analysis of the contrasting dynamics of successional (north-coast) forests and cyclically replacing (west-coast) forests. So far, nine sets of observations over 6.6 years have been analysed, and a further analysis is planned to start in 1986, covering another three sets of observations to 21 years. Both the Kolombangara and Sungai Menyala forests are under threat of conversion to more lucrative land-use, the much-discussed fate of many tropical rainforests, despite the contribution they have made to ecological understanding of forests[2,8-11], which itself underpins silviculture. The longer the studies continue, the more they reveal.

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FINEGAN REPLIES—I thank Whitmore for pointing out this gap in my review[1]. The studies he cites are interesting and illuminating and deserve a wider audience (although I was unable to find Whitmore's ref. 5 and am unable to comment on it)

One point concerning long-term studies is worth making here. Two of those cited by Whitmore[2,3] provide structural and floristic description only, and therefore cast very little light on the mechanisms of the successions which are clearly occurring. Measures of species abundance against time can be used to support almost any mechanistic model of succession, from the initial floristic composition ideal to a number of composite processes, as shown in my Fig. 4[1]. No amount of description, whether inferred or obtained directly, will overcome this problem; hence the last paragraph of the review. Whitmore's studies at Kolombangara exemplify the sort of approach which is needed[4].

I did not discuss cyclic replacement because there is a clear ecological basis for separating this process from the successions I review, involving differences in initial environmental conditions and sources of colonizing individuals. As Whitmore himself has pointed out, pioneer trees participate only infrequently in cyclic replacement patterns[4,5]. I should have restricted my formal definition of succession by referring to processes occurring in cleared areas large enough to permit the establishment of a vegetation dominated physiognomically by pioneer trees.

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Edited by Michael Gibbons and Bjorn Wittrock
Longman 1985 Pp 173. Pbk £9.95, $15

"SHOULD university teachers or researchers display patriotism by undertaking work for the armed forces which will inevitably be secret and unpublishable?". "Should they help pay for their institutions in hard times by making contracts with industrial firms whereby publication of research must be delayed, perhaps for a year or two, until intellectual property rights have been safeguarded by patents?". "Is public funding of university work best guided by simply choosing the best men, and leaving them to choose their research topics as best they can?". "Would the best women, if so funded, choose differently from the best men?". "For such privileges, who are the best people and how do you choose them?".

These are good questions, the resolution of which is of great importance for the maintenance of democracy and survival of freedom of inquiry and speech. It is therefore eminently reasonable to convene a conference to discuss them in detail. One would invite people from a cross-section of backgrounds university teachers currently doing research in the humanities, engineering and science (and, if possible, across more than one field), practising men of business and public affairs; and some military people, to relate their problems and experiences. One would cover diplomacy, matters of public order, economics, investment, taxation and technology, with science and philosophy bulking large in the background. There would doubtless be difficulties of language and psychology, and many important differences in priorities and chosen compromises, but the result would be very fruitful.

Conversely, it might seem one-sided to conduct such a conference in the virtual absence of the practitioners of science, and to publish essays only by (admittedly able and clever) university teachers whose specialism is mainly the study of those who study engineering and science, in the absence of practitioners of finance, law, psychology and medicine. And it might seem confusing to use the title *Science as a Commodity*

There used to be a gibe that you could always find the Germans in a crowd by inviting everyone to choose between two doors marked "Heaven" and "Lectures on Heaven". Excessive specialist theorizing is now spread throughout the Anglo-Saxon world, to the extent that it is no longer fair to pick on the Germans Those of us who have worked hard to encourage interest in broader matters in the choice, conduct and exploitation of research, feel a little frustrated because success in drawing attention to this synthesis has created another band of specialists, engaged not in practice, but only in a new branch of theory

There are wise thoughts in this book, the published record of the meeting, but there is also an overdose of theology, and repetitious concern with a few causes célèbres (such as Faust, Bacon, the Manhattan Project — described rather inaccurately by one of the contributors — and the arrangements between Hoechst and Mr Whitehead on the one hand, and the universities in Boston, Massachusetts, on the other) And there is very little about the actual products, processes and services which most illuminate the connection between free inquiry and practical application (or, which comes first), or whether the great increase in society's investment in technology seems to have helped or hindered such basic research as the astronomical study of the Universe, its cosmological interpretation, the structure of matter and energy, and the origins of life and inheritance. It may be that the practical men (inside and outside the universities) were in fact invited but would not come; if so, their contributions should have been sought by other means, and not beaten into shape with theory.

The unconfused title for the book could well be *Technology as a Tradeable Investment* or *Should Scientists do Secret Military Research?*, but these would not sound sufficiently mysterious and profound Professor Wittrock, in his summing up on p. 156, says "Science has not become a commodity; it always was". Professor Salomon, on p. 78, says "Science as a commodity? Only half a century ago, such a question would have appeared as a provocation, and a century ago, as an aberration". This apparent disagreement between gurus stems, of course, from different interpretations of the word "science", and the problem is intensified for two other reasons: first, the words science, technology and research are repeatedly used interchangeably. Secondly, in this context the term "commodity" is less appropriate than the term "investment". The trading of investments is a rather different process from trading in consumer goods. Technology, rather than science or research, is our most comprehensible kind of intellectual investment. It has been traded always, but most particularly since the patent system was introduced four centuries ago to *prevent* (not assist) secret operation; the method was the award of a temporary monopoly in return for disclosure, to allow the recoupment of the investment cost Inventive applied science can sometimes be traded as a component of technology, but the ways of nature cannot be closed to astronomers, high-energy physicists or geneticists. These subjects can be kept secret only for as long as there is military control over the instruments needed for the work (rockets to launch the satellite: detectors to measure the radiation). By contrast a commercial firm would want to go public and sell the gear

So the real problem is that of the *military* restraint of the dissemination of *technology*, with its attendant economic penalty for all, and not that of the selling of university science by commercial interests. Commerce requires the most temporary protection of knowledge that will make long-term research and development worth while, and the earliest practicable publication Men of good will such as Sir Cyril Hinshelwood, President of the Royal Society from 1955 to 1960, regarded applied research, of whatever kind, as one of the most valuable sources of ideas for scientific discovery, they sought to participate in both discovery and invention. When military secrecy hindered this natural exchange, they sought means for distilling the clues for science into publishable form.

Once this confusion is cleared up, the book becomes a useful quarry with some interesting thoughts on a variety of matters. For instance it is hard not to agree with Helga Nowotny that women would choose different research topics from men, and hard not to conclude that both science and technology are impoverished by the small number of influential women involved. But the real need is for much more study of individual examples, for example to help decide whether peacetime science and the technology-based business that funds it depends heavily, lightly or not at all on science conducted for military purposes. Did polythene, radar and the gas turbine (all invented in peacetime) require wartime funding to reach peacetime use? Was anything at all invented in wartime, does war merely accelerate and enable the development of peacetime ideas that were languishing? More broadly, would cephalosporin have been discovered by a firm, and could the semi-synthetic penicillins have been developed in a university? More pragmatism, please, before the next bout of analysis □

Duncan Davies, formerly Chief Scientist and Engineer at the Department of Industry, is Chairman of the ceramic development company BCRA Ltd and Visiting Professor at the University of York

Awash in an ocean of information

David Smythe

The Geology of the Atlantic Ocean.
By K. O. Emery and Elazar Uchupi.
Springer-Verlag 1984 Pp 1,050 DM 360, $104.80.

THE Atlantic Ocean has grown a few centimetres wider in the past few years. It has also undergone a change in location. The North Atlantic is nowadays thought of as the segment between Europe and Greenland, and the Africa – North-America segment is now the Central Atlantic. The South Atlantic remains as before, between Africa and South America. This sensible shift in nomenclature avoids such possible infelicities as "southern Central North Atlantic", or even "northern Northern North Atlantic". Emery and Uchupi's encyclopaedic compilation, some eight years in the making, sticks to the older (oceanographic) rather than the new (plate tectonic) Atlantic, and barely reaches Iceland. The book is therefore incomplete, which is a pity; many of the current problems in understanding the complex plate history north of 50°N or so have arisen through an artificial division of expertise and interest north and south, respectively, of the Greenland–Scotland Ridge.

Eleven pairs of large, loose-leaf charts cover the region 60°N to 60°S They include bathymetric, physiographic, sediment isopach, and tectonic maps Each chapter is ocean-wide in scope, ranging from history, through physiography, geology and evolution, to a final chapter on geography and economics, including waste disposal and hydrocarbons. The most up-to-date and comprehensive of them are the accounts of syn-rift and drift sediments — subjects on which the authors are long-established authorities. These chapters, making up some 40 per cent of the book, draw upon many references of 1980–1983 vintage, and take advantage of good-quality reproduction or re-drawing of original figures.

Where the discussion strays beyond the book's title — both sideways and downwards — I have increasing reservations. Some sections have a definite air of obsolescence. Why, for example, write about the North Sea at all if you have to lean heavily on review papers, one or two of which were mediocre even when they were read at the first major conference on the North Sea in 1974? Why try to write about the Neolithic and Early Bronze Age sailors of north-west Europe (incorrectly referred to as Celts) if you neglect their knowledge of navigation? They were able to predict complex lunar motions and presumably, therefore, the powerful Atlantic tides on which their maritime civilization depended. The North Sea has become a mature oil province since the early 1970s, and in the same period the whole new science of archaeoastronomy has blossomed

The chapter on internal igneous structure begins at the core and works upwards through geomagnetism, the mantle, heat flow and seismicity, petrology and so on to the ocean crust itself. On the way up there is a short excursion to the Moon. The heart of the subject — the structure, petrology and magnetic properties of oceanic crust — is dealt with in rather cursory manner; for example, there are no diagrams here showing the great variety of evolutionary models that have been thought up for the basaltic Layer 2

Even more unfortunately, an important section on the geomagnetic polarity reversal timescale picks upon one of the least satisfactory revisions for the late Mesozoic and Cenozoic, which was published in 1980. An entire industry of reversal timescale "improvements" has sprung up in the United States in the past few years, but Emery and Uchupi perpetuate an inexplicable omission by the specialists of any reference to the superb work of Hailwood and co-authors, which appeared in the Initial Reports of the Deep Sea Drilling Project in 1979 — hardly an obscure source. Six years ago you could have taken Hailwood's scale, added it to the Gubbio timescale for the late Cretaceous, run it through a pocket calculator to update the radiometric decay constants, and you would have had a "working" timescale for the past 100 million years or so, essentially as good as any of today's efforts. The moral of this example seems to be that you have to publish loud, fast and often in order to get cited, which will merely hasten the day when we are all drowned by the rising sea-level of publications.

Emery and Uchupi have obviously had difficulty in keeping their ambitious project watertight in the decade since they laid its keel. Although they have made a valiant effort, I think that the scope of the subject, at least in the terms that they see it, is now simply too broad for any two people to tackle successfully. □

David Smythe is a Principal Scientific Officer at the British Geological Survey in Edinburgh.

New journals review

On 26 September *Nature* will publish the fifth annual review supplement devoted to science journals Publishers and learned societies wishing to submit material should refer to last week's issue of *Nature* (p 607) or contact the Book Review Editor (London 836 6633)

BWP four

John Andrews

Handbook of the Birds of Europe, the Middle East and North Africa. The Birds of Western Palearctic. Vol.IV: Terns to Woodpeckers.
Chief editor Stanley Cramp.
Oxford University Press: 1985.
Pp.960 £60.

THE latest volume of "BWP", as it has come to be known, describes a further 113 species of birds from the 760 or so which occur in the Western Palearctic. Commencing with the terns, skimmers and auks, it progresses through sandgrouse, pigeons and parrots — whose sole representative is the introduced ring-necked parakeet — to cuckoos, owls, nightjars, swifts, kingfishers, bee-eaters, roller and hoopoe, concluding with the woodpeckers.

Each species entry runs to about 6,000 words. As well as giving field identification characteristics, it provides detailed descriptions of plumages necessary for the identification of specimens in the absence of reference skins, and data on moult measurements, weights, structure and geographical variation. The colour plates, all of them by artists with field experience of most if not all of the subject genera, show the main plumages, including downy young (and eggs) with an acceptable consistency of style.

Once the text moves on to material derived from research it is inevitably more varied in quality and comprehensiveness. The habitat descriptions enable one to look for a given bird in the right place but are often insufficient, even when considered together with information on food, to guide either habitat management or conservation judgements on the implications of subtle changes in land use. In general, more is known of breeding requirements than of wintering habitat usage, and that too is reflected in the book.

Distribution maps show separately the world and Western Palearctic ranges, while the text concentrates upon historic changes in range (which yet often remain inexplicable). Population size is usually based on national estimates, which are notoriously questionable.

The bulk of the text is devoted to social patterns and behaviour, and breeding biology. In bringing together all of this material, the book — and the series — has value not only in its own right but as a basis for comparison which may in time extend our knowledge of "why" as well as "what". By implication, this massive and invaluable exercise in ornithological publication raises the question of whether the work should be allowed to end with the appearance of each volume or whether some continuing process of updating is practicable and worthwhile. □

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, Bedfordshire

Culture of genetics

Peter Goodfellow

Mammalian Cell Genetics.
By Martin L. Hooper.
Wiley: 1985 Pp.175. £63.25, $49.95.

AT THE same time as the molecular revolution in genetics, a quieter revolution has occurred in the genetics of cultured cells. Older techniques, such as variant isolation and production of somatic cell hybrids, have been improved and supplemented with methods such as DNA-mediated gene transfer, expression of bacterial genes in mammalian cells, microcell transfer of chromosomes and chromosome-mediated transfer of sub-chromosomal fragments. In *Mammalian Cell Genetics* Dr Hooper has provided a commentary on the whole of modern somatic cell genetics, designed to be read in conjunction with the primary literature.

Guides such as this can either be Talmudic, expanding and clarifying an obscure text or, like the synopsis of an opera, condense the story while emphasizing the highlights. By the author's stated intention, the book falls into the latter category. It does not purport to tell you how to do an experiment but informs you of the experiments that have been done. As such it will provide novices with an overview of the possibilities available before they delve into more specialist publications.

A good commentary should be comprehensive and emphasize the right points. *Mammalian Cell Genetics* is comprehensive for techniques up to 1983, but the tables of cell lines available should not be regarded as exhaustive and events of the past two years have somewhat overtaken the tables of molecular vectors The choice of highlights is more open to argument and largely a matter of personal opinion. I would have emphasized human gene mapping, monoclonal antibodies and isolation of oncogenes by DNA transformation of cultured cells. Perhaps as a consequence of his apparent determination to be succinct, Dr Hooper treats the first two of these achievements as derivatives of the esoteric, and often arcane, truths of chromosomal segregation and extinction of "luxury" functions in hybrid cells. Formally he is correct, but, for me, it counts as a failing. Limitations on length probably also explain the absence of some essential practical details, such as the amount of transforming DNA a mammalian cell can take up and incorporate into its genome or the maximum size of individual DNA molecules which can be incorporated. Also missing is debate about the major unsolved problems in mammalian cell genetics; for example, many people would like to obtain homologous recombination between exogenously introduced DNA and the equivalent recipient cell gene.

The book is a helpful guide and any postgraduate student of mammalian genetics would benefit from reading it. Unfortunately, the absurd price means that our hypothetical student will either have to spend a week's wages or read somebody else's copy. I would suggest the second alternative. □

Peter Goodfellow is Head of the Human Molecular Genetics Laboratory at the Imperial Cancer Research Fund Laboratories, London

Colourful behaviour

M. E. G. Evans

Insects in Camera.
By Christopher O'Toole.
Oxford University Press: 1985. Pp. 154. £14.95, $29.95.

THIS is not just another book of pretty pictures of animals. Rather, it is a collection of superb colour photographs by Ken Preston-Mafham in which are captured the behaviour of living insects. Christopher O'Toole's text introduces and explains the photographs so that we have a rather unusual combination of linked illustrations and text, describing not merely a series of insect types but their great diversity of behaviour patterns.

The question posed is "How are insects adapted to survive in a hostile world?" The answer, of course, is "very well", but this book delves into the underlying reasons. There are seven sections, each of which is introduced by a short essay. The rest of the text consists of long legends to each of the photographs, in which the species and their systematic positions are noted, and their activities described. The photographs are not just of insects seen against typical backgrounds; some are unique, for instance the picture of a hovering muscid fly introducing her offspring into driver ant society by the precision bombing of an ant column with her eggs.

A disadvantage of breaking up the text into a series of short paragraphs is that the book is necessarily somewhat fragmented; there are nearly as many stories as there are pictures. However, my main criticism is that where there are three or four prints to a page the detail cannot be appreciated. I would have preferred none of the photographs to have been less than half-page size, even at the price of fewer of them. Nevertheless, this is a stimulating account of insect behaviour and adaptation, with an intelligent text elaborating upon the excellent pictures. For many readers it will be literally an eye-opener. □

M E. G Evans is a Senior Lecturer in Zoology at the University of Manchester

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Personal atmosphere for the physicist

J. R. Milford

Physical Meteorology.
By Henry G. Houghton.
MIT Press 1985 Pp.442. $37.50 £41.95.

FOR Professor Henry Houghton's colleagues and former students the publication of *Physical Meteorology* will be an event of some note It will also enable a much wider range of atmospheric physicists to benefit from his wisdom The qualities of a great teacher shine through this book, with scholarship, clarity of argument, enthusiasm and a welcome readiness to discuss points of uncertainty. He is also ready to say when an exact theory is inapplicable to a real, untidy atmosphere, for instance when we consider the scattering of radiation.

The use of the book as a text to support other people's courses will be restricted, however, by a number of its characteristics. First, it is only for competent physicists, assuming as it does some prior knowledge of Raman scattering, or of what is meant by local thermodynamic equilibrium, to give just two examples. Secondly, the choice of material is very personal, as indeed is made clear in the preface. The main sections consist of four chapters on radiation in the atmosphere and three on cloud physics these are preceded by an opening chapter on the atmospheric aerosol This is logical enough, but there is only minimal explanation as to how this information may be used later in the book and there are in fact few references back to it. The final chapters on optical phenomena and atmospheric electricity are also self-contained

The book thus covers the topics which are traditional in texts on atmospheric physics Professor Houghton admits to doubts on the title, and it seems to me that *Physical Meteorology* is now a misnomer: operational meteorologists will not find much of the theory behind their practice here. The turbulent transfers of energy which bulk large in much of our science find no mention, and surely the links with large-scale energetics merit discussion if meteorology is included in the title?

In smaller matters the choice of material is again unashamedly (and excusably) personal. However, more applications of the existing material could have been included: illustrations of scattering or anomalous propagation of radar would have helped readers to make links with specialist studies without any risk of trespassing on them. The relevance of the radiation chapters to remote sensing could have been brought out too, in contexts other than temperature sensing with the 15μm CO_2 band Even in this section the paragraph on the non-American method is so brief as to be quite unhelpful.

Textbooks which include problems at the end of each chapter are always attractive to other teachers, but those included here are less useful than some since they are mainly designed to stimulate open-ended discussions. They provide no help to the struggling lecturer who needs to know whether he has got it right

As I read the book I was occasionally irritated by misprints, and, more often, by the adherence to old-fashioned units, even when a change would be trivial: why not replace $°C^{-1}$ by K^{-1}? Also, the basic radiation and derivations are not entirely clear, mainly due to Professor Houghton's refusal to use the term "radiance" Some diagrams raise questions I would like answered, such as Fig 7.14 which includes collision efficiencies for drops rising to a value of ten in a very curious fashion. But one should not be put off by such deterrents, or by the absence of references after 1980, for here there is much insight and illumination of the principal parts of atmospheric science □

J R Milford is a Senior Lecturer in the Department of Meteorology at the University of Reading

Fresh information

- *Biotechnology Engineers. Biographical Directory*, edited by O R Zaborsky and D K Zubris, containing details of 236 "key individuals" in North America Publisher is Omec International, Washington, DC, price is $29.95.
- *International Directory of Contract Laboratories* (in toxicology), compiled by Edward M Jackson Publisher is Marcel Dekker, price is $49.75 (North America), $59.50 (elsewhere)

Psychological notes

Eric Clarke

The Musical Mind: The Cognitive Psychology of Music.
By John A. Sloboda.
Clarendon: 1985. Pp.291. £20.

THE power of music to move people's minds, and the ability of a few minds to produce performances and compositions of great profundity, have raised questions that can be traced from Plato's *Republic* to the current Oscar-award-winning film *Amadeus*. Since the beginnings of modern psychology they have also been a focus of attention for those attempting to understand musical cognition with more rigorous methods. Until recently the psychology of music could not claim to be a major area of research, but two new journals on the subject and a steady stream of books and articles indicate that this is changing.

Most of the literature has, however, held more interest for the experimental psychologist than for the musician, concerned as it is with highly reduced "musical" materials investigated under extremely constrained (and unmusical) conditions. It is therefore a breakthrough that a book has now appeared which brings together recent work in a way that demonstrates its significance for musicians, whilst in no way compromising the psychological theory on which it is based.

In the preface to *The Musical Mind*, John Sloboda makes it plain that the primary aim of his book is to bridge this gap, and he is to be congratulated on doing so. Sloboda's cognitive approach, and his desire to address matters that preoccupy musicians, have resulted in a book that covers many areas usually absent from writings on the psychology of music. Thus while there is a substantial chapter on listening to music, there are also accounts of musical learning and development, musical performance, and composition and improvisation. The section on composition is an excellent attempt to unravel the psychological processes involved in this least-understood area of musical cognition, using the compositional sketches of various composers (Beethoven, Mozart, Stravinsky), and the written accounts of Sloboda's (and another composer's) compositional methods. It is Sloboda's willingness to make use of evidence from a wide variety of sources that allows him to delve into areas previously considered too private to be investigated, and which brings an engaging freshness to the book.

Besides these four chapters, the book is outstanding for the subjects treated in the second and last chapters — music, language and meaning, and music, culture and biology, respectively. The second chapter draws together some of the recent thinking on the formal similarities between music and language, and considers the psychological processes that they may share. The last chapter, which looks at the psychological issues raised by cross-cultural studies of music, and examines the neurological and broader biological bases for music, is an important reminder of how ethnocentric most work on the psychology of music is, and of the insights to be gained by widening the cultural perspective.

There are some weaknesses: the comparison of the work of the linguist Chomsky and the musicologist Schenker is too simplistic; the discussion of musical meaning is taken less far than it might have been; and a consideration of the psychological questions stemming from developments in contemporary music would have been welcome. But the clarity of Sloboda's writing and his numerous suggestions for further research will make his book essential reading for anyone, student or researcher, interested in how minds and music interact. □

Eric Clarke is a Lecturer in the Department of Music at the City University, London.

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New Technology in Biotechnology

The Nature *conference under the above title takes place in London on 25–27 June. Alongside the conference is an exhibition of laboratory equipment.*

Exhibitors and stand numbers

1 Alfa Laval
2 V A Howe
3. Anachem
4. ISC
5 Du Pont
6 Nature
7 New Brunswick
8 & 17. Wisepress
9. & 10. L H Fermentation
11 Flow Laboratories
12. S G.I.
13 Roth
14 Lab Mate
15 Kruss
16. Cambridge University Press
17. Wisepress
18 Bioengineering
19 AMS
20 Uniscience
21 University of Surrey
22. Heraeus
23. & 24 Commonwealth Agricultural Bureaux
25. Leicester Biocentre
26. Anglian Biotechnology
27. Becton Dickinson UK
28. Bactomatic
29. Celltech
30 Apcel
31. Elsevier
32 & 33. Applied Biosystems
34. MBR Bioreactor AG
35. British Library
36. Carl Zeiss Jena
37 Hobsons
38 LEEC
39. Janssen
40 Amicon

The three-day conference and exhibition take place in the Novotel, Hammersmith. Details of the exhibits on each stand are given in the exhibition catalogue.

● The Commonwealth Mycological Institute (CMI) is one of four institutes and ten bureaux of the Commonwealth Agricultural Bureaux. It offers a range of services to biotechnologists, as well as mycologists and specialists in related disciplines. A key area of the institute's activities lies in consultancies and contract research With some 300,000 reference specimens of microfungi (plus 11,000 in the culture collection), a substantial library, online database, and access to electron microscopes, computer and chemotaxonomic and biochemical facilities, the institute is well-placed to help solve problems involving microfungi. In addition, support from the Department of Trade and Industry to the culture collection and industrial services enables the institute to provide new and improved services to industry, and to build up biochemical data on strains held.
Reader Service No. 100.

● New Brunswick Scientific has developed a completely new series of pilot-scale fermentation systems designed for mammalian cell culture in bulk suspension and using microcarriers, and for bacterial cell culture. Of particular interest to users of pilot-scale fermenters are the design features in the standard open frame skid concept used by all NBS systems: all piping including filters, valves, regulators and steam traps is housed within the open frame skid, which makes installation and maintenance simple and trouble-free. At the laboratory scale, New Brunswick Scientific will be showing a new fermenter for shear-sensitive cultures, which uses a rotating lift tube for agitation, reducing shear forces on the culture to a minimum. A full range of monitoring and control instruments is available to complement both pilot and laboratory-scale fermentation systems. New Brunswick has recently acquired Biosearch, a company which manufactures a complete range of instruments and chemicals for the synthesis of oligonucleotides and peptides. Both the DNA and peptide synthesizers are fully automated, allowing researchers previously inexperienced in organic chemistry to successfully synthesize the most complex sequences. The instrument software ensures optimum conditions for each coupling once the sequence has been entered via the user-friendly keyboard.
Reader Service No. 101.

• The Bactometer, on the Bactomatic stand, is capable of handling 1–512 samples simultaneously, and performs total and selective counts in the range of 1 to 10 million organisms automatically, without the need for tedious procedures such as serial dilutions and plate counts. Results are typically available in 1–8 h. Based on impedance technology, the computer-assisted data system can display impedance growth curves which have been applied to optimization of a fermenter media phase sensitivity and culture metabolism studies, antibiotic/preservative/vitamin assays and sterility testing. Also on the Bactomatic stand is the Vitale AMS system for the rapid, automated identification of microorganisms. The computer-assisted system can identify between 1 and 240 organisms, typically within 4–10 h. The in-built database covers Gram-negative and Gram-positive bacteria, common pathogens and yeast, and can also determine MIC values and sensitivity for up to nine antimicrobial agents simultaneously.
Reader Service No. 102.

● Roth Scientific is marketing a series of versatile bench-top fermenters, engineered by Applikon of Holland. A feature of the systems is simplicity of use. Pilot-scale units are similarly available in volumes of 20l to 300 litres Applikon offers a choice of borosilicate glass fermenter vessels pilot vessels for bacterial culture have a ratio of height to diameter of 3:1 while those for animal/plant work are shorter and wider, with a 2:1 ratio. The range of vessels includes flat-bottomed, round-bottomed (supported in a tripod) and jacketed vessels all of which can be used to successfully grow bacteria, filamentous organisms, and plant and animal cells. Two forms of stirrer assembly can be specified — a mechanically driven lipseal assembly or a powerful magnetically coupled stirrer which remains coupled even at high speed or high viscosity Other products offered by Roth Scientific in the Biotechnology range are a versatile ultrasonic disintegrator, which is capable of processing four samples simultaneously, complete high-pressure liquid chromatography systems and a range of freezers and thermostatic baths.
Reader Service No. 103.

●Applied Biosystems has chosen the *Nature* exhibition for the launch of its new UK subsidary, Applied Biosystems Ltd. Among the facilities available are technical support and service laboratories, substantial stocks of reagents and solvents, and a specialist service for protein sequencing, peptide synthesis and DNA synthesis. At the exhibition, there will be on display three new products: the Model 120A PTH Analyser, a specially designed chromatography system for use either 'on-line' with the Model 470A gas-phase protein sequencer (also be on display) or as a 'stand-alone' system for the analysis of phenylthiohydantoin (PTH) amino acids All common PTH amino acids are resolved to better than 95%, with detection sensitivity of less than 1 picomole The fully automated Model 381A DNA synthesizer produces high-purity, defined-sequence oligonucleotides quickly and economically, with routine coupling yields of 98 – 100% and synthesis of oligonucleotides with over 100 bases The Model 380B DNA synthesizer has been developed as a successor to the 380A and has a new 'touch-screen' CRT control and built-in programming to allow the use of any synthesis strategy. Also on display will be the Model 430A solid-phase peptide synthesizer
Reader Service No. 104

● Bioengineering UK Ltd will be exhibiting a range of fermentation reactors which includes two new products: the paddle stirrer and visual safety reactor. The paddle stirrer is an agitation system which has particular application in the culivation of delicate cells. It can be used to convert a conventional stirred tank into a delicate cell reactor. The paddle stirrer system provides a novel solution to the problem of achieving a compromise between agitation effect and dissolved oxygen transfer, using an inversion of a rotational motion which simultaneously disperses and homogenizes. The stirrer transmits its energy mainly by impulse without shear and burbling. The visual safety reactor, or foil reactor, uses a foil cylinder of high mechanical strength in place of an equivalent glass cylinder. The advantage of the new reactor is that it eliminates the risk of accident when sterilizing glass vessels *in situ*. Visibility is better than with glass, and different foils can be experimented with to obtain novel configurations. The foil can be disposed of after use, thus eliminating the need for extensive cleaning. This concept also allows inexpensive development of 'tower'-type reactors and experimentation with air-lift systems.
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How to avoid the 19 critical mistakes in buying fermentation equipment

Make one mistake and you can have problems that include inadequate process control, permanent operator inconvenience and costly downtime.

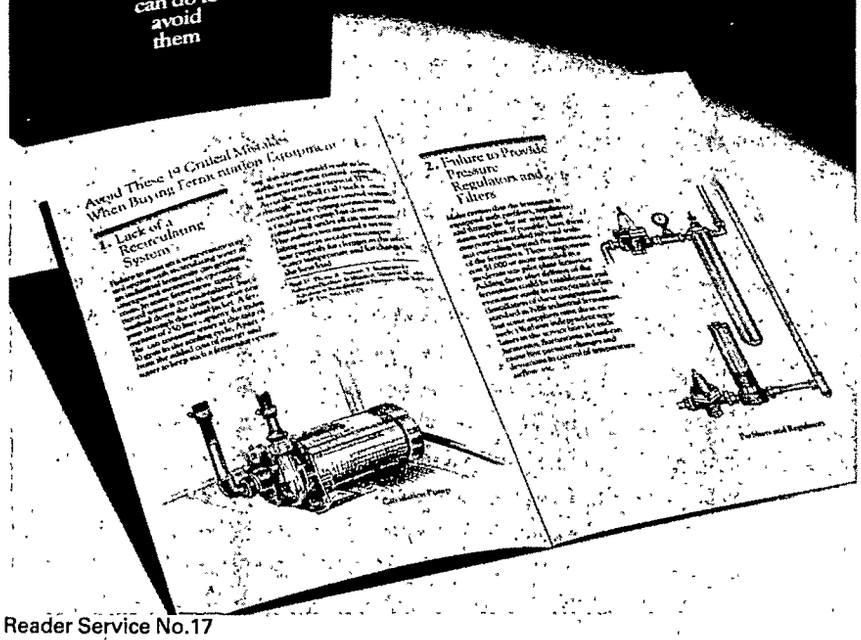
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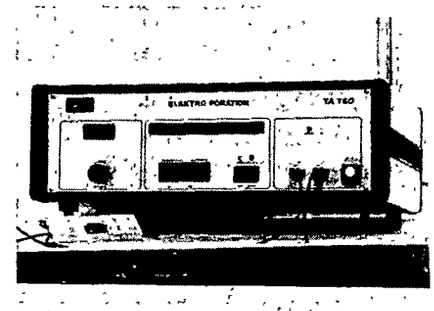


Reader Service No.17

● The Celltech stand features a range of immunoprocessing reagents based on monoclonal antibodies for the purification of α, γ and, most recently, β-interferon. Each kit provides single-step, high-yield purification. Celltech's range of monoclonal-based assays includes a gamma immunoradiometric assay (IRMA) for the assay of γ-interferon and NK2 and YOK IRMA for assay of different subtypes of α-interferon. *Abstracts in BioCommerce* (ABC), a commercial news index produced twice monthly by Celltech, is now available as an online computer database. At the Celltech stand the database will be demonstrated in action.
Reader Service No. 106.

● A new 2-litre airlift fermenter that will be ideal for monoclonal antibody production, media optimization and other similar applications is available from LH Fermentation. Based on a 2-litre total volume glass vessel, the fermenter has a working volume of 1,700ml with a removable glass draught tube and a stainless-steel top plate. This has ports for oxygen and carbon dioxide electrodes and other sensors. Temperature control is via thermostatically controlled water circulated around a jacket on the glass vessel; agitation is achieved on the standard unit by the 'airlift' principle with a glass sinter providing the gas dispersion. The LH Fermentation stand will also feature a new 30-litre airlift fermenter which is fitted with complete instrumentation and a gas blending system which can accommodate the culture of shear-sensitive animal and plant cells. And LH's new microprocessor-based fermentation control package can monitor eight parameters in single or multiple fermentation.
Reader Service No. 107.

●EBIP, the European Biotechnology Information Project, based at the British Library in London, and run with the help of European Commission funding, is investigating information provision in biotechnology across a broad front. It has produced a wide range of guides to information sources in key areas such as safety and regulations, business information and culture collections. EBIP is backed by the full resources of the British Library, and EBIP services are also available via the Online system DIMDI.
Reader Service No. 108.



The Kruss TA 750 'electroppration' apparatus for the transfer of DNA and other molecules through cell membranes Reader Service No. 109

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Aims and Scope

The journal provides a medium for the rapid publication of full-length articles as well as short communications on various aspects of biotechnology. Information on new or potential applications of biotechnological relevance in the following areas will be considered for publication:

- molecular biology
- genetics
- biochemical aspects of metabolism and its control, including viral, microbial, plant and animal cell systems
- process development and its control, including computer applications automation and the development of (bio)-sensors as applied to biosystems.

The overriding criteria for publication are originality, high scientific quality and interest to a multidisciplinary audience.

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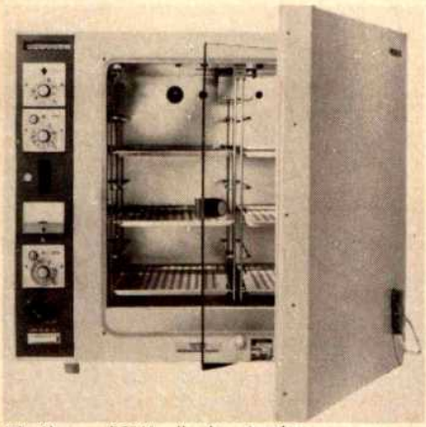
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Reader Service No.41



The Heraeus B5060 cell culture incubator.

● A new, sophisticated cell culture incubator, the electronically-controlled Heraeus B5061 'Auto-Zero', will be displayed on the Heraeus Equipment stand. The CO_2 control on the B5061 has an automatic self-adjusting measuring system to keep the operating parameters constant and reproducible, making manual recalibration of the CO_2 zero unnecessary. This is particularly helpful when the incubator is used for long-term cultivation and manufacturing. Additionally, in the O_2 version, the O_2 zero can be calibrated without interrupting the operation of the incubator. Also on show will be the Heraeus B5060 incubator which, like the B5061, is available with linings made in a choice of bactericidal and fungicidal copper, or stainless steel. With the CO_2 version of the B5060 the temperature and CO_2 content are adjustable with great accuracy, so that the optimum conditions for cell and tissue culture can be created. It has an electronic temperature controller and an adjustable temperature limit controller with a recording facility. An easily drained water bath provides constantly high humidity.
Reader Service No. 110.

● Laboratory and Electronic Engineering Co (LEEC) will be featuring their automatic CO_2 incubators. The GA3 is the large slimline version of the popular 150-litre model GA2, featuring a single chamber with double doors, and a capacity of 330 litres. Both incubators are designed for techniques of open dish tissue cell culture in which the *p*H of the medium is maintained in equilibrium with the incubator's CO_2 atmosphere. The incubator automatically controls the CO_2 concentration to a pre-set level, and a percentage CO_2 meter on the control panel monitors the output of a thermal conductivity detector cell which continuously analyses a gas sample from the chamber. LEEC are now offering a sterilization feature as an optional extra on both models. An additional boost heating element raises the chamber air temperature to a pre-set temperature of 90–100°C. Also on display will be a selection of compact incubators, tissue culture roller apparatus and drying cabinets.
Reader Service No. 111.

● Flow Laboratories Ltd will be exhibiting a selection of new and existing products for the following: laminar air flow and filtration, microplate technology and tissue culture and diagnostics. On display will be the complete new range of disposable membrane filters, including minifilters for use with syringes, and single or double capsules for the filtration of larger volumes of liquid; and new models of the Titertek enzyme immunoassay readers the Multiskan MCC/MCC340, a twin reader, with the capacity to read, shake and incubate two plates simultaneously; HTE Multiskan Plus with a barcoded microplate identification system; and Fluoroskan for fluorimetric enzyme immunoassays. All these microplate readers have a bidirectional computer control facility which enables them to be used for kinetic studies. There is also a new range of viral diagnostic reagents for use in complement fixation, enzyme munoassay and immunofluorescence techniques. Other products on show include a range of liquid-handling devices such as Titertek digital multichannel pipettes and a selection of disposable plastics, tissue culture media and glassware.
Reader Service No. 112.

● Becton Dickinson will exhibit the Falcon range of disposable tissue culture plastics and related products. All Falcon Labware products, from the smallest Petri dish to the largest roller bottle, are sterilized by gamma irradiation. Products featured will include Primaria, a surface-odified plastic substrate for primary cell culture, and the FAST system for simultaneous screening via ELISA of all 96 wells of a hybridoma culture plate for clones producing antibodies of desired specificity, together with disposable filter units, pipettes, multi-well plates and tubes.
Reader Service No. 113.

● The Cetus Pro/Pette, an instrument for the rapid and accurate processing of microtitre plates, will be the centre of Anachem's display. The machine was developed for general cell culture, hybridoma studies and ELISA applications. Plates can be processed either in rows or columns with tip change between rack movement if required. Single wells may be accessed, allowing transfer of samples from tubes to individual wells. The Pro/Pette can also empty and refill wells for serial dilutions or transfer between plates, again with optional tip change. From Gilson, there will be on display pipettes, dispensers, diluters and automatic liquid handlers with 'robotic arms'. Analytical and preparative HPLC equipment, together with examples of Anachem's range of columns and accessories, will also be shown.
Reader Service No. 114.

These notes are based on information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

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If interested, please write with full details to Managing Director, ACADE DIAGNOSTIC SYSTEMS S.A./N.V., Passage de la Vecquée, Box 4100, 1200 Brussels, Belgium. Telephone: 02/762.89.75. Telex: 65867. Replies may be in English, German, French or Dutch.

Reader Service No.69

LASERS SPECIAL ISSUE

The 25 July issue of *Nature* will feature specially commissioned articles on lasers and laser applications, including V. Letkhov on lasers in biology and medicine, S. D. Smith on optical computers, M. Key on X-ray lasers and the late A. Kaster on the early history of the laser.

The issue will also contain a Product Review section on lasers and related products. Manufacturers are invited to send press releases on products or on novel applications to the address below, to arrive not later than 4 July.

Nature (Product Review),
4 Little Essex Street,
London WC2R 3LF, UK.

June meeting

28-29 June, **Investigation and Belief — 3rd Annual International Conference on the Paranormal,** University College, London, UK (M. Hutchinson, Secretary, CSICOP, UK Branch, 10 Crescent View, Loughton, Essex IG10 4PZ, UK)

July meetings

8-12 July 1985, **7th International Meeting on NMR Spectroscopy,** Cambridge, UK (Dr J. Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN)

September meetings

1–4 September 1985, **First International Symposium on ESR Dating**, Ube, Japan (Prof. Ikeya, Secretary of the Symposium, Technical College, Yamaguchi University, Tokiwadai, Ube 755, Japan)

2–3 September 1985, **International Symposium on Impact Testing and Performance of Polymeric Materials**, Guildford, UK (Miss Rachael Alexander, Short Courses and Conferences Section, Bureau of Industrial and External Liaison, University of Surrey, Guildford, Surrey GU2 5XH)

3–6 September 1985, **Second International Symposium on the Synthesis and Applications of Isotropically Labelled Compounds,** Missouri, USA (Dr R. Wilk, Symposium Coordinator, School of Pharmacy, 5100 Rockhill Road, Kansas City, MO 64110 USA)

16–19 September 1985, **International Environment and Safety Conference**, Olympia, London, UK (D. Parker, Conference Coordinator, International Environment and Safety, Labmate Limited, Newgate, Sandpit Lane, St Albans, Herts. AL4 0BS, UK)

October meetings

7–9 October 1985, **International Symposium on Biotechnology in the Food Processing Industry**, Minnesota, USA (Lynette Marten, University of Minnesota, St Paul, Minnesota 55108 USA)

7–10 October 1985, **Electronic Imaging '85 – International Electronic Imaging Exposition and Conference**, Massachusetts, USA (R. Murray, Institute for Graphic Communication, 375 Commonwealth Avenue, Boston, MA 02115, USA)

8–10 October 1985, **International Conference on the Occupational and Environmental Significance of Chemical Carcinogens**, Bologna, Italy (Organising Committee, International Conference on Chemical Carcinogenesis, c/o Istituto di Oncologia, Viale Ercolani, 4/2, 40138 Bologna, Italy)

Please send 'Announcements' entries to: The Editor, Nature, 4, Little Essex Street, London WC2R 3LF, UK

20–23 October 1985, **Halifax '85–16th Annual Underwater Mining Institute**, Nova Scotia, Canada (H. Joseph, Ocean Mining Division, 355 River Road, Ottawa, Ontario, K1A 0E4)

November meetings

7-9 November 1985, **International Congress on Neo-Adjuvant Chemotherapy**, Paris, France (SOMPS-1st International Congress on Neo-Adjuvant Chemotherapy, Pavillion Jacquart, Hôpital de la Salpétrière, 47 Boulevard de L'Hôpital 75651 Paris, France).

19-21 November 1985, **International Conference on Advanced Welding Systems,** London, (The Conference Office, The Welding Institute, Abington Hall, Abington, Cambridge CB1 6AL, UK).

20-22 November 1985, **International Conference on Nitrates in Water**, Paris, France (G. Martin, Ecole Normale Supérieure de Chimie, 35000 Rennes-Beaulieu, France).

December meetings

2-4 December 1985, **International Conference on Spacecraft Structures,** Toulouse, France (CNES, Department des Affaires Universitaires 18, avenue Edouard-Belin 31055 Toulouse, France)

3–5 December 1985, **Ninth International Online Information Meeting**, London, UK (J. Mulligan, Learned Information (Europe) Ltd, Besselsleigh Road, Abingdon, Oxford OX13 6LG)

3-6 December 1985, **Fourth International Meeting on Biostereometrics '85,** Cannes, France (J. Prado, Coordinator Central du Congres, ANRT-Association Nationale de la Recherche Technique, 101, Avenue Raymond Poincaré, 75116 Paris, France)

4-5 December 1985, **International Conference on Electric and Magnetic Fields in Medicine and Biology,** London, UK (C. Dagnall, IEE Press Office, Savoy Place, London WC2R 0BL, UK).

11-13 December 1985, **IFAC Symposium on Modelling and Control of Biotechnological Processes,** Noordwijerhout, The Netherlands (IFAC/BIC '85 c/o Klvl, PO Box 30424, 2500 GK The Hague, The Netherlands).

Appointments

Dr Geoffrey Rivett has been appointed Senior Medical Officer at the Department of Health and Social Security. He will take charge of Med PCR division (Primary Care, Regional Medical Services) with effect from 1 July 1985.

The Council of the Royal Society has appointed **Dr P.T. Warren** as Executive Secretary of the society,on the retirement of **Dr R.W. Keay.**

Dr Lewis Roberts, Director of the UK Atomic Energy Authority's Energy Research Establishment at Harwell, has been appointed the first professor of Risk Assessment at the University of East Anglia, Norwich.

Two appointments have been made to the Medical Research Council with effect from 1 August 1985. **Professor Wing** will be Chairman of the MRC's Neurosciences Board, and **Professor Newsom-Davis,** the present Chairman of the Neurosciences Board, will be an independent scientific member.

Three new members have recently been appointed to the Engineering Council. They are **Mr Norman Holland**, UK Group Standards Manager of Philips Electronic and Associated Industries, **Sir Richard 'Brien**, Chairman of the Engineering Industry Training Board, and **Sir Robert Telford**, Life President of The Marconi Company Ltd.

Loughborough University of Technology has appointed **Professor John Phillips** as Vice-Chancellor from 1 January 1986, in succession to Sir Clifford Butler. Professor Phillips is at present director of research and Wolfson Professor in the Wolfson Institute at the University of Hull.

Awards

PROFESSOR Gert Utermann from the Institute for Medical Biology and Genetics of the University of Innsbruck has been awarded the Morgagni Medal for his investigations on the pathophysiology of familial hyperlipidaemias. The G.B. Morgagni Young Investigator Awards will be awarded to Dr G. Bolli of the University of Perugia and Dr E.Van Obberghen of the University of Nice.

The William Hopkins Prize of the Cambridge Philosophical Society has been awarded to Dr D.O. Gough of Churchill College for his pioneering achievements in the subject of helioseismology.

The University of Glasgow has appointed **Dr David W.T. Crompton** to the John Graham Kerr Chair of Zoology with effect from 1 October 1985. He is presently lecturer in parasitology in the University of Cambridge, fellow of Sidney Sussex College, Cambridge and adjunct professor in the division of nutritional sciences, Cornell University, New York.

The International Association of Gerontology has awarded the 1985 Sandoz Prize jointly to Sir Martin Roth, Professor of Psychiatry at Cambridge (UK), and Dr Karl Esser, Professor of Botany at the University of the Ruhr (FRG).

The Jung Foundation for Science and Research 1985 prize for Medicine has been awarded to three recipients. The prizewinners are: **Professor Hendrik Coenraad Hemker** — for work on the biochemistry of blood anticoagulation, **Professor Rudolph Pilchmayer** — for work on liver transplantation and **Professor Peter K. Vogt** — for work on the genetic analysis of retroviruses.

NATURE CLASSIFIED

AMERICAN OFFICES NEW YORK Garth Walkes 65 Bleecker Street, New York, NY 10012 — Telephone (212) 477-9600
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UNIVERSITY OF LONDON
THE LONDON HOSPITAL MEDICAL COLLEGE

MLSO

IN THE DEPARTMENT OF FORENSIC MEDICINE

A state registered MLSO is required to take charge of the Histopathology section. The postholder must have a wide knowledge of Histopathological techniques, including diatomaceous preparation with preparation of gross specimens as required for Court presentation. In addition the postholder will be responsible for the administration of toxicological samples and the conveying of same to Guy's Hospital for their analysis. Whitley salary scale £5,738 — £8,096 plus £1,084 London Weighting

Contact the Assistant Secretary (Personnel) LHMC Turner Street, London E1 2AD (01-377-8800 x 3/12) for an application form which should be returned within 21 days of this advertisement. (6840)A

THE UNITED MEDICAL AND DENTAL SCHOOLS

St Thomas's Campus

RESEARCH ASSISTANT
(possibly post-doctoral)

required to work on immunity to and the molecular biology of rubella virus. Virology experience essential; some knowledge of ELISA and PAGE techniques and use of monolonal antibodies an advantage.

Funding for post is currently being sought; anticipated start date early September.

Salary according to age and experience.

Applications with cv and names and addresses of two referees to Staffing Officer, Dean's Office, St Thomas's Hospital Medical School, London SE1 7EH, not later than 18th July, 1985 quoting ref: STH/V/38. (6859)A

IMPERIAL CANCER RESEARCH FUND
Developmental Biology Unit
Oxford

RESEARCH SCIENTIST

Applications are invited for a five year appointment with the prospect of tenure. Applicants should be specialists in any area of developmental biology, of senior postdoctoral status and able to plan and execute an independent research programme

The Unit which will open early in 1986 is situated in the Zoology Department of Oxford University The Director is Prof R Gardner and permanent staff members are Drs D Ish-Horowicz and J Slack

Salary range £11,675 to £14,925 (revision pending).

For further details write or telephone Ms S M Hurley, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX on 01-242 0200 ext 305 Please quote reference 82/85 (6819)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

required to work with Dr D S Bendall on an SERC-funded project to isolate and characterize ferredoxin-plastoquinone oxidoreductase, the key enzyme of cyclic photophosphorylation in chloroplasts The post will be available for three years from 1st October 1985 with salary in the range £7,980 — £8,920 (under review) according to age and experience

Applications (with curriculum vitae and names of two referees) should be sent to Dr D S Bendall, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW, before 19th July 1985. Further details from Dr Bendall, Tel: 0223-351781. (6834)A

New Technologies Manager

Clinical Reagents
c. £24,000 + car

My client is a leading international company providing specialised products for use in health care, life-sciences research and industry. Its firmly established Clinical Reagents Department, is embarking on a major expansion programme.

We are searching for a key manager for the department – a person combining research credibility with entrepreneurial flair. The New Technologies Manager will be expected to operate at the leading edge of the department, foraging inside and outside the company for new technologies that can be applied in clinical reagents development. This will obviously need an acute awareness of the state-of-the-art together with business acumen. The strength to 'product champion' an idea through to manufacture will be a key role.

The management responsibilities associated with this job are for some 40 professionally qualified scientists and 3 section leaders who operate from 'well-found' laboratories (new technology, instrumentation and clinical microbiology).

We would like to discuss this pioneering appointment with candidates aged between 30-40 who have several years' experience of clinical diagnostics, ideally including a knowledge of instrumentation. Recent experience will ideally have been with a diagnostic or a biotechnology company where the expertise could be applied to clinical diagnostics. High academic attainment is a pre-requisite.

If you feel you have the ability called for in this advertisement, we would like to take things further with you. In the first instance send your curriculum vitae in confidence, quoting reference NTM/BT, to Geoffrey King, Managing Director at the address below

This appointment is open to both men and women.

(6912)A

Cambridge Recruitment Consultants
International Biotechnology Division
1a Rose Crescent, Cambridge CB2 3LL Telephone 0223 311316

CANADA

IDRC/CRDI

The International Development Research Centre (IDRC) is a corporation funded by the Government and created by the Parliament of Canada to support research designed to adapt science and technology to the needs of developing countries.

For its Regional Office in Eastern and Southern Africa, situated in Nairobi, Kenya, the Centre is seeking a

Regional Representative for the Health Sciences Division

The representative will be required to identify research and research institutions in the health sector in Eastern and Southern Africa; assess research needs and capabilities, as well as constraints to the conduct of research in the region; work with researchers in developing research proposals, and monitor on-going projects.

Qualifications and Experience Required

- **Medical degree plus graduate training in Public Health or Tropical Medicine, *or* Doctoral degree in Public Health, Epidemiology or equivalent discipline.**
- **Ten years' experience in research and research administration;**
- **working experience in developing countries;**
- **knowledge of and contacts with governmental, university and private organizations in the health field in Eastern and Southern Africa;**
- **proven independent research capability in the health field;**
- **Excellent command of both spoken and written English and working knowledge of French.**

Working Conditions: Extensive travel required.

The successful candidate will be eligible for a generous overseas benefit and allowance package.

Interested candidates should send their résumé to: Competition BR/NTR, Human Resources Division, IDRC, P.O. Box 8500, Ottawa, Ontario K1G 3H9, before August 15, 1985. (NW1867)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Scientific Appointment in the Laboratory of Gene Structure and Expression

(Revised advertisement)

Applications are invited for a scientific position in the Laboratory of Gene Structure and Expression (Head: Dr F. G. Grosveld) in the field of studies on the regulation of gene expression. The candidate will be expected to carry out independent research in an area which is compatible with the programme of the Laboratory and should have relevant postdoctoral experience. Extensive experience with recombinant DNA technology and tissue culture techniques is definitely required.

Information about current activities in the laboratory and elsewhere in the Institute is available in the Institute's Annual Report which has been distributed to major Medical Libraries and can be obtained by post from the Director's secretary.

The position might be filled either by a career or a short-term appointment, according to the age and experience of the candidate. The salary will be at an appropriate point on the Council's range equivalent to University non-clinical academic staff scales, plus £1,233 London allowance and superannuation provision.

Applications should include curriculum vitae, list of publications, a brief statement of research plans (about 1000 words), and the names of three persons to whom reference may be made, and sent to the Director, National Institute for Medical Research, Mill Hill, NW7 1AA by 19th July 1985, quoting ref: GSE/11/C. (6854)A

NEUROENDOCRINOLOGIST

Department of Physiology

QUEEN'S UNIVERSITY

A vacancy exists for an experienced neuroendocrinologist. The preferred applicant should have the Ph.D and/or the M.D. degree, several years teaching experience and a record of independent research of high quality. The applicant will be expected to lead an active research program and to participate in undergraduate and graduate teaching. The facilities for research are outstanding and the appointment is a regular one under tenure regulations. The appointment will be made at a level appropriate to the candidate's qualifications

Applications are encouraged from candidates of either sex. Applicants should submit a curriculum vitae, a brief statement of their research interests and objectives, selected reprints, and the names of three referees from whom a confidential assessment of research and teaching abilities may be obtained to: Dr. V. C. Abrahams, Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6. (Second Advertisement). (NW1870)A

DIRECTOR

BLOOMFIELD CENTRE FOR RESEARCH IN AGING

AT THE LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

In Association With

MCGILL UNIVERSITY

The Lady Davis Institute will establish a new research group for studies in aging. Initially the group will consist of the leader and two junior investigators, with the focus of interest on various aspects of the biology of the aging process. It is anticipated that three more positions will become available for work in the psychosocial and epidemiological aspects of aging.

The successful candidate (M.D. or Ph.D.) must have a demonstrated research record of aging or a closely allied area. A tenure-track university appointment will be made at the level of Associate Professor or Professor. **Please submit a current curriculum vitae, a short description of personal research interests, a brief comment on the desired areas of expertise of the two junior investigators to be recruited, and the names of three referees by August 1, 1985, to: Dr. Norman Kalant, Director, Lady Davis Institute for Medical Research, 3755 Cote St. Catherine Road, Montreal, Que. Canada H3T 1E2.**

In accordance with Canadian immigration regulations applications from Canadian citizens and landed immigrants will be considered first. (NW1873)A

ETH ZÜRICH

Applications are invited for a faculty position in

Geology

at the Department of Earth Sciences of the University and the Swiss Federal Institute of Technology in Zurich.

The new professor will be expected to participate in the general and historical geology parts of the basic geology course, to teach historical and regional geology in advanced courses and to supervise diploma and doctorate level students in field work projects.

In research a particular interest in stratigraphy with interaction in sedimentology and tectonics is desired. Candidates should be willing and able to teach at all university levels and to cooperate with colleagues within and outside the department.

Applications with curriculum vitae and list of publications should be submitted before July 15, 1985, to:
The President of the ETH Zurich
Prof. H. Urspring
CH-8092 Zurich. (W1952)A

Associate Director Drug Delivery Research

Smith Kline & French Laboratories is establishing a multi-disciplinary research team of Ph.D. scientists to investigate fundamental approaches to the delivery of drugs. We are currently seeking an individual to lead the Physiochemistry Section and serve as an Associate Director in the Drug Delivery Department. Research activities emanating from this Section should provide a mechanistic understanding of solute transport to membrane surfaces and through biological membranes leading to development of strategies to control the luminal flux of drugs.

Specific areas of research may include: (a) mass transport of drugs across selected biological membranes, (b) characterization of those membranes and associated luminal contents with respect to biochemical activity and physiological factors, (c) carrying out mass transport studies using pharmacokinetic principles to define potential routes of administration, (d) developing carrier-based systems for targeting and/or protecting macromolecular drugs, and (e) defining interactions of polymeric-based delivery systems and/or carriers at biomembranes. Initial emphasis is to be placed on delivery of peptides and proteins.

The candidate should be a nationally or internationally recognized scholar with an established record of significant scientific achievement in areas impacting on drug delivery.

Presently located in Philadelphia, the Department will be relocating to a new state-of-the-art Research and Development facility near Valley Forge. We offer an excellent salary, benefits and relocation package as well as the opportunity for personal and professional growth. Please forward your resume to George P. Kissell, Jr., Manager of Personnel Relations, Smith Kline & French Laboratories, 1576 Spring Garden St., Philadelphia, PA 19101. (NW1884)A

SK&F
A SmithKline Beckman Company

RU leiden

UNIVERSITY OF LEIDEN

The Center for Bio-Pharmaceutical Sciences has a vacancy for a

full professor of biopharmaceutics (m/f)

IN PARTICULAR DRUG TARGETING

vacancy number: 5-162/2407

The Division of Biopharmaceutics of the Center is intended to conduct research on modern methods of drugs targeting **in vivo,** such as,
- coupling of drugs to macromolecular or colloidal systems, incorporating target tissue selective substrates (e.g. antibodies);
- selective bio-activation **in situ** using prodrugs and enzyme systems specific to target tissues.

The Professor of Biopharmaceutics will play a major rol in building up the Division's research and teaching.

The Center for Bio-Pharmaceutical Sciences is a new part of the Faculty of Mathematics and Natural Sciences. Its primary objective is to conduct multidisciplinary research into the production of drugs, and their disposition and effects in man and animals. In close relation to its research, the Center will also offer a 3 year undergraduate program (M.Sc.level) as well as a 4 year graduate program (Ph.D.level).
In addition to the Division of Biopharmaceutics, the Center contains Divisions of Analytical Chemistry, Pharmaceutical Technology, Pharmacognosy/Plant Cell Biotechnology, Pharmacology, Medicinal Chemistry and Toxicology.
All Divisions have 1 full professorial chair, 2 senior faculty positions, 4 positions for graduate research assistants, and 2,5 supportive functions (2.0 technical and 0.5 administrative). All positions in the Division of Biopharmaceutics are still vacant.
The profile of the Center has been specified in a brochure which may be obtained from the Secretary Advisory Committee (address below).

Candidates should have a vast experience relevant to the research objectives of the Division of Biopharmaceutics. They are expected to be able to stimulate research collaboration with other Divisions of the Center as well as with outside partners, including industry. Proficiency in management of multidisciplinary research is an asset. In addition, candidates should be prepared to engage in the design and teaching of undergraduate and graduate courses.

Candidates should also be willing to learn Dutch.

Candidates are invited to send a written application, with full C.V., to the Secretary of the Selection Advisory Committee, Mr O. H. Postma, P.O. Box 9502, 2300 RA Leiden, The Netherlands (phone 071-148333, ext. 4219 or 4788), within two months after the publication of this advertisement. Further information may be obtained at the same address from the Committee Chairman, Prof. dr H. E. Jungınger (phone 071-148333, ext. 3856 or 3850).

(W1958)A

UNIVERSITY OF FLORIDA
FLORIDA STATE MUSEUM

DIRECTOR

Applications are invited for the Directorship of one of the largest and most active university museums of natural history The FSM emphasizes research and exhibits on the natural and cultural history of Florida and Latin America

The FSM faculty consists of 24 salaried curators who also hold titular appointments in related teaching departments. Physically the Museum is a modern structure central to the campus with one floor for each of its three departments, Natural Sciences, Anthropology, and Interpretations The Museum serves dual functions as the Florida State Museum, with permanent and travelling exhibits and a statewide membership program, and as a University Museum, with strong research and educational missions.

The Directorship is equivalent to the rank of Dean within the University of Florida Applicants should have appropriate experience and vision to plan the Museum's long-range growth, to develop a statewide constituency and fund-raising campaign, to supervise the research and public education programs of the Museum; and to maintain liaison with officers and lawmakers in state government.

The position opens as of January, 1986 The salary is commensurate with qualifications and experience and competitive with that of other major museums Applications naming three references should be postmarked no later than October 31, 1985 to.

Dr. David Webb
Florida State Museum
Gainesville
FL 32611
USA

The search will be conducted under Florida's "Government in the Sunshine" Law The University of Florida is an Equal Employment Affirmative Action Employer.

(NW1877)A

Assistant Professors of Plant Pathology

University of California, Davis

Two 11-month (1 month vacation) tenure-track positions are available January 1, 1986, each with 20% teaching and 80% research responsibility

Vegetable Crop Pathologist: Develop an original, basic research program on biology and control of diseases of vegetable crops, including physiogenic diseases, and to participate in teaching Introductory Plant Pathology and a graduate course in the appointee's area of expertise.

Fruit Crop Pathologist: Develop an original, basic research program on the biology and control of viral, mycoplasma or fastidious procaryotic pathogens of woody perennial crops, and to participate in teaching Introductory Plant Pathology and a graduate level course in the appointee's area of expertise.

Both appointees will also participate in mission oriented research, University service, and graduate advising/training Applicants should have a Ph D. in Plant Pathology or related discipline with expertise appropriate to the position Send. (a) curriculum vitae, (b) official undergraduate and graduate transcripts, (c) list of official published work and reprints, (d) statement of teaching and re search interests, (e) names and addresses of three references to·

R. K. Webster, Chairman
Department of Plant Pathology
University of California, Davis, CA 95616
Telephone: (916) 752-0300

Application deadline: August 30, 1985

THE UNIVERSITY OF CALIFORNIA IS AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER

(NW1881)A

Harvard Medical School

PROFESSOR OF MICROBIOLOGY

There is now a search for a Professor of Microbiology and Molecular Genetics to carry out a strong research program focussed on the relationship between growth factors, their receptors, the control of cellular proliferation and oncogenesis at the Dana-Farber Cancer Institute. Interested individuals should send a letter and curriculum vitae to Doctor Samuel A. Latt, Genetics Division, Childrens Hospital, 300 Longwood Ave., Boston, MA 02115 Harvard Medical School is an Equal Opportunity/ Affirmative Action Employer.

(NW1890)A

Harvard Medical School

DEPARTMENT OF PHARMACOLOGY

Tenured Professorship – Applications are invited for the position of Professor in the Department of Pharmacology at Harvard Medical School. Candidates should have a strong background in neuropharmacology. The position will also involve active participation in the teaching of pharmacology to medical and graduate students Applicants should submit curriculum vitae and a statement of research interest to Doctor David Potter, Chairman, Pharmacology Search Committee, Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Harvard Medical School is an Equal Opportunity/Affirmative Action Employer.

(NW1883)A

Harvard Medical School

PROFESSOR OF PHYSIOLOGY

The Department of Physiology of Harvard Medical School is seeking to fill a tenured professorship in the field of Systems Physiology with special emphasis on the coordination of functions at different levels of biological organization in the intact organism The individual to be appointed will assume the following duties: carry out research in the field of Integrative Physiology, fulfill a major role in the teaching of Physiology to first year medical students and praticipate in the trainng of Ph.D. students. Qualified women and minority candidates are encouraged to apply Submit curriculum vitae to Doctor Elio Raviola, Chairman, Physiology Search Committee, Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115.

Harvard Medical School is an Equal Opportunity/Affirmative Action Employer

(NW1887)A

RESEARCH SCIENTIST

Bristol-Myers Company, a leader in industrial biotechnology and a major international manufacturer of pharmaceutical products, has an excellent opportunity in its Genetic Engineering Department for a Senior Research Scientist to provide support to our Genetic Microbiology Research Program

To qualify, the successful candidate must have a PhD in Microbiology or related discipline, with 2 or more years postdoctoral research in the molecular biology of antibiotic producing organisms Experience, documented by publications, should include studies of the genetics of streptomycetes, fungi and recombinant DNA technology as applied to gene cloning in streptomycetes Responsibilities will involve the development of microbial strains with improved production of antibiotics and the production of novel antibiotics

We offer a good starting salary, comprehensive benefits and a working environment conducive to your professional growth For prompt, confidential consideration, forward your resumé to Department Head, Genetic Engineering

(NW1886)A

BRISTOL-MYERS COMPANY
INDUSTRIAL DIVISION
P.O. Box 4755, Syracuse, New York 13221
An equal opportunity employer M/F

Forestry Commission —
Farnham, Surrey

Biometrician/Modeller

You would work with a small team of mathematical scientists concerned with modelling processes and systems occurring in the forest and forest industry The work involves liaison with project leaders (biologists, foresters, ecologists) formulating their problems in mathematical form, when necessary, and developing and fitting models which will aid in their solution The fields of application include deer population dynamics, forest windthrow, drainage and growth The main project at the current time is on the development of an integrated forest growth model based upon the competition/growth/mortality of a stand of individual trees You will be expected to make a substantial contribution to this work which will help to optimise forest management practices

You should have a good degree in mathematics or statistics and preferably a post-graduate degree in an applied mathematical subject A physics degree with good statistical knowledge is also acceptable

Appointment as Scientific Officer £5905-£8150 or Higher Scientific Officer £7435-£10,035 according to qualifications and experience For the higher grade, at least 2 years' post-graduate experience is required for those with a 1st or 2nd class honours degree and at least 5 years' experience for those with other qualifications SALARIES UNDER REVIEW.

For further details and an application form (to be returned by 12 July 1985) write to Mrs K Fielding, Forestry Commission Research Division, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH.

Please quote ref: SA/35/DM

The Civil Service is an equal opportunity employer

(6864)A

Scientific Civil Service

PROFESSOR AND CHAIRMAN

Department of Medical Microbiology and Infectious Diseases

The Faculty of Medicine, University of Alberta, invites applications for the position of Professor and Chairman, Department of Medical Micro biology and Infectious Diseases, the appointment to be effective July 1, 1986. The successful candidate will be expected to provide administra tive direction and leadership direction and leadership to an actively dev eloping department. In order to enhance undergraduate and graduate teaching as well as research, the current staff of 8 faculty members in Medical Microbiology is being increased by cross appointments of 10 faculty members in Infectious Diseases Medicine and Paediatrics. Current research interests are directed towards microbial pathogenesis. The activities of the University of Alberta Hospitals Microbiology Division are also integrated into the department.

Applicants should have an active research program and be able to attract extra-mural support. The programs of the Alberta Heritage Foundation for Medical Research provide an ideal opportunity to develop innovated research areas. The appointment will be at the professorial rank with salary communsurate with qualifications and experience. Applicants should apply enclosing a Curriculum Vitae and the names of three refer ees by August 31, 1985 to:

Dr Douglas R. Wilson
Dean, Faculty of Medicine
2J2 Walter Mackenzie Health
Sciences Centre
University of Alberta
Edmonton, Alberta T6G 2R7

The University of Alberta is an equal opportunity employer, but in accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW1893)A

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany, invites applicants for the following vacancy at the Laboratory's Outstation at the «Institut Laue Langevin» site in Grenoble, France

BIOPHYSICIST

The successful candidate will have the responsibility of an instrument of light scattering (photocorrelation and Brillouin scattering) This instrument is part of the set-up of the Outstation which is doing structural molecular biology using neutron diffraction, X-rays, EM and light scattering The job will include a service activity helping visitors It is also expected that the scientist will by involved in one of the existing programmes

The candidate should have a good background in physical chemistry

An above-average salary will be offered to the successful candidate Certain allowances are payable in addition, such as family allowance, children's allowance and non-resident allowance, depending on personal circumstances

An initial contract of 3 years' duration will be offered to the successful candidate This contract may be renewed, depending on circumstances at the time of review.

Please write briefly for application form, quoting reference n°85/14 to
EMBL,
Personnel Section,
Postfach 10 2209,
D-6900 Heidelberg.

(W1954)A

EMBL

CHAIRPERSON

Department of Physiology and Biophysics

THE UNIVERSITY OF TEXAS MEDICAL BRANCH AT GALVESTON

Applications and nominations are invited for the position of Chairperson of the Department of Physiology and Biophysics Candidates must be a highly qualified individual with nationally recognized credentials in research and senior academic accomplishments with administrative experience or potential The position offers excellent opportunity to develop an internationally leading adademic department due to outstanding resources, scientific environment and faculty positions.

Application or nomination should include a curriculum vitae. Address correspondence to· James C. Thompson, M.D., Chairman, Department of Surgery, The University of Texas Medical Branch, Galveston, Texas 77550.

The University of Texas Medical Branch is an Affirmative Action/Equal Opportunity employer (NW1856)A

YORK UNIVERSITY
Department of Chemistry
ANALYTICAL CHEMISTRY

Applications are invited for a tenure-track appointment at the rank of Assistant Professor. The applicant should have a Ph D. and a strong record of accomplishment in advancing a modern area of analytical chemistry preferably with interests related to atmospheric environmental chemistry. Dedication to excellence in research, as well as in teaching at the undergraduate and graduate levels will be expected

The closing date for applications is October 31, 1985

Applications consisting of a curriculum vitae, a summary of interests in, and plans for research and the names and addresses of three referees should be sent to: Chairman, Department of Chemistry, Room 128 Petrie Science Building, York University, 4700 Keele Street, North York, Toronto, Ontario, M3J 1P3 CANADA. (NW1871)A

The European Molecular Biology Laboratory, an international research institute situated in Heidelberg, West Germany, invites applicants for the following vacancy at the Laboratory's Outstation at the «Institut Laue Langevin» site in Grenoble, France

CRYSTALLOGRAPHER

The EMBL Outstation at Grenoble is developing structural biology using neutron beams An instrument has been built in cooperation with the Institut Laue Langevin which will be dedicated to low resolution crystallography (- 15 Å°) on complex objects Work is now being done on protein nucleic acid complexes and membrane proteins Projects are brought in by outside users or generated by in-house scientists Computing facilities are available, including a VAX 750 and a colour graphic display PS 300 and access to ILL DEC 10

The post is for an experienced protein crystallographer to develop low resolution neutron crystallography The person appointed will be expected to take responsibility both for instrumental aspects and to develop their own project (possibly in cooperation with an outside laboratory)

An above-average salary will be offered to the successful candidate Certain allowances are payable in addition such as family allowance, children's allowance and non-resident allowance, depending on personal circumstances

An initial contract of 3 years' duration will be offered to the successful candidate This contract may be renewed, depending on circumstances at the time of review

Please write briefly for an application form, quoting reference n°85/15 to (W1955)A

EMBL,
Personnel Section,
Postfach 10 2209,
D-6900 Heidelberg

EMBL

Re-Advertisement

HOUGHTON POULTRY RESEARCH STATION

Grant-Aided by the Agricultural & Food Research Council

IMMUNOLOGIST

Applications are invited from immunologists to join a group studying cellular interactions in the immune system of chickens with the long term objective of providing a rational basis for the development and presentation of vaccines, particularly novel vaccines produced by genetic engineering techniques The initial objective is the production and application of monoclonal antibodies specific for lymphocyte populations and subpopulations and the development of in vitro assays for soluble mediators such as interleukins

Applicants should have a first or upper second class honours degree in a biological subject and preferably a PhD, with at least two years relevant post-graduate experience.

The appointments will be made in the Higher Scientific Officer grade, salary: £7,435 × 6 to £10.039.

The position is available for a period of three years, commencing on a date to be arranged prior to October 2nd, 1985.

For further details and application forms please contact the Secretary, John Andrews at Houghton Poultry Research Station, Houghton, Huntingdon, Cambs, PE17 2DA, tel St Ives (0480) 64101. Closing date for applications is 12th July 1985.

The Houghton Poultry Research Station is an Equal Opportunities Employer (6802)A

KINGSTON POLYTECHNIC
School of Geology

SENIOR TECHNICIAN
Scale 4/5 £7,212 — £8,919
including London Allowance

A Senior Technician is required in the School of Geology to provide support in Mineralogy and Petrology, and structural Geology

Applicants should hold HND/C or degree in Geology or other related subject Preference will be given to those with suitable experience.

TECHNICIAN (Part-time)
Scale 2 £4,421.25 — £4,819.50
including London Allowance

The technician is required to work 27 hours a week to assist with support in Mineralogy and Petrology, and Stuctural Geology. Applicants should possess HND/C or degree in Geology or related subject. School leavers with relevant O/A levels will also be considered. Experience would be an advantage but is not essential

Application forms and further details from Staffing Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel 01-549 1366 ext 205 Closing date 5th July 1985 (6831)A

KING'S COLLEGE LONDON (KQC) (QUEEN ELIZABETH CAMPUS AT KENSINGTON)
LECTURESHIP IN MOLECULAR GENETICS

Applications are invited for the above Lectureship in the Biochemistry Department, tenable from 1 October 1985, or as soon as possible thereafter.

The appointee will be responsible for teaching aspects of Molecular Biology to undergraduates and MSc students and will be associated with the MRC Human Genetic Diseases Group under the direction of Professor D Robinson.

A research interest in the application of molecular genetics to the diagnosis, treatment or elucidation of human disease states is therefore desirable.

Salary will be within the range £7,520 — £14,925 per annum plus £1,233 per annum London Allowance. Universities' Superannuation Scheme contributions are payable.

Preliminary informal enquiries should be addressed to Professor D Robinson at the Biochemistry Department, Queen Elizabeth Campus at Kensington, Campden Hill Road, London W8 7AH

Application forms and written further particulars can be obtained from Mr S P Harrow, Assistant Registrar, King's College London, Strand, London WC2R 2LS (tel 01-836 5454 Ext 2689). Applications should be submitted, with the names of not fewer than two referees, not later than 22 July 1985. (6881)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF FORESTRY AND NATURAL RESOURCES

POSTGRADUATE RESEARCH ASSOCIATE

Applications are invited for the post of NERC funded Research Associate to study genetic variation in a Forest Pathogen. The work will involve the measurement of variation in morphology, isozymes, and tolerance to monoterpenes in the ascomycete fungus Crumenulopsis sororia, a pathogen of pines

The appointment is for a three year period from 1st September, 1985 on the 1B salary scale.

Applicants should hold, or expect to obtain a first or upper second class degree in an appropriate biological science

Applications, including a curriculum vitae and the names of two referees, should be sent as soon as possible to Dr R A Ennos, Department of Forestry and Natural Resources, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh, EH9 3JU

Please quote reference No 5258 (6851)A

THE UNIVERSITY OF NEW SOUTH WALES
Sydney, Australia
PROFESSOR
SCHOOL OF CHEMICAL ENGINEERING AND INDUSTRIAL CHEMISTRY
(Centre for Petroleum Engineering Studies)

The University of New South Wales has been designated by the Federal Government as the Centre for teaching and research in petroleum engineering in Australia. It is now in the process of establishing a National School of Petroleum Engineering that will serve the needs of the Australian Petroleum Industry. The University has established a Centre for Petroleum Engineering Studies which will offer undergraduate and postgraduate degrees in petroleum engineering commencing in 1986.

The Centre draws on existing expertise within the Schools of Chemical Engineering and Industrial Chemistry, Applied Geology and Mining Engineering in the Faculty of Applied Science. Applications are invited for appointment to a Chair. The successful applicant initially will be Director of this Centre, develop the undergraduate teaching program, contribute to research activity and extend existing links with industry.

Candidates should have a commitment to excellence in undergraduate and graduate education and have demonstrated research capacity and leadership in drilling, reservoir, production, or other areas of petroleum engineering. Industrial experience in oil and gas production is highly desirable.

Enquiries regarding the Chair may be addressed to the Dean of the Faculty of Applied Science or to Professor C J D Fell, Head of the School of Chemical Engineering and Industrial Chemistry, University of New South Wales, PO Box 1, Kensington, NSW 2033, Australia.

Salary. A$54,948

Subject to consent by the University professors may undertake a limited amount of higher consultative work.

The University reserves the right to fill any by invitation.

Applications close 16 August 1985.

For full information about conditions of appointment and method of application write to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Equality of employment opportunity is University policy.

(W1957)A

TECHNICIAN or EMBRYOLOGIST with experience in mammalian embryology, required to work for IVF clinic in Athens. Salary negotiable. Interested persons should write in confidence to Dr H G Massauras, 3 Marasli Street, Athens 106-76, Greece. (W1920)A

ONCOGENE SCIENCE INC.

Expanding staff at Long Island, NY research facilities due to advances in

Oncogene and growth factor research

to develop products for the early

detection -- monitoring -- treatment of human cancers.

— Positions Available —

DIRECTOR OF BIOCHEMISTRY

Responsible to direct and coordinate corporate biochemistry program. Ph D in Protein Chemistry — purification and sequencing, 8-10 years postdoctoral experience. Strong publication record highly desirable. Will contribute to development of diagnostic and pharmaceutical products.

PROJECT DIRECTOR

Responsible for formatting diagnostic tests for HTLV-III infection and oncogene activation. Test development, pilot and scale-up operations, standardization of appropriate QC procedures. Ph D. in Immunochemistry. 5-7 years postdoctoral experience in immunoassay, format design, product development.

MOLECULAR BIOLOGIST

To participate in projects aimed at determining the role of oncogenes in human cancer using cDNA cloning, in situ hybridization and bacterial expression of antigens. Ph D in Molecular Biology, although candidates with Masters degree and appropriate experience will be considered.

Be part of the excitement at OSI.

Send resume and personal data to Scientific Director, Oncogene Science Inc., 222 Station Plaza North, Suite 330, Mineola, NY 11501

Competitive salary. Liberal benefits. Equity potential.

Equal Opportunity Employer (NW1885)A

UNIVERSITY OF WESTERN ONTARIO
LONDON, ONTARIO
CANADA
POST-DOCTORAL POSITION FOR BIOCHEMIST

with experience in enzymology (phosphorylation preferred) or in cloning and sequencing DNA for studies on developing lung. Position funded by Canadian MRC. Minimum stipend approximately $20,000.

Send curriculum vitae and names of 3 referees to Dr Fred Possmayer, Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

(6841)A

ASSISTANT RESEARCH SCIENTIST

Research work on mechanism of folding and function of X-chromosome-linked enzyme, phosphoglycerate kinase, synthetic antibodies, genetically engineered protein to study relationship between biological functions and structures. Must have Ph.D. in Chemistry and be able to manipulate and maintain CD, NMR, UV-VIS and Spectroflourimeter.

Job site Duarte, California.
Salary: $25,000.00 per year.
Send this ad and your resumé to.

Job #5373, P.O. Box 865, Sacramento, CA 95804
not later than July 6, 1985

(NW1878)A

MOLECULAR BIOLOGIST

Tenure-track position at the assistant professor level for a molecular biologist. Applicant must have at least 2 years postdoctoral experience and demonstrated accomplishment in eukaryote gene expression studies. Expertise in recombinant DNA technology essential. Ph.D. or M.D. **Send curriculum vitae, a statement of research interest, and the names and addresses of 3 references to: Dr. Ralph Weichselbaum, Professor & Chairman, Dept. of Radiation Oncology, Biological Sciences Division, The University of Chicago, Box 440, 5841 S. Maryland Ave., Chicago, IL 60637.** An Affirmative Action Equal Opportunity Employer.

(NW1874)A

RESEARCH SCIENTISTS

California Biotechnology Inc., an established biotechnology company, is seeking scientists at the Ph.D. and M.S./B.S. levels to participate in exciting research programs. Qualified applicants should have experience in one of the following areas:

Protein and peptide chemistry with broad expertise in the purification, characterization, structural analysis, or synthesis of novel proteins and peptides.

Monoclonal antibody production with experience in both *in vivo* and *in vitro* techniques of hybridoma production.

Molecular pharmacology to study ligand-receptor interactions and coupling of receptors with intracellular mediators of action.

Molecular biology with experience in recombinant DNA techniques and the expression of foreign genes in bacteria, yeast, or mammalian cells.

We offer a comprehensive benefits package and competitive salaries. For consideration, please submit a curriculum vitae, a list of publications and the names of three references to:

CBI

Dr. John Shine
Director of Research
California Biotechnology Inc.
2450 Bayshore Frontage Road
Mountain View, CA 94043

(NW1880)A

PROFESSORIAL POSITIONS

Neurobiology Center
Mount Sinai School of Medicine
the City University of New York

Mount Sinai School of Medicine of CUNY announces a new initiative in molecular genetics. The Neurobiology Center plans to appoint groups of research scientists who are creatively attacking important biological problems using the most advanced techniques in molecular genetics.

We are seeking (an) established investigator(s), who will assume a leadership role, as well as applicants for assistant or associate professorships.

Please send inquiries and curriculum vitae to:

Dr. Kenneth L. Davis
Dept. of Psychiatry
Mount Sinai School of Medicine
1 Gustave L. Levy Place
New York, New York 10029 (NW1868)A

HERIOT-WATT UNIVERSITY

Department of Physics and Department of Electrical & Electronic Engineering

RESEARCH ASSOCIATE

Application of Optical and Electro-Optical Techniques to Computing

Applications are invited for the above interdepartmental research post, funded by Burroughs Machines Ltd and aimed at studying the likely future impact of laser, nonlinear optical and electro-optical technology on computer and information processing hardware

The succesful applicant will be based in the Physics Department and will work within a large group funded by the SERC, Industry and the EEC, working on nonlinear optics, optical bistability and all-optical computing concepts He/she will be expected to carry out experimental research of relevance to both these topics and digital electronic computers, such as the use of fibre-optic or holographic techniques for realising optical interconnections etc.

Candidates must have obtained a Ph D. in a field relating to at least osme of the areas indicated above The post will be tenable for an initial period of three years and the salary will start in the range £7,980 to £9,390 (under review) and includes yearly increments

For further details contact Dr. Andy Walker (031 449 5111). Application forms are available from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX quoting Reference No. 55/85.

(6872)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF ORGANIC CHEMISTRY

PROJECT ASSISTANT

Applications are invited for the above post in the Wolfson Unit for Computer Aided Design of Organic Synthesis The work requires a good knowledge of Organic Chemistry and involves the development of a reaction indexing database for use with a new computer program called ORAC

A good honours degree in Chemistry is required An interest in chemical applications of computers would be an advantage

The post is available immediately for a fixed term of up to one year. There may be the possibility of a further fixed-term contract thereafter.

Salary within the range £6,600-£8,920 on the IB Grade for Other Related Staff (£6,600-£10,330) (under review) according to qualifications and experience.

Informal enquiries may be made to Dr A P Johnson (tel 0532 431751 ext 6071/6058)

Application forms and further particulars may be obtained from the Registrar. The University, Leeds LS2 9JT, quoting reference no. 45/39 Closing date for applications 5 July 1985 (6875)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

MRC PROTEIN PHOSPHORYLATION GROUP

RESEARCH ASSISTANT

(Postdoctoral or Postgraduate)

The successful candidate will work with Dr Grahame Hardie on the regulation of hepatic fatty acid synthesis by protein phosphorylation The study is supported by the MRC for 3 years and will involve studies of the phosphorylation of enzymes in iso lated hepatocytes treated with hormones or other agents (see Eur J Biochem (1984) 140, 325-333) The MRC Protein Phosphorylation Group consists of the laboratories of Professor P Cohen, Dr T Cohen and Dr Hardie and is extremely well equipped for this type of study The position is available immediately but persons who can start at any time up to January 1986 are invited to apply The appointment, which will be for one year in the first instance, will be on the Research Grade IA (£7,520 — £12,150) or IB (£6,600 — £8,920) depending on age, qualifications and experience

Informal enquiries may be made to Dr Hardie at the Department of Biochemistry (Dundee 23181 ext 4253) Applications, including curriculum vitae and the names of two referees, should be sent to the Personnel Officer, The University, Dundee, DD1 4HN as soon as possible. Please quote Ref. EST/70/85J. (6853)A

QUEEN MARY COLLEGE, UNIVERSITY OF LONDON

SCHOOL OF MATHEMATICAL SCIENCES

POSTDOCTORAL RESEARCH ASSISTANT IN APPLICATIONS OF COMPUTER ALGEBRA IN CATASTROPHE THEORY

Applications are invited for a research assistant to work for two years with Dr F. J. Wright, commencing as soon as possible after October 1, 1985. Experience in at least one of Computer Algebra and Catastrophe/Singularity Theory is desirable Salary on the Research and Analogous 1A scale £7,520-£12,150 per annum plus £1,233 London Allowance.

Further details may be obtained from Dr. Wright

Applicants should sent a curriculum vitae including list of publications (two copies) (and copies of any that are particularly relevant) and the names of two referees to the Assistant Personnel Officer, Queen Mary College, Mile End Road, London E1 4NS, and quote ref 85/43/N. The closing date is July 31, 1985.

Students interested in studying for a Ph.D. in this area are also invited to contact Dr Wright directly.

(6857)A

THE ROYAL VETERINARY COLLEGE

University of London

DEPARTMENT OF ANATOMY

POSTDOCTORAL RESEARCH ASSISTANT IN CELL BIOLOGY/ BIOCHEMISTRY

To work with Dr J. Bee on modifications of cell:cell interactions *in vitro* during loss of the cartilage phenotype and their implications to osteoarthritis Applicants should have an interest in cell biology or biochemistry of the cell surface, particularly intercellular junctions and cell communication. Experience in immunological methods is desirable, but not essential.

The appointment is funded by the Nuffield Foundation initially for twelve months with possible extension to three years.

Salary on the Universities' Research Scheme Grade 1A scale. Starting salary not less than £8,753 per annum including London Allowance. Superannuation under the Universities' scheme.

Application form obtainable from Assistant Secretary (Personnel), the Royal Veterinary College, Royal College Street, London NW1 0TU (Tel: 01-387 2898). (6856)A

STAFF RESEARCH ASSOCIATE MICROBIOLOGIST

Conducts research on particular aspects of physiological and morphological development of Bdellovibrio bacteriovirus, in collaboration with principal investigator. Must have demonstrated working knowledge of modern biochemical, microbiological & molecular biology techniques including isotope labelling & counting; ultracentrifuge; gel electrophoresis procedures & asceptic techniques; specimen preparation for electron microscopy thin sectioning techniques and modern electron microscope) in addition to experience in working with two-membered cultures. Ph.D. req. $1700/mo. Job located at educational institution in Los Angeles. **Send this ad and your résumé to Job #1724, P.O. Box 865, Sacramento, CA 95804 no later than 7/5/85. Must show you have the legal right to work.** (NW1875)A

POSTDOCTORAL RESEARCH POSITION in the University Medical Center Basic Science Department. Studies in cellular immunology of the function and development of human thymocytes and T-lymphocytes and on gene expression of lymphokine receptors. Experience in immunology, biochemistry and/or molecular biology desirable. Send résumé to: Joan Harmon, Personnel Department, NYU Medical Center, 560 First Avenue, New York, NY 10016. An Affirmative Action/Equal Opportunity Employer M/F. (NW1888)A

RESEARCH POST FOR A PHARMACOLOGIST

Horsham, West Sussex

A new position has arisen in the Pharmaceuticals Division of CIBA-GEIGY PLC for a Pharmacologist to join a group undertaking research into cardiovascular disease and its treatment.

The successful applicant will hold a degree in Pharmacology, or a related biological science with appropriate post-graduate experience in Pharmacology. A high degree of practical skill and the ability to work effectively within a multidisciplinary research group will be essential in this post.

An attractive starting salary is supplemented by a range of fringe benefits and conditions of employment which one would expect of a major international employer.

To help us assess your suitability for this post, please send in a letter of application together with a cv to:–

Miss S J Byrne, Personnel Officer, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex RH12 4AB.

(6909)A

CIBA–GEIGY

EXCITING RESEARCH OPPORTUNITY IN WEST GERMANY

CELL BIOLOGIST

With experience in light and electronmicroscopic techniques and their application to immunological and biochemical studies at the cellular and sub-cellular level. The ideal candidate should have an MD and/or Ph.D, and postdoctoral experience.

In addition to the researcher's salary, this federally funded position includes salary for a Technician, start-up funds and an appropriate supply budget.

Please send CV and references to:
Dr. Rolf Kinne
Director Max-Planck-Institut fuer Systemphysiologie
Rheinlanddamm 201
4600 Dortmund 1, West Germany. (W1953)A

POSTDOCTORAL POSITIONS
HOWARD HUGHES MEDICAL INSTITUTE/ PHILADELPHIA

Fully funded postdoctoral positions in molecular biology and molecular genetics are now available at the Howard Hughes Medical Institute of the University of Pennsylvania. Active research projects include: transcriptional and translational control of eukaryotic gene expression, mechanisms of cellular transformation and gene amplification, molecular mechanisms of chromatin inactivation and the application of molecular genetics to studies of inherited disease.

For infomation please contact:
Dr. Steve Liebhaber, Coordinator
Howard Hughes Medical Institute
Department of Human Genetics
Medical Labs Building/G3
University of Pennsylvania School of Medicine
Philadelphia, PA 19104 (NW1869)A

UNIVERSITY OF ST ANDREWS
Department of Physiology and Pharmacology

(a) LECTURESHIP IN PHYSIOLOGY
(b) WELLCOME LECTURESHIP IN HUMAN MUSCLE PHYSIOLOGY

(a) Applications are invited for an established Lectureship in the above Department. Applicants should have a good research record in any field of experimental Physiology The Department occupies sites at the Bute Medical Building and Gatty Marine Laboratory. The successful candidate will be based at the Gatty Marine Laboratory and should have research interests which make full use of the facilities provided He/she will be required to contribute to the teaching of general Physiology to students. Current research interests of the Department include ion trans-port, neuropharmacology, muscle and environmental physiology.

(b) Applications are also invited from candidates under 35 years of age with a degree in Physiology or a related discipline, for a Lectureship in Human Muscle Physiology funded by the Wellcome Trust. The appointment is available for 3 years in the first instance renewable for a further 2 years The successful applicant will be required to contribute to the teaching of human and general physiology to students of physiology, pharmacology and physical education. The emphasis of this appointment will be on research and candidates are required to submit a 500 word summary of their proposed research schemes.

Salary at appropriate point on scale £7,520 — £14,925 per annum, starting salary probably not above £10,720 plus USS.

Further information about both posts may be obtained from the Department.

Applications (two copies preferably in typescript) in the form of *curriculum vitae*, statement of research interests and the names of three referees, should be lodged with the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ, NOT LATER THAN 5 JULY 1985. Candidates should indicated clearly which post(s) they are applying for.

(6844)A

BRISTOL POLYTECHNIC

SCIENCE DEPARTMENT

LECTURER II/SENIOR LECTURER IN PHYSICS

Applications are invited from good honours graduates in Physics or Applied Physics with post-graduate or industrial research experience Applicants should have an interest in aspects of Physics and Engineering related to scientific instrumentation and microprocessor technology. He/she will be encouraged to maintain a research activity in this or a related area

SALARY SCALE: LII £7,548-£11,175 (bar)—£12,099 per annum
SL £11,175-£13,128 (bar)—£14,061 per annum

The appointment will be made on the appropriate scale according to relevant previous service/experience. (Progression from the LII scale to the SL scale is in accordance with the provisions of the Burnham Further Education Report).

For further details and an application form, to be returned by 8 July 1985, please contact the Personnel Office, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol or ring Bristol 656261, Ext 216 or 217.

Please quote Reference Number L/61 in all communications

Avon County Council is an Equal Opportunities Employer (6901)A

Applications are invited for a position in neurophysiology at the Assistant Professor level in the Department of Physiology at the University of Manitoba Faculty of Medicine. Candidates should have a PhD or equivalent, with two or more years of post-doctoral training. Preference will be given to individuals with experience and a continuing interest in electrophysiological analysis of neural networks and/or neurotransmitter actions using *in vitro* or *in vivo* systems. Send curriculum vitae, an outline of future research plans, and arrange for three letters of reference to be forwarded to. **Dr. Henry G. Friesen, Professor and Head, Department of Physiology, The University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0WE Canada.** Deadline for receipt of applications is 1 September 1985 In accordance with Canadian immigration requirements this advertisement is directed to Canadian citizens and permanent residents. (NW1857)A

ORGANIC GEOCHEMIST

STANFORD UNIVERSITY

The School of Earth Sciences is intent on adding a new program in organic geochemistry and is seeking an individual with a solid geologic perspective to lead this effort Current research in all four departments in the School including Applied Earth Sciences Geology, Geophysics, and Petroleum Engineering involves work on sedimentary systems and we view organic geochemistry as an emerging tool of fundamental importance to these studies We also think that organic geochemistry constitutes a field of especially exciting potential for the future studies of the origin and fate of organic matter in modern and ancient sedimentary environments chemical fossils, sedimentary hosted mineral deposits petroleum geochemistry as well as applications to study of the tectonic and sedimentary evolution of continental margins

Applicants for this tenure-track position should have an established research record involving application of organic geochemistry to geological questions, an enthusiasm for teaching, and a clear ability to garner and maintain support for their research program We invite applications from both junior and senior scientists but we are most interested in finding a mid-career scientist of international reputation who is stimulated by the challenge of establishing a new program within the unusually diverse intellectual and geological environment offered by the San Francisco Bay region, Stanford University, and the School of Earth Sciences

Applicants should send a letter outlining their research and teaching interests along with vita including the names of three individuals familiar with their work to

Prof. James C Ingle, Chairman, Search Committee,
Department of Geology, School of Earth Sciences,
Stanford University, Stanford CA 94305

The level of this appointment is open and may be at Assistant, Associate, or Full Professor depending upon the individual selected Application deadline is September 30 1985

Stanford University is an Equal Opportunity Employer and we especially encourage applications from women and minority students (NW1882)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

Central Public Health Laboratory

NATIONAL COLLECTION OF TYPE CULTURES

PLASMID COLLECTION

A Basic Grade MICROBIOLOGIST

(1st or 2nd class honours degree or postgraduate qualification) is needed by the National Collection of Type Cultures (NCTC) to complete a team of three whose task is to establish a service supply collection of plasmid bearing strains The work entails preservation, characterization, quality control and cataloguing and will initially concentrate on enterobacterial strains Previous experience in plasmid work an advantage.

NHS conditions of service apply

Salary scale £6,559 — £8,725 + £648 London Weighting

Further details and application forms from the Personnel Departments Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT (6832)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE

Tenovus Institute for Cancer Research

RESEARCH TECHNICIANS

required in the above Institute for work on various projects concerned with cancer research, some of which may have NHS involvement.

Salary scales and points will be dependent upon qualifications and experience, ranging from £4,531 to £7,159 per annum

Applications, in the form of a curriculum vitae with names and addresses of two referees, should be sent to Mrs Margaret Lewis, Tenovus Institute for Cancer Research, Heath Park, Cardiff, CF4 4XX as soon as possible. (6868)A

UNIVERSITY OF OXFORD

Dyson Perrins Laboratory

ORGANOMETALLIC CATALYSIS IN ASYMMETRIC SYNTHESIS

Applications are invited for post-doctoral work on a project concerned with the development of asymmetric catalysis and its use in the preparation of a range of optically active compounds, starting on October 1st 1985, or as soon as possible thereafter Those interested should write to Dr J M Brown, Dyson Perrins Laboratory, Oxford, OX1 3QY, UK, enclosing a full curriculum vitae and the names and addresses of two referees. Appointment will be for one year at this stage with the possibility of renewal, on the salary scale £7,520 — £7,980 pa, plus USS. (6865)A

UNIVERSITY OF SURREY

ROBENS INSTITUTE/ DEPARTMENT OF PHYSICS

WELLCOME LECTURESHIP

Applications are invited from medically orientated scientists preferably with a medical qualification, for the post of Wellcome Lecturer The post will be concerned with the development of Magnetic Resonance Imaging Techniques for the investigation of occupationally related disease. The successful candidate who will be based in the Robens Institute of Industrial and Environmental Health and Safety, will work in conjunction with the Department of Physics and will join a dynamic interdisciplinary team Facilities within the NMR Laboratory include a 1.5T intermediate scale imaging apparatus and a 0.15T whole body system The appointment is for three years, extendable to five years subject to review

Applicants should be under 35 years of age and should not be currently holding a tenured University post

Salary will be in the range £7,520 — £14,925 per annum (under review), according to age, qualifications and experience Superannuation under USS conditions.

For an informal discussion of the post please contact Dr L Hawkins (Robens Institute) or Dr D Taylor (Department of Physics) on Guildford (0483) 571281.

Further particulars are available from the Academic Registrar (AA), University of Surrey, Guildford, Surrey, GU2 5XH, or telephone Guildford (0483) 571281, Ext 633 Applications from men and women, in the form of a curriculum vitae, including the names and addresses of three referees, should be sent to the same address by 12 July 1985 quoting the reference 399/H (6835)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

RESEARCH ASSISTANT IN BIOCHEMISTRY

Applications are invited for the above post, funded by the Multiple Sclerosis Society for two years. The successful applicant will be involved in the analysis of lipid peroxidation products in blood and CSF with a view to the development of a possible diagnostic test for MS. Some experience of HPLC, although not essential, would be an advantage.

Starting salary £6,600 pa on the 1B scale, plus USS

Applications, including full details of qualifications and experience plus the names of two referees, should be sent by 4th July 1985 to Dr M I S Hunter, Department of Biochemistry and Microbiology, University of St Andrews, Irvine Building, North Street, St Andrews, Fife, KY16 9AL, from whom further particulars may be obtained. (6886)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Research School of Chemistry

POSTDOCTORAL RESEARCH POSITIONS

Applications are invited from suitably qualified men and women for appointment to postdoctoral research positions in the Research School of Chemistry

Current major research interests in the School are:

ORGANIC CHEMISTRY

(Professor A L J Beckwith, Professor L N Mander, Mr R W Rickards, Dr J K MacLeod, Dr L Radom): including free radical reactions; mechanistic and synthetic organic chemistry, reactive intermediates; organic and bio-organic chemistry of biologically active compounds, ICR and mass spectrometry; ESR spectroscopy; theoretical organic chemistry.

INORGANIC CHEMISTRY

(Professor B G Hyde, Professor A M Sargeson, Dr M A Bennett, Dr G B Robertson, Dr S B Wild): including solid state inorganic chemistry; synthesis, structure and reaction mechanisms of transition metal complexes; biomimetic chemistry; organotransition metal chemistry; X-ray crystallography; resolution and reactions of chiral arsines and phosphines.

PHYSICAL AND THEORETICAL CHEMISTRY

(Professor J W White, Dr J Ferguson, Dr R Bramley, Dr L R Brown, Dr T R Welberry, Dr D J Evans): Novel materials; low dimensional phenomena including interfaces; neutron scattering; spectroscopy and photochemistry; photophysics and magnetic resonance; NMR spectroscopy, structure and dynamics of macromolecules; X-ray diffraction and theoretical studies of disordered materials, liquid state chemical physics

CONDITIONS OF APPOINTMENT Appointments will be made at the levels of Postdoctoral Fellow and Research Fellow. The level appropriate for each appointee will be determined by the University and will depend on qualifications and experience Appointment as Postdoctoral Fellow will be for one or two years; appointment as Research Fellow will be for three years in the first instance Salary will be within the ranges: Postdoctoral Fellow $A22,614 — 25,905 pa; Research Fellow $A26,236 — 34,467 pa Current exchange rates are $A1:Stg 54p. $US 68c. $C 90c Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra Eligible appointees will be required to join the Superannuation Scheme for Australian Universities Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote reference number RC 30.5.1 when submitting applications and when requesting further particulars, which are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or from the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia.

There is no application form. Applicants should supply to the Acting Registrar of the University by 30 August 1985 a curriculum vitae, a list of publications, and statement of research interests, together with two passport-sized photographs, the names and addresses of three academic referees and the probable data on which the Postdoctoral Fellowship or Research Fellowship, if awarded, could be taken up.

The University is an equal opportunity employer. (W1961)A

SHROPSHIRE HEALTH AUTHORITY

THE ROBERT JONES & AGNES HUNT ORTHOPAEDIC HOSPITAL

Post Doctoral Principal Biochemist

DEPARTMENT OF RHEUMATOLOGY

Experience in protein biochemistry and immunology essential.

As well as providing a quality service of immunological investigations, there is an additional research element. Both aspects are closely integrated with clinical material.

Applications, stating age, qualifications and experience, together with the names of two referees should be sent to the Unit Administrator, The Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire, within three weeks of this advertisement Further information may be obtained by contacting Dr D J Ward, Consultant Rheumatologist. (6884)A

THE UNIVERSITY OF MANCHESTER

POSTDOCTORAL RESEARCH POST IN THE

DEPARTMENT OF ANATOMY

Applications are invited for this three-year post, funded by the Medical Research Council, to undertake experiemental studies into Parkinson's Disease The work will involve stereotaxic surgery, deoxyglucose metabolic mapping, receptor binding autoradiography and neuroanatomical tracing techniques. Previous experience in one or more of these would be a distinct advantage The post is available from October 1st, 1985.

Initial salary up to £8,920 pa. Superannuation.

Applications, including the names of two referees, should be sent as soon as possible to Dr A R Crossman, Department of Anatomy, The Medical School, The University, Manchester M13 9PT (6839)A

Imperial Tobacco Limited, the UK's leading tobacco products manufacturer, seeks a suitably qualified scientist to join its Research Team.

INDUSTRIAL TOXICOLOGIST

Bristol **£11,500–£13,800**

This position offers an excellent opportunity for a young graduate specialist with flair and creative skills to act in an advisory/consultative capacity on a wide range of the Company's activities including Health and Safety at Work. The successful candidate will also be expected to keep abreast of any new developments and applications in methodology and mathematical modelling.

Candidates should ideally have a post-graduate qualification or at least a first-class honours degree in physiology, pathology, genetics or other relevant discipline, together with recent experience in toxicological research.

We offer a progressive salary within the range £11,500–£13,800 dependent on experience, together with an excellent range of benefits and staff facilities.

Please send full CV or write/telephone for an application form to A. W. L. Mortimore, Personnel Services, P.O. Box 244, Hartcliffe, Bristol BS99 7UJ. Tel. Bristol (0272) 781111 Ext 2206

IMPERIAL TOBACCO LIMITED

Imperial Tobacco Limited

Imperial Tobacco is an equal opportunity employer.

ETH ZÜRICH

Applications are invited for faculty positions in

Plant Biochemistry

at the Swiss Federal Institute of Technology in Zurich.

One or two faculty positions are available in the Faculty of Natural Sciences. The successful applicants are expected to develop their own research program and to participate in teaching basic biology to students of biology, forestry and agricultural engineering, rural engineering and pharmacy. For the advanced biology students the new professors will teach the field of their own research.

The applicants should have a Ph.D. and should have performed several years of independent research, preferably in the fields of plant physiology or developmental plant biology. They should be talented and willing to teach at all university levels.

Applications with curriculum vitae and list of publications should be submitted not later than July 15, 1985, to:

The President of ETH Zurich
Prof. H. Ursprung
ETH-Zentrum
8092 Zurich
Switzerland. (W1959)A

RESEARCH ASSOCIATE and RESEARCH SPECIALIST

positions for conducting research on genetic aspects of biodegredation of hazardous chemicals. Postdoctural Research Associates (Ph D) must have experience in recombinant DNA techniques as well as in DNA sequencing.

One Research Associate will work jointly with the University of Illinois at Chicago and an environmental research team in microbial ecology-biotechnology at the U.S EPA Laboratory in Gulf Breeze, Florida The other Research Associate and the Research Specialist (B S) will work jointly with the EPA Hazardous Waste Engineering Laboratory in Cincinnati, Ohio.

Send resumés and other details to

A.M. Chakrabarty
Department of Microbiol.
Univ. of Illinois at Chicago
Health Sciences Center
Chicago, Illinois 60612

The University of Illinois is an EEO/AA Employer (NW1894)A

CENTRAL MANCHESTER HEALTH AUTHORITY
ST MARY'S HOSPITAL
DEPARTMENT OF MEDICAL GENETICS
Immunogenetics Laboratory
GRADUATE SCIENTIFIC OFFICER

Required to work with Dr G M Taylor on the development of Immunogenetic techniques for the diagnosis of Down's Syndrome, funded by N W Regional Health Authority The project involves the analysis of cell-surface antigen expression by Down's cells.

Experience in any of the following would be advantageous; preparation of monoclonal antibodies, cell culture, cytogenetics or fluorescence flow cytometry

The position is available for one year in the first instance, with an extension up to three years. Salary up to £7,311 pa according to qualifications and experience.

Applications with names of 2 referees and informal enquiries to Dr G M Taylor, Department of Medical Genetics, St Mary's Hospital, Whitworth Park, Manchester M13 0JH Tel: 061-276 6472/6499. Closing date 30th June 1985. (6900)A

UNIVERSITY COLLEGE CORK
RESEARCH POSITIONS IN MICROBIOLOGY/ BIOTECHNOLOGY
POSTDOCTORAL RESEARCH ASSOCIATES

(2) to work on projects involving molecular genetics of plant-microbial interactions —

(A) to investigate genes involved in carbon metabolism in Rhizobium (Genetic Engineering);
(B) to investigate the regulation of carbon metabolism in Rhizobium and plant growth promoting Pseudomonas spp. (biochemical-genetics/physiology)

Positions are available from 1 July 1985 and salary will be in the range IR£8,596-IR£11,173 (less PRSI contribution) according to experience

Applications including a C.V and the names of two referees should be sent as soon as possible to Dr F. O'Gara, Microbiology Department, University College, Cork, Ireland, from whom further particulars are available. (6903)A

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF BIOCHEMISTRY
TWO POSITIONS

are available in a group working on the DNA sequences that direct heat-shock activation of the yeast gene for 3-phosphoglycerate kinase. Both are tenable initially for 3 years and are funded by the SERC Biotechnology Directorate. One appointment will be made at

Post-Doctoral (RESEARCH ASSISTANT IA)

level and the other at

TECHNICIAN (GRADE 3)

level. Experience in cloning and/or M13 sequencing would be preferable.

Applications for either the post-doctoral or technician posts should be sent to Dr P W Piper, Department of Biochemistry, University College London, Gower Street, London WC1E 6BT (Tel: 01-387 7050 extn: 291), to arrive not later than 31 July 1985. They should include a cv and the names and addresses of two referees (6858)A

LEUKAEMIA RESEARCH FUND CENTRE AT THE INSTITUTE OF CANCER RESEARCH:
TECHNICIAN/RESEARCH OFFICER

required to help organise a molecular biology laboratory and actively participate in its research programme into Cellular and Molecular Biology of Human Leukaemia. Experience in recombination DNA techniques essential. Candidates should have a degree, HNC or equivalent qualification.

Salary will be in the range of £5,738 — £8,096 + £1,042 pa. London Weighting allowance, depending on qualifications and experience. Applicants are advised that in the majority of the Institute's premises smoking is prohibited

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 301/B/86. (6862)A

THE UNIVERSITY OF MANCHESTER
Theoretical Chemistry Group
ICI JOINT RESEARCH SCHEME

A Postdoctoral position is available for a two year period for a theoretical chemist or physicist to participate in a fundamental quantum-statistical study of the effect of high pressures on the electronic stucture of molecules and molecular processes occuring in detonation. The work will be under the direction of Professor W Byers Brown and will be carried out in collaboration with the Detonation Research Group, ICI plc, Ardeer. Applicants should have experience of computational quantum chemistry techniques and preferably also with statistical perturbation theories. Salary on the IA Range for Research and Analogous Staff (£7,520 — £12,150 pa) according to age, qualifications and experience.

Scientific enquiries and applications to Professor W Byers Brown (061-273-7171 Ext: 5286), Department of Chemistry, The University, Manchester M13 9PL. (6894)A

UNIVERSITY OF THE WEST INDIES
Mona, Jamaica
SENIOR LECTURER/ LECTURER IN ORGANIC CHEMISTRY

Successful applicant will be expected to participate in the teaching and organisation of the courses in the Department and to contribute to its research programme.

Salary scales (under review): Senior Lecturer J$29,532 × 825 — 33,657 × 924 — 36,429 (B) × 924 — 37,353 × 987 — 38,340 Lecturer J$23,688 × 768 — 28,296 (B) × 825 — 33,246.

Up to five full economy class passages plus baggage allowance up to US$900 on appointment and normal termination. Special allowance up to US$300 for shipment of academic books and teaching/research equipment on appointment Unfurnished accommodation at 10% of pensionable salary or housing allowance of 20% of pensionable salary. UWI contribution of equivalent of 10% of salary to Superannuation Scheme. Subsidized Health Service. Annual Book Allowance Annual Study and Travel Grant for self, spouse and three children. Transportation allowance to persons who own and operate a motor vehicle.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status, and the names and addresses of three referees should be sent as soon as possible to the Campus Registrar, UWI, Mona, Kingston 7, Jamaica, West Indies. Applicants in the UK should also send one copy to the Secretary General, Association of Commonwealth Universities (Appts). 36 Gordon Square, London WC1H 0PF. Further details obtainable from either source. (W1965)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF ZOOLOGY
RESEARCH ASSISTANT (GRADE 1B)

Applications are invited for an SERC-funded Research Assistantship to use DNA "mini-satellite fingerprinting" in studies of the ecology and behaviour of vertebrates.

Applicants should have some laboratory experience of DNA technology and should have or expect to obtain a good honours degree in an appropriate field of Biological Science. The post is for one year, commencing as soon as possible after July 1, 1985. Salary £6,600-£8,920.

Applications, together with a *curriculum vitae* and the names of two academic referees, should be sent as soon as possible to Professor H. C. Macgregor, Department of Zoology, University of Leicester, Leicester LE1 7RH.

Further information can be obtained by phone from Dr T. Burke at 0533-554455 extns. 323 or 115. (6874)A

SCHOOL OF HERBAL MEDICINE — PHYTOTHERAPY
PART-TIME LECTURESHIPS IN PHYSIOLOGY AND PATHOLOGY

Two lecturers are required for one day per week to lecture in physiology and pathology to students studying for the Diploma in Phytotherapy (Dip Phyt). The posts would be of particular relevance to lectures interested in the application of scientific disciplines to the study of phytotherapy

For further details apply to: The Principal; School of Herbal Medicine — Phytotherapy, 145 Forest-Road, Tunbridge Wells, Kent. (6849)A

POSTDOCTORAL MOLECULAR BIOLOGIST

Position available immediately to join active group studying the molecular genetics of RNA splicing in *E coli* Background in RNA processing and/or microbial genetics desirable Experiments conducted in a well-equipped research facility Salary competitive and commensurate with experience

Send curricumum vitae, description of future professional goals and names of three references to

Dr Marlene Belfort
Wadsworth Center for Laboratories and Research
New York State Department of Health
Empire State Plaza
Albany, NY 12201

(NW1892)A

UNIVERSITY OF NOTTINGHAM

LECTURESHIP IN PHYSICS

NMR IMAGING

Applications are invited for a Lectureship in Physics. The post is designed to strengthen research in the applications to medicine and biology of nuclear magnetic resonance imaging techniques The person appointed will work closely with Professor Mansfield on a large project supported by the Medical Research Council and the D H S S Experience in NMR is desirable but not essential. Knowledge of digital-electronics,. computer control, fast data processing, image analysis would also be relevant The lecturer will be expected to contribute to teaching of these as well as traditional physics subjects

Salary will be in the range £7,500 to £14,925 per annum.

Further particulars and application forms, returnable not later than 5 July 1985, may be obtained from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD Ref No. 1001. (6878)A

VIRAL IMMUNOLOGIST

The Lady Davis Institute for Medical Research, in affiliation with McGill University, has a position for an independent investigator at the Assistant Professor level. Candidates should hold an MD or PhD degree and have at least two years of post-doctoral experience.

Please send curriculum vitae, list of publications, a short description of proposed research and names of three referees to Dr N Kalant, Director, Lady Davis Institute for Medical Research, 3755 Cote St, Catherine Road, Montreal, Quebec, Canada H3T 1E2.

In accordance with Canadian Immigration regulations applications from Canadian citizens and landed immigrants will be considered first. (NW1872)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSIOLOGY

Applications are invited for the post of

LECTURER IN PHYSIOLOGY

The Department is particularly seeking a physiologist who is a veterinary graduate. It would, however, also be interested in receiving applications from candidates with a medical degree or a research interest in cardiac muscle. The salary will be within the range £7,520 - £14,925 per annum together with superannuation.

Applications including the names and addresses of two referees, should be sent by 28th June 1985, to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH, from whom further particulars may be obtained. Please quote reference TLJ. (6772)A

MOLECULAR IMMUNOLOGY POST-DOCTORAL SCIENTIST

Salary from £9,000

Celltech, a leader in the field of biotechnology, has achieved its present success in the space of five years. Pioneers in several areas, our achievements are based on the foundations of scientific excellence, innovation and commercial expertise.

Celltech's Molecular Immunology group is expanding its activities in the molecular cloning and expression of immunological targets, principally antibodies. We are looking for a Molecular Biologist/Immunologist, either a post-doc or a graduate expecting soon to finish a PhD. Experience in molecular or cellular immunology would be desirable

We are an ambitious and forward looking company, which places as much emphasis on its people as its products. You will work in a small friendly team, gaining invaluable broad-based experience, with plenty of opportunity for advancement.

Please send a detailed c.v. to Jane Walley, Celltech Ltd., 244–250 Bath Road, Slough, Berks SL1 4DY, quoting Ref. 268.

CELLTECH

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

DIVISION OF IMMUNOLOGY

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT

to join a group working on the immune response to neurotropic viruses. The successful applicant will work on the immune response to Theiler's virus infection — an unusual persistent virus infection of the central nervous system. Candidates should hold or expect to obtain a doctorate in the field of cellular immunity, and have an interest in viral immunology.

The appointment, which is financed by a grant from the Multiple Sclerosis Research Fund is for 3 years.

Age range 24-27, Salary range £7,520–8,450 under review.

Formal applications, including the names and addresses of two referees should be sent to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. Telephone enquiries to Dr A Nash (0223) 64631 (6870)A

UNIVERSITY COLLEGE CORK

RESEARCH POSITIONS IN MICROBIOLOGY/ BIOTECHNOLOGY

POSTDOCTORAL RESEARCH ASSOCIATES (2)

To work on projects involving molecular genetics of plant-microbial interactions —

(a) To investigate genes involved in carbon metabolism in rhizobium (Genetic Engineering):

(b) To investigate the regulation of carbon metabolism in Rhizobium and plant growth promoting pseudomonas SPP (Biochemical-Genetics/ Physiology)

Positions are available from 1 July 1985 and salary will be in the range - IR£8,596 — IR£11,173 (less PRSI contribution) according to experience.

Applications including a cv and the names of two referees should be sent as soon as possible to Dr F O'Gara, Microbiology Department University College, Cork, Ireland, from whom further particulars are available. (6885)A

GREATER GLASGOW HEALTH BOARD

Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ

DUNCAN GUTHRIE INSTITUTE OF MEDICAL GENETICS

PRINCIPAL SCIENTIFIC OFFICER

This is a new fixed term appointment for three years in the first instance made through a grant from New Developments in Health Care in order to develop molecular genetics laboratory services within NHS in Scotland The successful candidate will be expected to help in the development of DNA Analysis for the diagnosis and carrier detection of genetic disease

Candidates should have an honours degree in Genetics, Molecular Biology or related discipline Postgraduate experience in molecular genetics is essential.

Application Form and Job Description available from Personnel Office, Royal Hospital for Sick Children, Yorkhill. Telephone 041 339 8888 extension 517

Closing Date 5 July 1985.

(6833)A

UNIVERSITY OF DUBLIN
Trinity College

DEPARTMENT OF MICROBIOLOGY

RESEARCH FELLOW

Applications are invited for the above post in the Department of Microbiology at Trinity College, Dublin.

The appointment which will be tenable for a period of two years, will involve molecular cloning and site-directed mutagenics of virulence determinants of bovine isolates of **Staphylococcus aureus.**

Salary: IR£8,500 per annum.

Intending candidates should submit a full curriculum vitae, together with the names of two referees to

Dr. T. J. Foster,
Department of Microbiology,
Moyne Institute,
Trinity College, Dublin 2

to arrive not later than **31st July, 1985.** (6887)A

ASSISTANT PROFESSOR

Applicants are being sought to fill an assistant professorial position to work on leishmaniasis in the Department of Medicine, Division of Tropical Medicine, of Harvard Medical School.

Applicants should have expertise in cellular immunology and experience in working with these organisms The person would be expected to establish a vigorous research and teaching program

Applicants should send a curriculum vitae, bibliography, copies of publications, names and addresses of three referees and a summary of both research and teaching objectives to

John R David, M.D.,
Seeley G. Mudd Building
250 Longwood Avenue
Boston, Massachusetts 02115

Harvard is an equal opportunity/ affirmative action employer Applications from women and minorities encouraged (NW1889)A

UNIVERSITY OF KENT
AT CANTERBURY

Biological Laboratory
Readership/Senior Lectureship in Mammalian Biochemistry

Following the election of Dr R B Cain to a Chair in the University of Newcastle, applications are invited for the above post The requirement is for a biochemist with a strong research record who will take a leading role in planning and implementing developments in mammalian biochemistry. The post is offered on a permanent basis and the salary will be on the Reader/Senior Lecturer scale: £14,135 — £17,705 per annum.

Forms of application and further particulars of the post may be obtained from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, CANTERBURY, Kent CT2 7NH.

Closing date 19 July 1985. Please quote ref A42/85/N (6899)A

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for an SERC funded post to work on the mechanisms by which globular proteins fold into their active state. Spectroscopic, scattering, hydrodynamic and monoclonal antibody techniques will be used to study naturally occurring, mutant and recombinant proteins The appointment is for three years tenable from September 1985 or soon after

Salary will be up to £7,980 per annum on the Range 1A salary scale: £7,520 — £12,150 per annum according to age, qualifications and experience.

Candidates with PhD or expecting to have gained a PhD by the autumn should send applications with curriculum vitae and the names and addresses of three referees to Professor R H Pain, Department of Biochemistry, University of Newcastle upon Tyne, NE1 7RU by July 18th. (Telephone 0632 328511 Ext 3437). (6891)A

UNIVERSITY OF BRISTOL
Tsetse Research Laboratory, Langford
ENTOMOLOGIST

Applications are invited for a three year postdoctoral appointment funded by the Overseas Development Administration at the Tsetse Research Laboratory, Langford (12 miles south of Bristol) The successful candidate will be expected to undertake laboratory research into the sexual behaviour of tsetse flies in relation to visual and chemical stimuli with a view to improving trapping techniques in the field

Considerable automation of data collection is anticipated and interest/ experience in electronics and microcomputers will be an advantage Position available from 1st October, 1985 Salary on 1A Scale within range £7,520 — £8,920 according to age.

Reply, with curriculum vitae and the names of two referees, by 31st July 1985 to Dr P A Langley, Tsetse Research Laboratory, Langford House, Langford, Bristol, BS18 7DU (6846)A

BIRKBECK COLLEGE
(University of London)
POSTDOCTORAL RESEARCH ASSISTANT
LABORATORY OF MOLECULAR BIOLOGY, DEPARTMENT OF CRYSTALLOGRAPHY

Applications are invited to work on a project funded jointly by the SERC and Pfizer UK ltd, to study cAMP phosphodiesterase enzymes from heart muscle as a basis for rational drug design The project will involve protein purification, microsequencing, cDNA preparation and computer graphics modelling The applicants should have experience in at least two of these areas The post is available immediately

Salary on the RA1A scale for research staff (£7,520 — £12,150 + £1,233 pa London Allowance)

Applications including curriculum vitae and the names of two referees should be sent to the Assistant Secretary (Personnel), (PRC), (N), Birkbeck College, Malet Street, London WC1E 7HX by 30th June 1985. Enquiries to Professor T L Blundell, FRS, at the above address or on 01-580 6622 ext: 284. (6883)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
Keppel Street, WC1E 7HT.
PREVENTIVE TERATOLOGY UNIT

Applications are invited for a
MEDICAL LABORATORY SCIENTIFIC OFFICER IN BIOCHEMICAL TERATOLOGY

to work on congenital malformations associated with environmental agents

The appointment is for 14 months at a salary, depending on age, experience and qualifications, on a scale which rises to £6,726 plus £1,042 London Weighting per annum

Applications, stating qualification and experience and the names of two referees, should be sent to the Assistant Personnel Officer at the School, quoting reference (N1). Closing date July 10th 1985. (6855)A

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF BACTERIOLOGY AND VIROLOGY
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for this post to work on the obligate intracellular prokaryotic, Chlamydia trachomatis. This MRC funded study involves an investigation of intracytoplasmic events related to persistence of infection within cultured eukaryotic cells. Applicants with training in either cell biology or microbiology will be considered; experience of cell culture techniques is highly desirable Initial salary range £7,520 — £8,450 pa Superannuation Position available from October 1985 for 3 years Enquiries for further details, and applications including CV and names of 2 academic referees, to Dr Shirley Richmond, Department of Bacteriology and Virology, The University, Manchester M13 9PT (6892)A

MONASH UNIVERSITY
Melbourne, Australia
CHAIR OF EARTH SCIENCES

Applications are invited for the position of Professor in the Department of Earth Sciences An innovative scientist with a strong research reputation is sought to guide future development

Established in 1972 Earth Sciences has gained recognition for the quality of its graduates and its research With ten academic and eight technical staff, it covers a wide range of teaching and research areas The Department is well equipped for experimental and analytical studies and has strong field-based interests. Involved in joint research with other university departments, industry, and government agencies, it attracts funding from both Australian and overseas granting bodies The international postgraduate research group includes six post-doctoral workers, thirty PhD and ten MSc students

Areas covered by the Department's current activities include igneous, metamorphic, and are petrology/geochemistry, structural geology, sedimentology and volcanology palaeoclimatology, palaeontology, isotope and solution geochemistry, regional geology, and tectonics. A geophysics teaching and research program will commence shortly with the appointment of two academic staff Workers in the above fields, or in any of the more quantitative aspects of the earth sciences, will find a stimulating research environment, but applications from those with other interests will be considered The successful applicant must have outstanding leadership qualities and the ability to interact positively with industry

Enquiries about the Department may be directed to Dr Ian Nicholls, Acting Chairman

Salary, $A54,948 pa. Superannuation, travel and removal allowance, and temporary housing assistance.

Information on application procedure and further particulars may be obtained from the Registrar, Monash University, Clayton, Victoria 3168, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF

Applications should reach the Registrar not later than 23 August 1985 Council reserves the right to make no appointment or to appoint by invitation at any stage

An equal opportunity employer (W1960)A

Please mention
nature
when replying to these advertisements

POSTDOCTORAL POSITION

available immediately in research-intensive department for Ph.D. in biochemistry, cell or molecular biology, or immunology Research deals with the mechanism of action at the receptor and postreceptor level of polypeptide growth factors that induce cellular transformation and differentiation.

Experience in the following areas is desirable: Receptor biochemistry; hybridoma technology, protein and peptide purification, hormonal regulation of gene expression

Send curriculum vitae and names of three references to.

Dr J. Massague
Department of Biochemistry
University of Massachusetts Medical School
55 Lake Avenue North
Worcester, Massachusetts 01605

An Equal Opportunity/Affirmative Action Employer. (NW1891)A

RESEARCH POSITIONS

1) Postdoctoral Associate and 2) Research Assistant (BS or MS) positions available in a research group working with RIBOSOMAL PROTEIN GENES AND cDNA EXPRESSION LIBRARY from higher plants DNA cloning and/or sequencing experience required. Write to: Dr Alap Subramanian, Group Leader, Max-Planck-Institut fur Molekulare Genetik, Abt Wittmann, Ihnestr 63, Berlin-Dahlem, Fed Rep Germany. (W1933)A

GRANTS & SCHOLARSHIPS

IMPERIAL CANCER RESEARCH FUND

BURSARY FOR TRAINING IN RESEARCH

Applicants are invited for an ICRF Bursarship tenable for 3 years from October 1985 for full time students leading to a higher degree in biochemistry. The bursarship offers the opportunity to join an active cell membrane research group in the Department of Biochemistry, at the Royal College of Surgeons, in collaboration with the Cell Surface Biochemistry Laboratory, ICRF

The research topic, which will be jointly supervised by Professor N Crawford and Dr M Crumpton (ICRF), involves investigating procedures for permeabilising blood cells and their use as carrier vehicles with encapsulated drugs and other agents which activates macrophages for sustained tumourcidal effects

Applications are invited from students, no more than 25 years of age on October 1st who hold, or expect to obtain a First or Upper Second Class Honours degree in Biochemistry or Cell Biology. The award carries a grant of £4,865 with possible additional allowances.

Further details and application forms can be obtained from: Miss S M Hurley, Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PN.

Please quote reference number: 85/85.

The forms should be completed and returned by 31st July 1985. (6904)H

ASSISTANTSHIPS

UNIVERSITY OF CAMBRIDGE

UNIVERSITY CHEMICAL LABORATORY

POSTDOCTORAL ASSISTANTSHIP

Applications are invited from molecular biologists for work on the cloning and expression of the genes for the key enzymes on the biosynthetic pathways to haem, vitamin B_{12} and related macrocycles This will be in collaboration with Dr C Abell and Professor A R Battersby

The appointment from a mutually agreed date in 1985 is for up to 2 years at a starting salary within the Scale £7520 (for 24 years of age) to £8920 (for 27 years of age) with an increment after one year. Applications with curriculum vitae and 2 referees' names should be sent as soon as possible to Professor A R Battersby, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW (6812)P

UNIVERSITY OF ABERDEEN

DEPARTMENT OF MICROBIOLOGY

POSTGRADUATE RESEARCH ASSISTANT

MOLECULAR MECHANISMS OF POTASSIUM TRANSPORT IN ESCHERICHIA COLI

The above SERC-funded Research Assistantship is offered for three years in the laboratory of Dr Ian Booth. The project will involve the use of advanced genetic techniques and gene cloning to study the mechanisms of regulations of potassium accumulation in E coli.

Salary on Range IB scale for Research and Analogous Staff £6,600 per annum, (scale under review).

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 11 July 1985 (Ref No ED/006). (6888)P

SEMINARS & SYMPOSIA

AN INTERNATIONAL SYMPOSIUM ON PULMONARY EMPHYSEMA AND PROTEOLYSIS

14 to 16 January 1986
Pasadena, California

State-of-the-art presentations will be given on the protease-antiprotease balance in the lung, oxidant-antioxidant interactions and their influence on proteases, cigarette smoke components, tissue injury and inflammation, therapeutic approaches to emphysema, and genetic polymorphisms and deficiencies and their effect on disease. A full symposium volume will be published.

Attendance is limited. For more information and application, please write to: Charles Mittman, M.D., City of Hope Medical Center, 1500 East Duarte Road, Duarte, Calif. 91010.

(NW1876)M

FIRST ANNOUNCEMENT

4th International Symposium on the Biology of the

'VASCULAR ENDOTHELIAL CELL'

Noordwijkerhout, The Netherlands, 19-23 August 1986

This meeting is the fourth in a series, which began with a symposium at Cold Spring Harbor in May 1980. It will be organised in co-operation with the Netherlands Organization for Applied Scientific Research (TNO).

Chairman: P. Brakman, Ph. de Groot, V. W M. van Hinsbergh, W J C. Melgert, J. A van Mourik

In conjuction with the symposium a display of industrial firms will be organized.

Papers can be presented either by post or orally.

For information write to

TNO Corporate Communication Department, P.O. Box 297, 2501 BD DEN HAAG, The Netherlands (W1963)M

UNIVERSITY OF ABERDEEN

DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE

RESEARCH ASSISTANTSHIP

THE INFLUENCE OF DIETARY POLYPHENOLS ON THE FIBRINOLYTIC OF MAN

Applications are invited for the post of Research Assistant to work with Professor D Ogston and Dr S B Wilson. The project will involve an assessment of the influence of polyphenols present in food and beverages on the fibrinolytic system of man and an identification of the compounds concerned. The post has been funded by the Scottish Home and Health Department for two years Applicants should have an Honours degree in Biochemistry or a related topic and may register for a higher degree if so desired

Salary within range £6,600-£7,980 per annum on the Range 1B Scale for Research and Analogous Staff with appropriate placing (scale under review)

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 12 July 1985 (Ref No EL/008). (6902)P

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANTSHIP

tenable for two years in the first instance at the Post-Graduate/Post-Doctoral level to work in collaboration with Dr C A B Jahoda and Dr R F Oliver on a skin biology project with applied potential.

We are seeking a biochemist with interest or experience in growth factors to study the control of cell proliferation in hair growth (ref. Nature, 311, 560-62, 1984).

Salary will be dependent of qualifications, age and experience within the range £6,600 — £8,920 per annum (pay award pending).

Applications containing a full curriculum vitae and the names and addresses of 3 referees should be sent as soon as possible to the Personnel Officer, The University of Dundee, Dundee, DD1 4HN. Please quote reference EST/60/85(J) (6843)P

continued on page 18

FELLOWSHIPS

ALL SOULS COLLEGE
RESEARCH FELLOWSHIPS

All Souls College intends to make elections to two Research Fellowships in the course of the academic year 1985-6.

The Research Fellowships are open to men and women whose work lies in following fields of study: Law, History, Philosophy, Politics, Economics, English Language and Literature, Classical Studies, Mathematical Sciences. The College would not normally elect to a Research Fellowship any person who will have reached the age of 40 on 1st March 1986.

The Research Fellowships will be tenable for a period of seven years in the first instance, and may be extended for a further period of not more than three years. On the expiry of the Research Fellowship, a Research Fellow who produces evidence of satisfactory achievement may apply to be considered for election to a Senior Research Fellowship.

Further particulars, including details of stipend and terms of appointment, and application forms, may be obtained from the Warden's Secretary, All Souls College, Oxford. Applications, on the application form, must reach the Warden not later than 19th October, 1985. (The envelope containing the application should be marked "Research Fellowship".) Applicants must ensure that references, from not more than three referees, also reach the Warden by 19th October 1985. (6873)E

BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY
FELLOWSHIP

The British Journal of Experimental Pathology is offering a Fellowship in the field of Experimental Pathology, to be awarded competitively at the end of 11985 and to be held during the years 1986, 87 and 88 The objective is to enable younger academic pathologists, whether medically qualified or not, to be freed from service, teaching and administrative duties fsor a period of three years Preference will be given to applicants under the age of 35 years The Fellowship carries a stipend commencing at the bottom of the Lecturer scale and an allowance for research expenses will also be made

The closing date is 8 August 1985 Further particulars may be obtained from

The Secretary to The British Journal of Experimental Pathology
Professor N Woolf
c/o The Bland-Sutton Institute of Pathology
The Middlesex Hospital Medical School
London W1.

The Fellowship is confined to applicants from institutes within the United Kingdom (6869)E

EMBO
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
Autumn 1985 — Awards
Next deadline: 15 August 1985

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1985. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 21 October 1985.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R Germany. (W1893)E

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

POST DOCTORAL FELLOWSHIP

Applications are invited for a Research Fellowship funded by the Medical Research Council to study recognition signals in adenovirus replication. The applicant should prefereably have some experience in either cloning or hybridoma technology and will become part of the Molecular Virology Group in the Department. The project will be under the supervision of Professor W C Russell from whom further information may be obtained (Telephone 0334 76161 ext 295).

The appointment will be for 3 years, starting as soon as practicable. Starting salary at appropriate point within range £7,520 to £10,330 per annum on the 1A Research scale, plus superannuation.

Applications (two copies preferably in typescript) with the names of two referees should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ to arrive not later than 2nd July 1985 (6813)E

UNIVERSITY OF BERN
POST-DOCTORAL FELLOWSHIP
YEAST MOLECULAR GENETICS

This position opens on 1st October 1985 for a duration of two years with possible extension The holder of this position is expected to develop his own project in the frame of our group working on genetic recombination in S pombe Funding is from the Swiss National Science Foundation

Please send curriculum vitae, publication list and the names of two persons for references to Dr J Kohli, Institute of General Microbiology, Baltzerstr 4, CH-3012 Bern, Switzerland. (W1956)E

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
POST-DOCTORAL FELLOWSHIP (MRC) IN HUMAN PHYSIOLOGY

The appointed person will join a small group under the leadership of Dr C T M Davies working on the physiology and biochemistry of elderly human muscles within the Department of Physiology and Pharmacology.

Post-graduates with appropriate experience of physiology interested in this Fellowship should write in the first instance to Professor P H Fentem, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, enclosing a curriculum vitae and the names of two referees (6895)E

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

JUNIOR RESEARCH FELLOWSHIP

Applications are invited from Graduates or students obtaining a good honours degree this summer in Biochemistry or related subjects for a 3 year Junior Research Fellowship. The successful candidate will register for a higher degree in the University of London. The project concerns the Biochemistry of blood platelet activation. There will also be an opportunity to apply biochemical techniques to an investigation of abnormal platelet behaviour.

A curriculum vitae and the names of 2 academic referees should be sent to Dr K R Bruckdorfer, Department of Biochemistry and Chemistry, RFHSM, Rowland Hill Street, London NW3 2PF, as soon as possible

Further details are available on request. (6896)E

MERTON COLLEGE, OXFORD
JUNIOR RESEARCH FELLOWSHIPS AND SENIOR SCHOLARSHIPS 1986

The college proposes, if candidates of sufficient merit present themselves, to elect to two or three Junior Research Fellowships and to two or three Senior Scholarships in 1986. These awards are open to both men and women Candidates for Junior Research Fellowships must be under the age of 27 on 1 October 1986, candidates for Senior Scholarships must be under the age of 26, or, in the case of Oxford graduates, must not have exceeded 18 terms from the date of matriculation

Details of the awards and of the method of applying for them may be obtained from the Warden's secretary, Merton College, Oxford, OX1 4JD. The closing date for applications is Thursday 17 October 1985 (6842)E

POSTDOCTORAL FELLOWSHIP

available in immunology

Current emphasis is the study of the scrapie prion protein Techniques will include chemical and recombinant DNA synthesis of peptides, production of monoclonal antibodies, and protein immunochemistry.
Candidates must have a Ph D , M.D , or D V.M. degree and be a U S. citizen or a permanent resident
Send curriculum vitae and three letters of reference to.

Dr Stanley Prusiner,
Department of Neurology,
HSE 781,
University of California,
San Francisco,
California 94143.

The University of California is an Affirmative Action/Equal Opportunity Employer (NW1879)E

The John Hopkins University
Department of Anesthesiology and Critical Care Medicine

POST DOCTORAL FELLOWSHIP IN RESEARCH

Applications are invited for this position available immediately to study the molecular biology of human cytochrome P450. The applicant must be creative and highly motivated to carry out independent experimentation utilizing human cytochrome P450 under study.

M D or Ph D. required with 1–2 years experience in molecular biology.

Contact: Roy C. Levitt, M.D., The John Hopkins University, Department of Anesthesiology and Critical Care Medicine, Blalock 1410, Baltimore, Maryland 21205. Telephone (301) 955-7609. (NW1822)E

CANOLFAN ASTUDIAETHAU TIROEDD CRAS
COLEG PRIFYSGOL GOGLEDD CYMRU
UNIVERSITY COLLEGE OF NORTH WALES
CENTRE FOR ARID ZONE STUDIES
THE ROBERTSON FELLOWSHIP

Applications are invited for a Fellowship, initially for one year, commencing September/October, 1985 from scientists with an interest in General Agriculture and especially in Animal Husbandry of arid and semi-arid areas.

The successful applicant will contribute to the teaching of postgraduate and undergraduate courses and participate in the development of the Centre, including work on overseas contracts.

A basic salary in the region of £12,500 pa will be offered, but may be supplemented by other earnings.

Further details from: Professor R G Wyn Jones, Centre for Arid Zone Studies, University College of North Wales, Bangor, Gwynedd.

Applications (two copies), giving details of age, education and experience, together with the names and addresses of two referees, should be sent to the Personnel Office, University College of North Wales, Bangor, Gwynedd. LL57 2DG. (6860)E

nature classified

NEW YORK OFFICE
Garth Walkes
65 Bleecker Street
New York, NW 10012
Tel: (212) 477-9600

TORONTO OFFICE
Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1L1
Tel. (416) 690-2423

LONDON OFFICE
4 Little Essex Street
London WC2R 3LF
Tel 01 240 1101
Telex 262024

STUDENTSHIPS

UWIST
University of Wales

APPLIED CHEMISTRY
RESEARCH STUDENTSHIPS IN BIO-INORGANIC CHEMISTRY
(Fixed-term 3 years)

To work in the field of chemical speciation modelling. Research areas include the speciation and bioavailability of trace metal ions in food stuffs, the interaction of pharmaceutical agents with metal ions and the chemical speciation of radioactive waste disposal systems.
Details from Professor David R. Williams, Department of Applied Chemistry, UWIST, PO Box 13, Cardiff CF1 3XF

RESEARCH ASSOCIATES
(fixed-term 2 years)

Chemical modelling of trace metal ions in natural waters, including the chemical speciation of radioelements in disposal systems.
Salary. Within Range 1A Research and Analogous Staff.
£7,520-£12,150 per annum
Requests (quoting Ref C72) for details and application form to Staffing Office, UWIST, PO Box 68, Cardiff CF1 3XA.
Closing Date: 25 July 1985.
(6837)F

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place, London W2 1PG
SERC CASE STUDENTSHIP

Applications are invited for a SERC Case Studentship in collaboration with Shell Research Limited (Dr D H Hutson) for research (leading to a PhD degree) into the effect of environmental conditions on the metabolism of chemicals by fish.

Applicants should have, or expect ot obtain, a first or second class honours degree in biochemistry, or chemistry, and have an interest in chemical and environmental toxicology.

Applications, including the names and addresses of two referees, should be sent to the Assistant Secretary (Personnel) at the above address by 3 July 1985. For further details of the studentship contact Dr P Millburn tel no: (01) 723-1252 ext 118 (6907)F

ROYAL HOLLOWAY AND BEDFORD COLLEGES
(University of London)
DEPARTMENT OF BIOCHEMISTRY
SERC/CASE STUDENTSHIP IN HERBICIDE BIOCHEMISTRY

Research studentship available in October 1985 to study the biochemical basis of herbicidal action of bleaching nitrodipheny-lethers, in collaboration with Shell Research Ltd. The project will involve the use of protein affinity labelling in purified plant membrane fractions. Candidates should expect to have a First or Upper Second Class honours degree in Biochemistry or a related subject.

Applications, including cv and the names of two referees, should be sent to Dr J R Bowyer, Department of Biochemistry, Royal Holloway and Bedford Colleges, Egham Hill, Egham, Surrey TW20 0EX (Tel: 0784 34455, ext: 259a) (6879)F

THE UNIVERSITY OF SHEFFIELD
RESEARCH STUDENTSHIPS IN PHYSICS AND GEOPHYSICS

leading to the degree of PhD, are available in the following areas.
Condensed Matter Physics
Metallic Glasses
Amorphous Semiconductors
Magnetism and Resonance Studies
Isotope Geophysics
Space Geophysics
Theoretical Physics

Applications are invited from graduates with first or upper second class degrees in Physics, Chemistry, Geology or Engineering
For further details please contact Professor I.G. Austin, Physics Department, Sheffield University, Sheffield S3 7RH. Telephone (0742) 78555 Ext 4351. Quote ref R300/G. (6906)F

UNIVERSITY OF DURHAM
SERC CASE RESEARCH STUDENTSHIP
The Mode of Action of a New Chemical Hybridising Agent.

Graduates in Plant Sciences, or other suitably qualified applicants, are invited to apply for the above studentship leading to the degree of PhD. The work will be carried out in collaboration with Shell Research Ltd, Sittingbourne, Kent.

The project will be centred upon a combined biochemical and biophysiological study of pollen development and behaviour in a variety of species, as modified by a new chemical hybridising agent. The work will involve autoradiography, microscopy (using newly developed epifluorescent techniques), amino acid and protein analysis

Enquiries are invited to Dr P Gates, Department of Botany, Science Laboratories, South Road, Durham, DH1 3LE (tel. 0385 64971), from whom further particulars may be obtained (6910)F

UNIVERSITY OF ABERDEEN
DEPARTMENT OF ENGINEERING
A MAJOR IMPACT IN ELECTRONICS

The University of Aberdeen, as part of a major new initiative in Electronics, is looking for research students for the following SERC CASE awards Commencing October 1985.

Knowledge Representation Using Massively Parallel Processors

in collaboration with the Admiralty Research Establishment (Portsmouth, UK) and Carnegie Mellon University (Pittsburg, USA)

A Reconfigurable, Fault Tolerant Network using Spread Spectrum Code Division Multiple Access Techniques

in collaboration with the Admiralty Research Establishment (Portsmouth, UK) and Netek Corporation (Chelmsford, Massachusetts, USA).

A methodology for the Design of Digital Systems

in collaboration with Fairchild Semiconductor (Reading, UK) and the Knowledge-Based VLSI Design Group at Xerox PARC (Palo Alto, USA)

High Level Applications Support for VLSI Systems

in collaboration with Fairchild Semiconductor (Reading, UK)

Control Protocols for a Packet Radio System

in collaboration with the Royal Signals & Radar Establishment (Malvern, UK)

Further particulars may be obtained from Professor C T Spracklen, Department of Engineering, University of Aberdeen, Natural Philosophy Building, King's College, Aberdeen. AB9 2UE (6908)F

UNIVERSITY OF READING
SERC-CASE STUDENTSHIP

Applications are invited for an SERC-CASE Studentship, in co-operation with IQ(Bio) Ltd of Cambridge, tenable for 3 years from October 1985 in the Department of Microbiology Applicants must have, or expect to receive this summer, a 1 or 2i honours degree in biochemistry or a closely related subject and have a particular interest in enzymology The successful applicant will investigate novel ultrasensitive enzyme-labelling systems for enzyme-linked immunosorbent assays (ELISA) and will register for a higher degree A minimum of 3 months will be spent at the IQ(Bio) Research Laboratories during the 3 years The value of the Studentship is £3,335 pa tax free (under review), plus University fees

Applications with full curriculum vitae including a telephone number and two academic referees should be sent as soon as possible to Dr L J Zatman, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ (6852)F

Southampton THE UNIVERSITY

INSTITUTE OF CRYOGENICS
CASE RESEARCH STUDENTSHIP

Applications are invited for an SERC/British Gas CASE studentship to study instabilities in cryogenic liquids using LDA, microthermometer and flow visualisation techniques

Apply to
Professor R.G. Scurlock
Institute of Cryogenics
University of Southampton
Southampton SO9 5NH
(6893)F

RESEARCH STUDENTSHIP

Applications are invited for an MRC research studentship, tenable in the MRC Cell Biophysics Unit at King's College London, for work in one of the following areas: Muscle structure on cross-bridge kinetics; cellular developmental neurobiology; cell movement; structure of the cyto skeleton

Applicants should send a copy of their curriculum vitae, and ask two academic referees each to forward an assessment, as soon as possible to Mr D M Drummie, King's College London, 26-29 Drury Lane, London WC2B 5RL (6890)F

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF BOTANY

SERC-CASE STUDENTSHIP NITRATE REDUCTION IN PLASTIDS OF HIGHER PLANT ROOTS

Applications are invited from graduates and final-year undergraduates for a 3 year studentship to be supervised by Dr M J Emes (Department of Botany, University of Manchester) and Dr D P Hucklesby (Long Ashton Research Station, Bristol) The aim of the project is to identify and characterise the natural electron donor for nitrite reductase in roots, and the person appointed will receive training in the techniques of protein purification, immunobiochemistry, and organelle isolation Applicants should have, or expect to obtain, a First or Upper Second class Honours degree in Biochemistry, Biology or Botany and have an interest in plant metabolism and enzymology

Applications containing a curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr M J Emes, Department of Botany, The University, Manchester M13 9PL (6838)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MICROBIOLOGY

Applications are invited for a

POSTGRADUATE RESEARCH STUDENTSHIP

The research project will involve investigation of the molecular genetic and physiological basis of virulence in pathogenic Neisseria.

The studentship is funded by MRC and is for three years, starting on 1st October, 1985 Applicants should have a First or Upper Second Class Degree in Microbiology, Biochemistry, Genetics or a related discipline.

Informal enquiries may be made to Dr J R Saunders, Tel: 051-709 6022 Ext: 3159.

Applications together with the names of two referees should be received as soon as possible by The Registrar, The University, PO Box 147 Liverpool, L69 3BX

Quote Ref. RV/117/N. (6867)F

ROBERT GORDON'S INSTITUTE OF TECHNOLOGY, ABERDEEN

SCHOOL OF PHARMACY RESEARCH STUDENTSHIP

Applicants with 1st or 2nd Class Honours Degree in Pharmacy or Pharmacology to study the mechanisms of action of possible antithrombotic drugs

Project funded by the British Technology Group and supervised by Dr G M Smith

Application forms from: The Personnel Department, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen, AB9 1FR (6866)F

UNIVERSITY OF LEICESTER

Department of Genetics

Applications are invited for a SERC research studentship in Genetics tenable for 3 years from October 1985. The award is 'earmarked' by SERC for research in the genetic analysis of Drosophila behaviour Applicants should have or expect to have a degree (1st class or 2/1) in a Biological subject and have an interest in molecular, genetic or evolutionary aspects of behaviour.

The Department is well equipped for research in genetics and has excellent facilities for molecular biology

Enquiries and applications may be made to Dr C P Kyriacou, Department of Genetics, Adrian Building, University of Leicester, Leicester LE1 7RH (6811)F

UNIVERSITY OF LEICESTER

SCHOOL OF BIOLOGICAL SCIENCES

(BOTANY DEPARTMENT)

SERC — CASE STUDENTSHIP (ICI Corporate Biosciences Group)

An SERC-CASE Studentship in collaboration with Dr W Schuch of the ICI Corporate Biosciences Group is available to work on a project concerned with the molecular biology of phytochtome action in light-grown plants, with particular respect to the control of growth and lignification in crop plants experiencing mutual shading. The work will involve the use of cDNA probes to estimate PAL mRNA levels and to measure specific transcription rates.

Applicants having, or expecting to achieve, at least an Upper Second Class Honours Degree in an appropriate subject should submit a curriculum vitae, with the names of two referees, as soon as possible to Professor H Smith, Department of Botany, University of Leicester, University Road, Leicester. (6863)F

UNIVERSITY OF BRISTOL

DEPARTMENT OF BOTANY

Applications are invited for an

SERC RESEARCH STUDENTSHIP

commencing October 1985 to work in one of the following areas of plant science: physiology/biochemistry, molecular genetics, ultastucture, mycology or phycology. Candidates should possess or expect to obtain at least an upper second class honours degree in a Biological Science or related subject.

Applications, which should include a cv and the names of two referees should be sent as soon as possible to Professor A E Walsby, Department of Botany, University of Bristol, BS8 1UG. (6897)F

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

SERC STUDENTSHIP

October 1985

Applications are invited for a studentship expected to lead to a Ph.D. degree for research on inherited biochemical lesions resulting in ketoacidosis in humans. The work will involve cultured skin fibroblasts from affected and unaffected individuals.

Applicants will need a first or upper second class honours degree in Biochemistry or a closely related subject

Applications including curriculum vitae and names of two academic referees to Dr B. Middleton, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH (6877)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF PHARMACOLOGY

MRC RESEARCH STUDENTSHIP

Applications are invited for MPhil/PhD work on electrophysiological investigations (particularly single ion channel recording) in peripheral ganglion cells and/or CNS neurons

Send CV and names of two referees to Professor D Colquhoun, Dept of Pharmacology, University College London, Gower Street, London WC1E 6BT. (6882)F

UNIVERSITY OF OXFORD

Dyson Perrins Laboratory (Organic Chemistry)

SERC POSTDOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited for postdoctoral research assistantships to work on the Isolation, Characterisation and Modification of Beta-Lactam Biosynthesis Enzymes from Cephalosporium and Streptomyces One post will involve enzyme isolation, kinetics, enzymatic synthesis of novel Beta-lactam antibiotics etc and the other one will involve the application of Recombinant DNA techniques. Candidates with experience of enzymology, molecular biology or bioorganic chemistry will be preferred The appointments are on the RA1A scale with starting salary up to £8,920 pa plus USS according to age and experience and would be initially for one year from October 1985 with the possibility of extension

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Prof J E Baldwin, Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY (6848)P

CITY OF LONDON POLYTECHNIC

Post-Doctoral Research Assistantship in Dendrochronology

Applications are invited for an SERC-funded post-doctoral Research Assistantship to be held jointly by the City of London Polytechnic and The Queen's University, Belfast. The successful applicant, who will be required to work for two years on the dating of buildings in north-west France by dendrochronology, will be based largely in London, but must expect to spend some time in Belfast and significant periods each year in the field in France. Previous experience of dendrochronology work is highly desirable Starting salary: £8,586 pa including London Allowance (pay review pending)

Further information and an application form may be obtained from Professor G I Meirion-Jones, Department of Geography, City of London Polytechnic, Old Castle Street, London E1 7NT, tel. 01-283 1030 ext 464. (6880)P

UNIVERSITY OF SOUTHAMPTON

Clinical Pharmacology/ Medicine 1

SERC (CASE) STUDENTSHIPS

Applications are invited from good honours graduates in pharmacology, biochemistry or physiology for the following SERC (CASE) studentships to study:

1. The biochemical pharmacology of dispersed human skin mast cells (in collaboration with Roussel Laboratories Ltd)

2 The development of bronchial hyper-reactivity in the guinea-pig (in collaboration with Roussel Laboratories Ltd)

3 The oxidative metabolism of arachidonic acid in human and primate mast cells (in collaboration with Fisons Pharmaceuticals PLC).

The studentships are available for 3 years from October 1st 1985 and the successful applicants are expected to register for a higher degree. Further information and applications, including curriculum vitae and the names of two referees to: Dr M K Church, Clinical Pharmacology, Southampton General Hospital, Southampton, SO9 4XY. Telephone 0703-777222 ext. 4312. Applications close June 30th 1985. Please quote reference No AJS/85/40/NA. (6836)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GENETICS

NERC RESEARCH STUDENTSHIP

Two studentships are available from October 1985 to work on the following projects

Dr A B Tomsett / Dr D A Thurman: Genetics of heavy metal tolerance in Aspergillus nidulans. This project aims to isolate resistant mutants from laboratory strains, to determine the genetic and physiological basis of these tolerances and to compare them with other fungal species isolated from metal-contaminated environments.

Prof D A Ritchie/Dr P Strike: The distribution of heavy metal detoxification genes in aquatic bacteria. This project aims to use previously cloned mercury and tellurium resistance genes as probes to investigate the presence of similar genes in a wide variety of bacteria from habitats around the River Mersey and to identify new genetic elements associated with these resistances Contact Dr P Strike for further details (051 709 6022 ext 3113).

Applicants should have, or expect to obtain, a 2.1 or 1st class Honours degree in Genetics, Microbiology, Biochemistry or Molecular Biology. Applications, including a CV and the names and addresses of two referees, should be sent as soon as possible to The Registrar, The University, PO Box 147, Liverpool, L69 3BX

Quote Ref: RV/114/N. (6845)F

UNIVERSITY COLLEGE LONDON

MRC NEUROIMMUNOLOGY PROGRAMME and ICRF TUMOUR IMMUNOLOGY UNIT

Department of Zoology

TWO RESEARCH STUDENSHIPS

Applications are invited for the above studentships to study

a) the molecular basis of cell-cell interactions in the development of the peripheral nervous system using monoclonal antibodies and cell culture. The MRC group comprises approx. 15 scientists and research students working together in the field of developmental neurobiology.

b) cell surface molecules involved in the activities of B lymphocytes using monoclonal antibody and molecular techniques. The ICRF group comprises approx 25 scientists and research students working in a wide range of immunological areas.

Candidates should hold or expect to receive a first or upper second class honours degree.

Applications should include a C.V. and the names of at least two academic referees and be sent to:

a) Dr A. W. Mudge and
b) Dr M. J. H. Ratcliffe
Dept. of Zoology
University College London
Gower Street
London WC1E 6BT

(6871)F

UNIVERSITY OF WALES COLLEGE OF MEDICINE

DEPARTMENT OF PATHOLOGY

PhD STUDENTSHIP (CRC FUNDED)

Applications are invited from graduates holding or expecting to obtain a 2(i) degree or higher in biochemistry, molecular biology, or related subjects, to work for 3 years on the molecular basis of thyroid cancer, using both an existing experimental model and human thyroid tumours.

The successful candidate will join an established group funded largely by the Cancer Research Campaign, and will gain experience in a wide range of recombinant DNA techniques, including gene transfer.

This post is available from September 1st. Further information can be obtained from Drs D or V Wynford-Thomas (Tel No. 0222/ 755944 Ext 3440)

Applications, in the form of a curriculum vitae with the names and addresses of two referees, should be submitted to the Registrar and Secretary, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN (quoting Ref No P1/47) within two weeks of the appearance of this advertisement (6898)F

Teesside Polytechnic

Department of Chemistry

Department of Chemical Engineering

(NORTH EAST BIOTECHNOLOGY CENTRE)

SERC CASE STUDENTSHIP

A SERC Case Studentship is available to commence in September 1985. The overall aim of the project is the design of an industrial process for the selective hydrolysis of natural plant lipids using lipase. The award is in collaboration with *Croda Universal Ltd.* who will enhance the grant payable to the successful applicant.

Candidates should hold, or expect to gain a good Honours degree in Chemical/Biochemical Engineering or Biotechnology.

For further details and application forms please write to:

Graham Street, NEBC, Department of Chemical Engineering Teesside Polytechnic, Borough Road, Middlesbrough, Cleveland. TS1 3BA. Telephone: (0642) 218121 ext. 4284 within two weeks of the appearance of this advertisement. (6850)F

LIVERPOOL POLYTECHNIC

School of Pharmacy

CASE STUDENTSHIP

STRUCTURE-ACTIVITY STUDIES ON ALPHA-BLOCKING AGENTS

Applications are invited for a CASE studentship, in collaboration with Reckitt and Colman Pharmaceutical Division, for research into the relationships between molecular structure and pharmacological activity in a series of alpha-adrenoceptor antogonists. Applicants should hold, or expect to gain this year, at least an upper second class honours degree in an appropriate discipline

Please write giving full details to

Dr J. C. Dearden, School of Pharmacy, Liverpool Polytechnic, Byrom Street, LIVERPOOL L3 3AF (Tel 051 207 3581 Ext. 2066).

Liverpool Polytechnic is an Equal Opportunity Employer and welcomes applications irrespective of race, sex, marital status or disability (6876)F

UMIST

NEUROBIOLOGY/ NEUROCHEMISTRY

Applications are invited for a PhD CASE Studentship (sponsors SERC and Dow Chemical Co) to work on the action of neuronal 5 hydroxytryptamine in the insect nervous system The project will involve electrophysiological techniques in the locust (in particular intracellular recording) neuroanatomical techniques, pharmacology and possibly biochemical analysis. Applicants must be of at least upper second class quality.

For further details write to Dr N M Tyrer, Department of Biochemistry and Applied Molecular Biology, UMIST, PO Box 88, Manchester M6O 1QD. Please quote reference BIO/S13. (6905)F

Southampton THE UNIVERSITY

Department of Chemistry

Applications are invited from students holding, or expecting to hold, a good Honours Degree in Chemistry for a

STUDENTSHIP

funded by ICI Organics Division. The project involves the electrosynthesis of reactive metal alkyls

Applications should be sent to:

Dr Derek Pletcher, Department of Chemistry, The University, Southampton SO9 5NH

Tel: 0703 559122 ext. 3370

(6861)F

COURSES & CONFERENCES

ADVANCED COURSE IN FLOW CYTOMETRY: ANALYSIS AND FLUORESCENCE ACTIVATED CELL SORTING

Paris, September 9–14, 1985

Sponsored by. The Cancer Research Association (ARC) - Jacques Crozemarie, President, and the "Centre Technique pour le soutien de la Recherche sur le Cancer des Laboratoires et Formations du Centre National pour la Recherche Scientifique" (C N R S.)

A Course in Flow Cytometry will be offered with special emphasis on cell biology Themes will include Multiparameter cell sorting, cell lineage studies, tumor cells characterization, gene amplification selection, cloning of genes by transfection, somatic cell genetics, hybridoma variant selection, liposome endocytosis, cell cycle analysis, Brd U and cytokinetic studies, mathematical modeling of the cell cycle, computer system for multiparameter data analysis, cytochemistry and new fluorochromes, karyotype analysis, pumped dye lasers, emission anisotropy

The Course will consist of lectures, laboratory demonstrations and problem solving sessions

Course Directors. Harry Crissman (Los Alamos National Laboratories), Claude Rosenfeld (INSERM, Villejuif, France)

Organizers: L A. Herzenberg (Standford University, USA), P Le Bouteiller (Centre d'immunologie de Marseille-Luminy, France), Z. Mishal (C N.R.S., Vilejuif, France), M. Monsigny (Centre de biophysique moléculaire, Orleans, France), J.L. Roti-Roti (Washington University of St. Louis, USA)

Z Darzienkiewicz, P Dean, M Goldberg, J. Gray, P Horan, C Julien, M Loken, H S Micklem, K Muirhead, D.R. Parks, A Truneh

Course tuition $500 (5 000 FF) includes laboratory fees, manual, coffee breaks, lunches and an optional Flow Cytometry Certificate

Send C.V and tuition deposit $50 (500 FF) to the local Course Director (max 50 persons)

Dr Claude Rosenfeld,
INSERM Unite 253,
Groupe Hospitalier Paul Brousse,
16 bis avenue Paul Vaillant Couturier,
94804 Villejuif, France

Application deadline June 30 1985, priority given to early applicants. **(W1919)C**

KINGSTON POLYTECHNIC — PART-TIME MSc

EARTH SCIENCE and the ENVIRONMENT
7th INTAKE. JANUARY 1986

Applications are welcomed from graduates with Honours degrees or equivalent qualifications in Geology, Geography or related subject areas

For further information contact Dr R T J Moody,
School of Geological Sciences, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366.
(6889)C

SECOND AND FINAL ANNOUNCEMENT

The 1985 TNO-ISIR meeting on the Interferon System
1985 Annual Meeting of the ISIR
Holiday Inn, Clearwater Beach
Florida, USA
13-18 October 1985

Organizing Committee W E. Stewart II, Chairman; H Schellekens, Co-Chairman, K Cantell, Co-Chairman; E. Borden, I Gresser, H K. Hochkeppel, J. Kawade, H Kirchner, W. J C Melgert, Ms K Zoon

Deadline: Registration September 1, 1985. For information write to:

TNO Corporate Communication Department
P.O. Box 297
2501 BD DEN HAAG
The Netherlands (W1964)C

PROPOSALS

CALL FOR PROPOSALS TO HOUSE THE WORLD DATA CENTRE FOR COLLECTIONS OF MICROORGANISMS OF THE WORLD FEDERATION FOR CULTURE COLLECTIONS

Because of the impending retirement of the Director, the World Data Centre (WDC) must be relocated and offers are sought from institutions willing to house and develop this important information centre The WDC is part of the World Federation of Culture Collections (WFCC) and is responsible for the collection, storage and distribution of information about culture collections internationally. It forms an essential resource for the support and development of microbiology in research, education and industry

Detailed information on requirements is available in the Request for Proposals document available from the WFCC's Steering Committee for the WDC or The President members of the WFCC. Applicants are required to provide a detailed specification of mechanisms for implementation of the databank, qualifications, resources and policy. This, or letters of intent, must be sent to the President WFCC by August 1st. Applicants will be assessed by the Steering Committee and recommendations made to the Executive Board of WFCC, which will make the final decision. Relocation must be completed by July 1986 and every assistance will be given to the successful applicant to complete a smooth transition of this important centre.

Steering Committee contacts· Dr M Krichevsky, Microbial Systematics Section, National Institute of Dental Research, National Institutes of Health, Building 10, Trailer A, Bethesda, Maryland 20205, USA; Dr R Hill, National Collection of Type Cultures, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT; Dr R E Stevenson, American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, USA and Dr H Sugaware, Kenkyuu-jouhoushitu, Riken, 2-1 Hirosawa, Wako-shi, Saitama 351, Japan.

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Deadlines: abstracts 15 August 1985; registration 1 September 1985.

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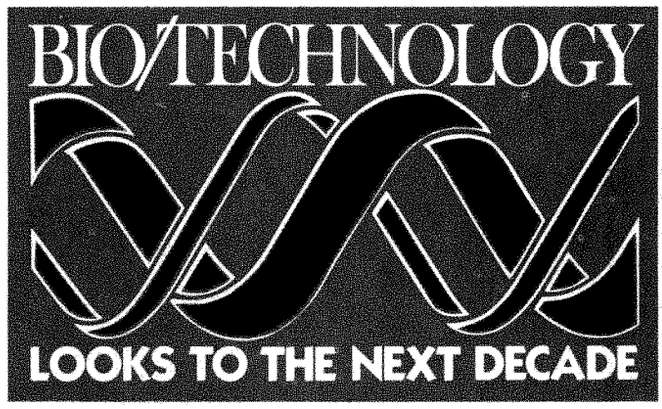
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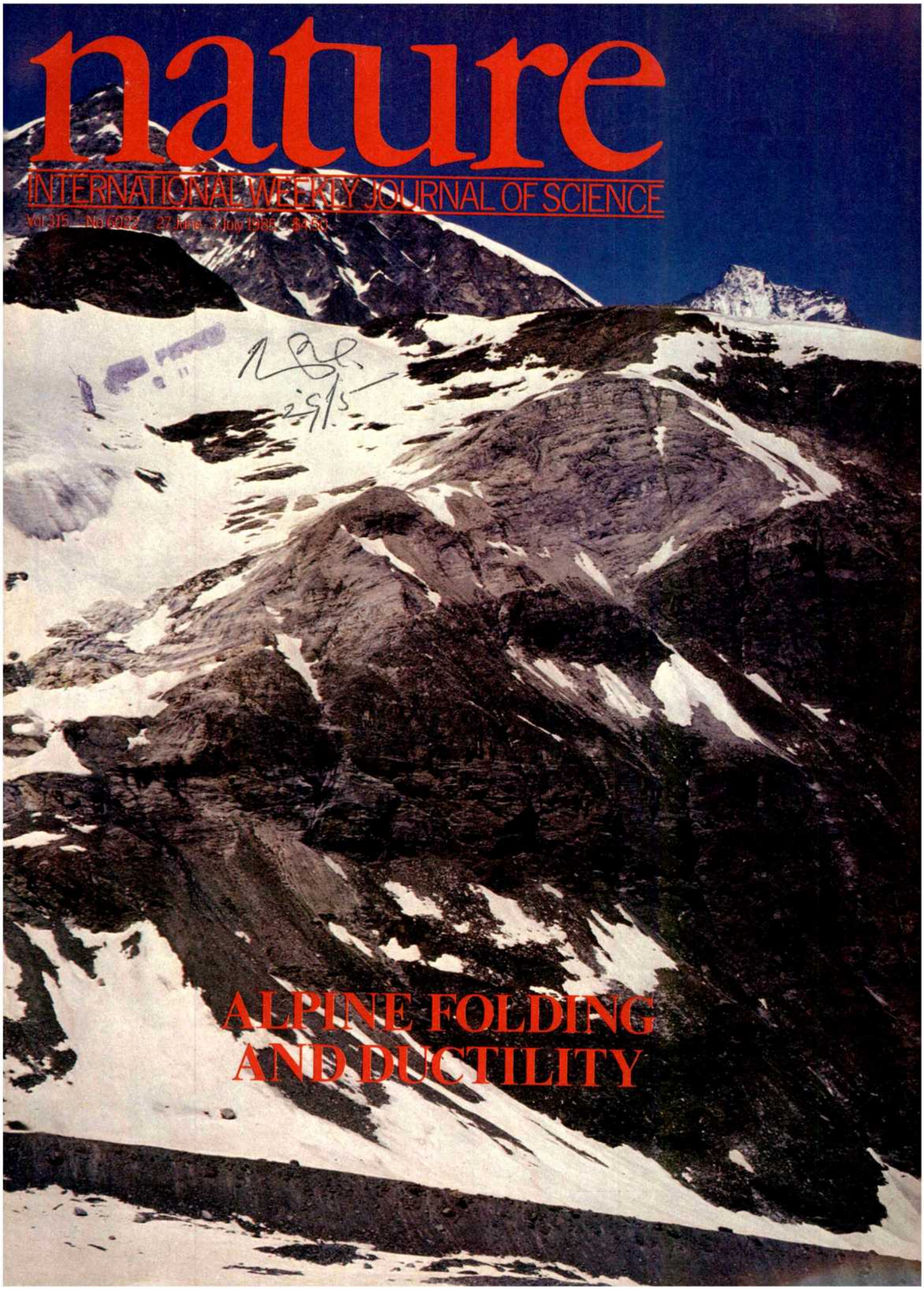
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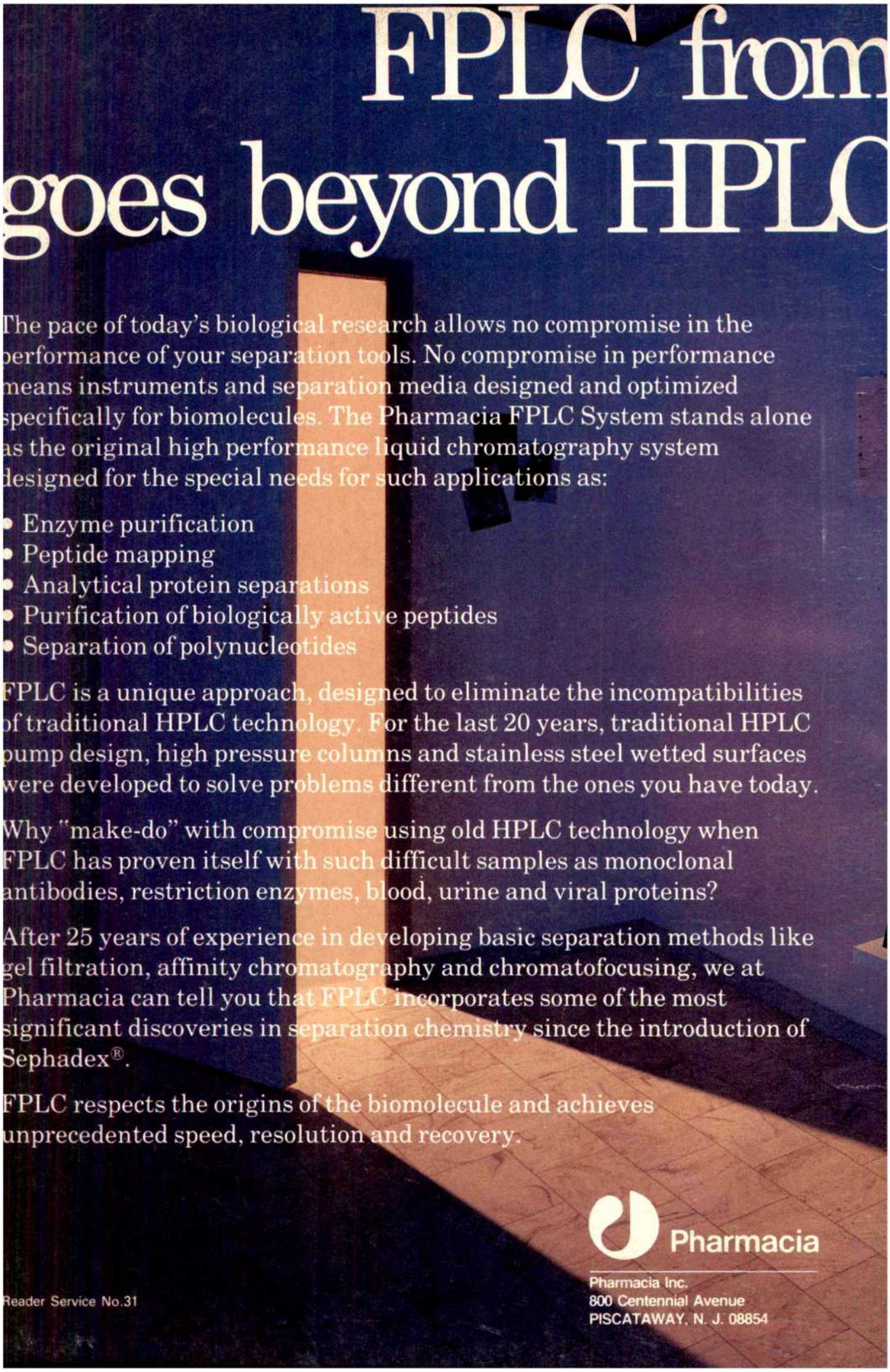
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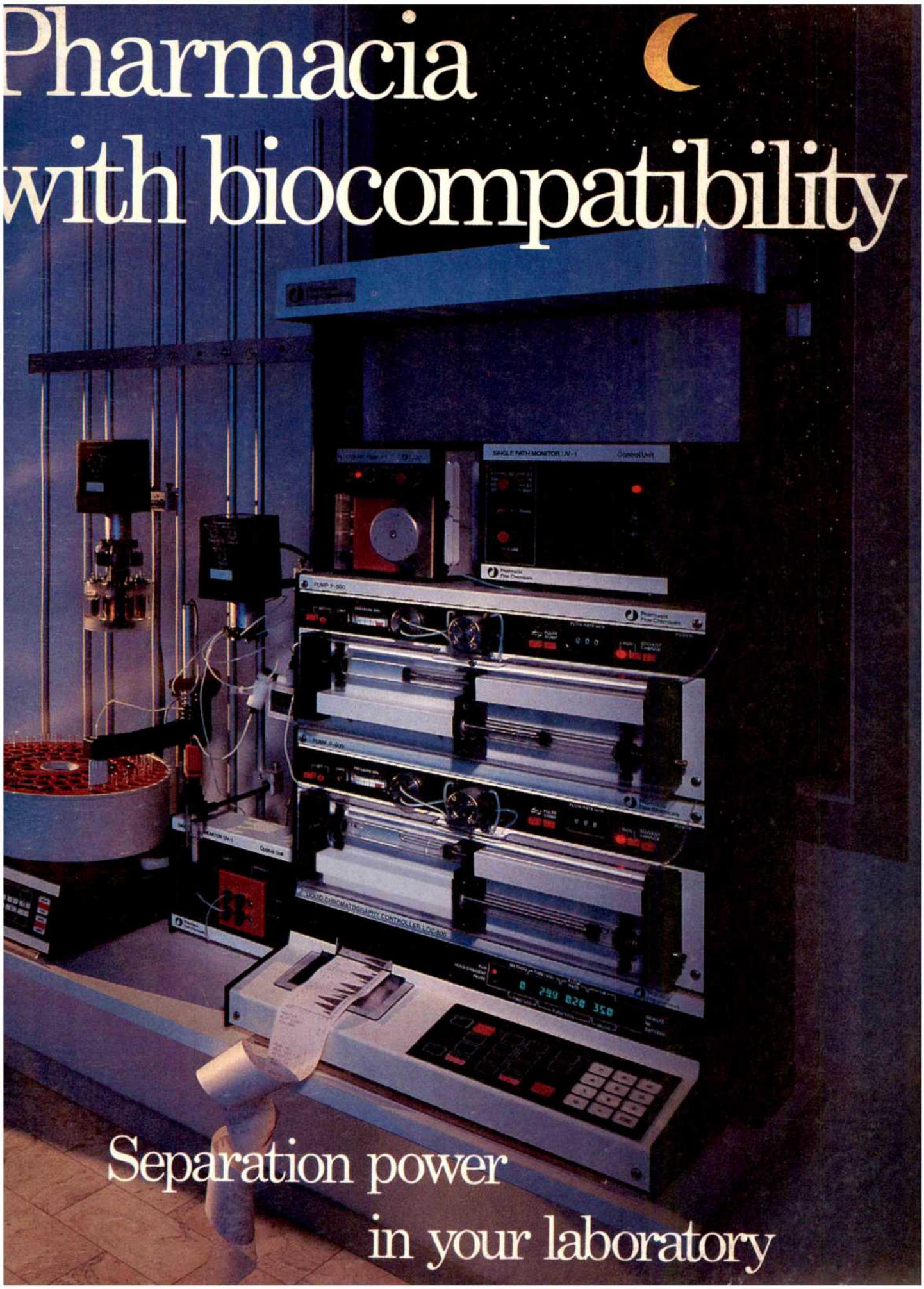


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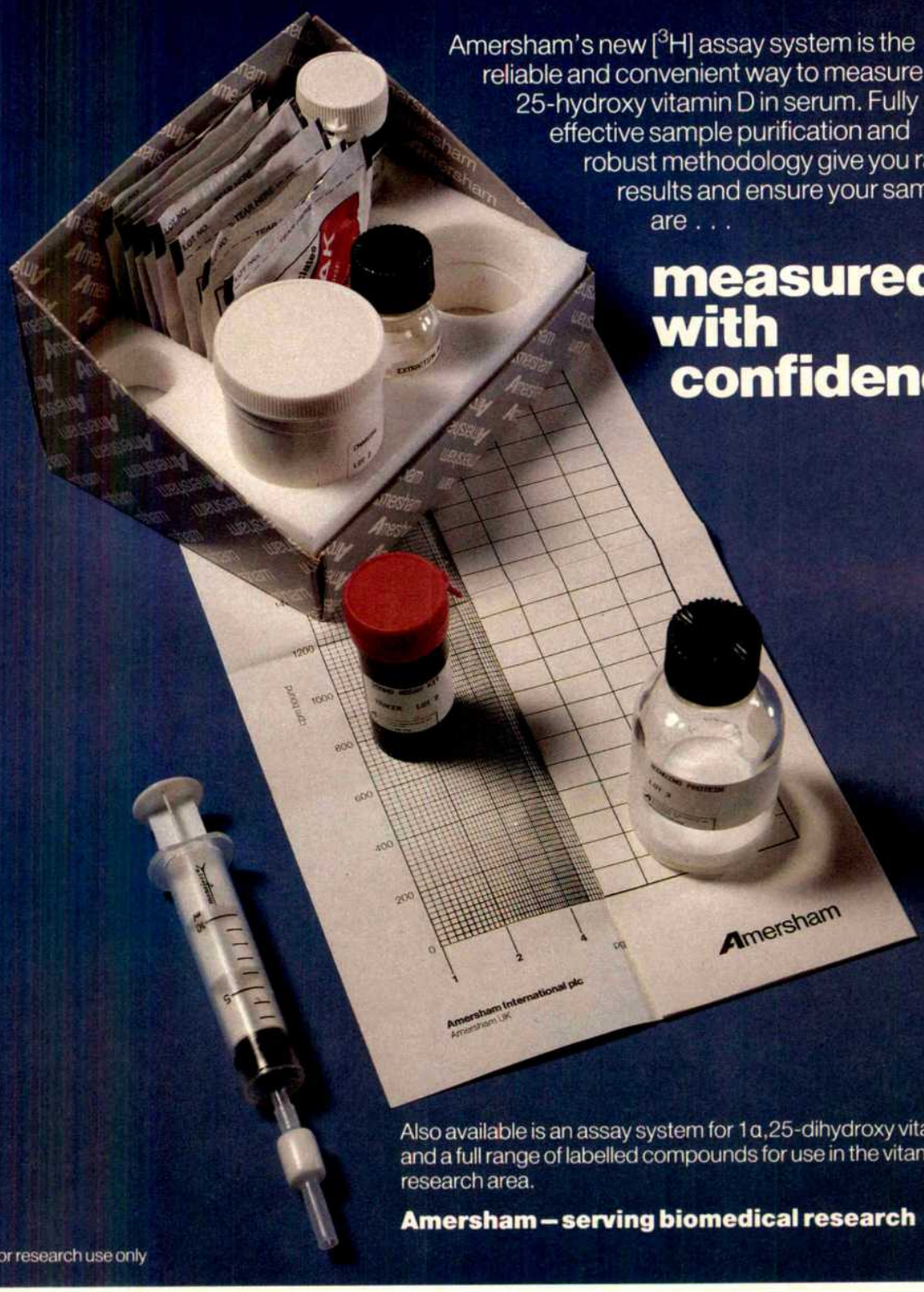
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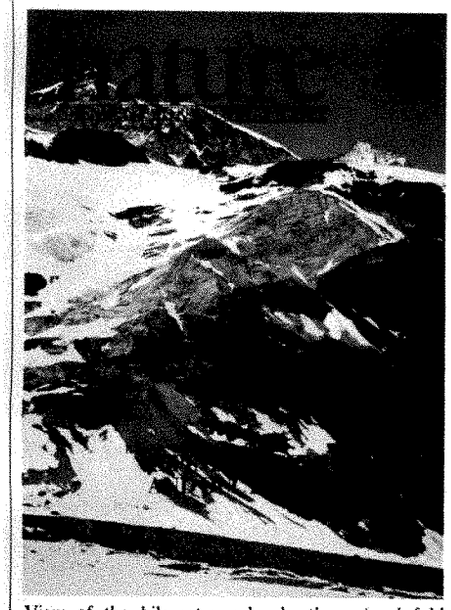
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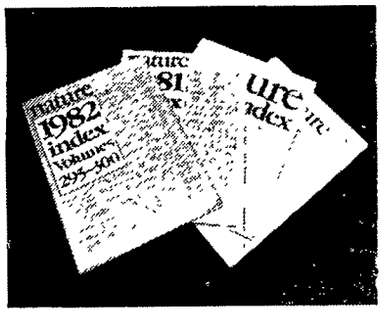
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J. Gerhart (U. of California at Berkeley)	Early development—Xenopus
C. Nüsslein-Volhard (Tübingen)	Early development—*Drosophila*
C. Goodman (Stanford)	Development of invertebrate neural systems
H.R. Horvitz (MIT)	Caenorhabditis

DAY 2: TUESDAY, OCTOBER 8

CELLS

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M. Davis (Stanford)	T-cell receptors
U. Storb (U. of Washington at Seattle)	B cells
J.M. Bishop (U. of California at San Francisco)	Oncogenes
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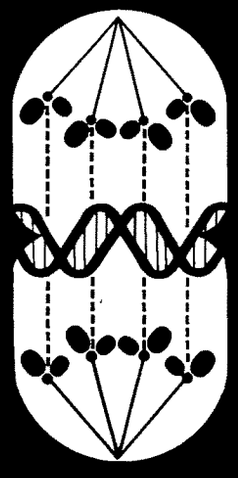
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GENES

Speakers	*Topics*
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D. Hogness (Stanford)	*Drosophila* homoeotic genes
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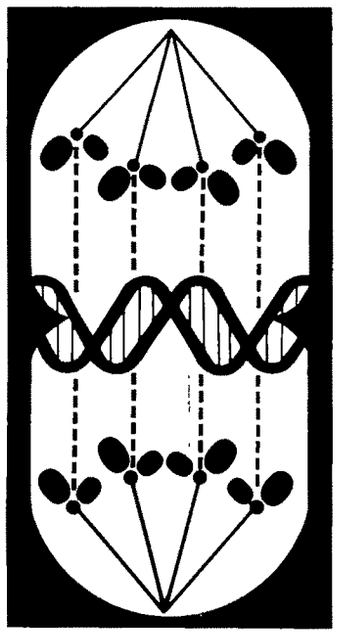
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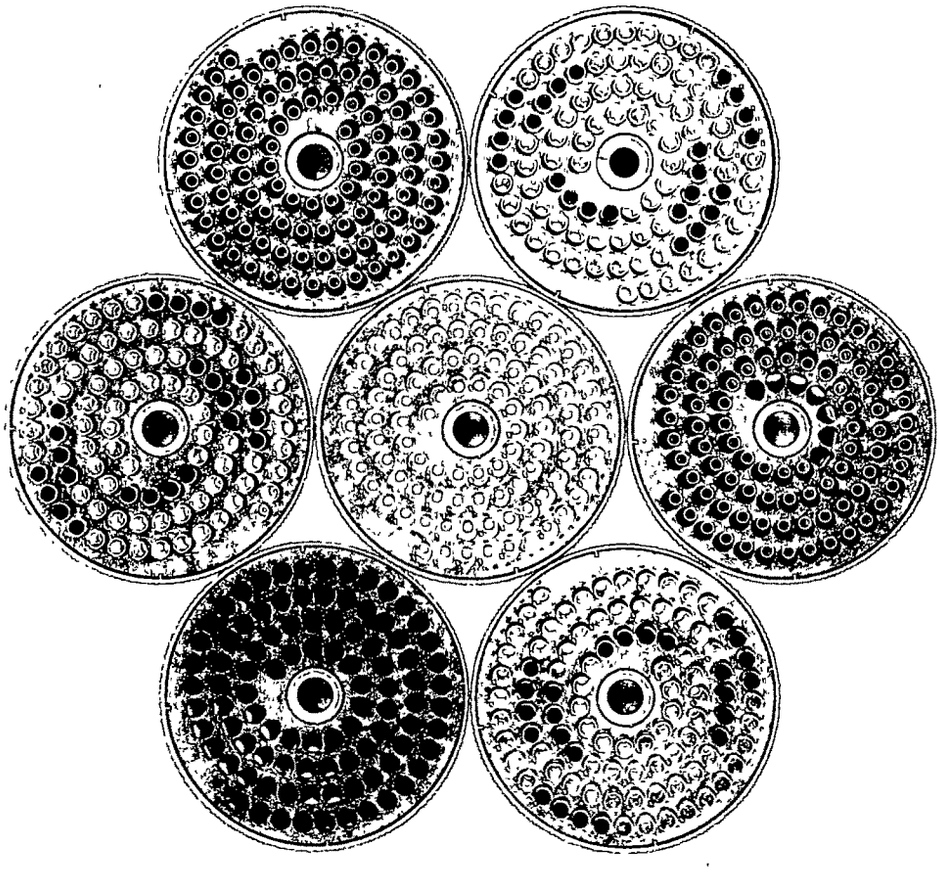
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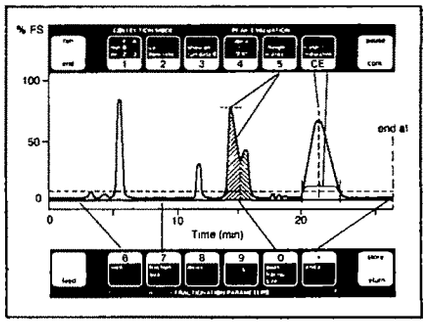
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NATURE VOL 315 27 JUNE 1985

Put the horse before the cart

This week's European summit meeting at Milan is an opportunity both for stimulating technical collaboration and to create a muddle. Governments should temper enthusiasm with cold calculation.

EUREKA, President François Mitterrand's way of saying that he has seen the light for Europe, will occupy the European heads of government at their meeting in Milan this weekend. The plan is to invest common resources in technological developments that will help to bridge what Europeans used, in the 1960s, to call the "technology gap" between themselves and the United States. (Nowadays, Japan is as much in people's minds.) President Mitterrand has been preaching strength through technological development ever since he took office four years ago, but his most recent advocacy of this cause appears to have been stimulated by the fear that the US Strategic Defense Initiative, whatever its military implications, cannot fail to widen the gap between the competence of European companies and those in the United States. The discomfort of many European governments with the military and political implications of the US proposals has given Eureka a more hearty welcome than the earlier French initiatives (see *Nature* 6 June, p.443) It remains to be seen whether wrangles over budget problems and the high price of European grain will allow the heads of government time to put flesh on the bones of Mitterrand's dream. The big danger is that ill-considered enthusiasm will point this important enterprise in the wrong direction.

Here is a cautionary tale. While very little has been said of the way in which Eureka would be put into effect, one theme of the past few weeks has been that European governments should settle on a few major technical objectives and then pool resources so as to make them come to pass. What kinds of projects? On the face of things, a scheme to equip the air forces of Western Europe with a military aircraft designed to take over from those now in service would seem to be an obvious (if unambitious) candidate. As it happens, this is a scheme on which five European governments (Britain, France, Italy, Spain and West Germany) have already embarked. So what are the enthusiasts for Eureka to make of the meeting last week in London, when the governments concerned failed to agree on the specification of the aircraft they would jointly build, and on a scheme for sharing the construction work among themselves? That does not promise well for the simpler goal-oriented versions of Eureka, although there is a final meeting next month, at which something may be salvaged.

The lesson from last week's meeting is that it will make no sense for European governments to invest in the improvement of technology if they do not at the outset agree on the partition of the benefits. The temptation will be to attempt to divide the spoils in proportion to the investments made by the several governments, but attempts to legislate for chauvinistic equity along these lines are doomed to failure. Xenophobia being what it is, even within the European Communities, at least one but most probably all partners would finish with a sense of having been short-changed. The other more logical basis for mutual support for technological developments would be to say that the benefits of the investments proposed could be exploited by whichever European companies first had the wit to recognize their utility. In the long run, Europe as an economic entity would benefit, but some parts of it more than others. Equity would then require that there should be mechanisms for redistributing wealth among European states, which amounts to saying that Europe should function as a federated state Politically that is a long way from reality. If Eureka is to succeed this side of infinity, there will have to be some halfway house.

Much will depend on the kinds of projects on the agenda. So far, there have been only general statements of what might be done. People such as President Mitterrand have been talking of the further development of integrated semiconductors, of machines with artificial intelligence and, more recently, of the exploration of the deep oceans. On one view, the particular choice of projects to support is almost immaterial. Provided that the objective is sufficiently ambitious, the enthusiasts say, and Europe's engineers sufficiently committed, there will be a host of incidental technical developments which cannot fail to benefit European industry as a whole. This is the argument by which the 1960s Apollo programme to send people to the Moon was justified not by its ostensible objectives but by its technological spin-off. What this week's summit should remember is that such arguments are an assault on reason and a recipe for extravagance. If the technologies of, say, welding and refrigeration need to be improved, why not mount market-oriented developments in those fields? Especially when one of Europe's problems is a shortage of skill, the consequences of too much ambition could be a damaging diversion of resources.

Eureka should begin from a careful analysis of why Europe fails at present. The most obvious weakness is that Europe is not yet a common market. One result is that companies cannot accurately tell what research and development is likely to meet market needs. Another is that there are too many small companies duplicating each other's efforts. Eureka needs a way of circumventing these constitutional obstacles, perhaps by allowing separate companies to collaborate in transnational research and development partnerships that could be blessed with common funds. To work on what? The heads of government at Milan will no doubt be flush with ideas. They should keep them to themselves. Politicians and civil servants have a poor record as entrepreneurs What is needed is a mechanism by which groups of companies can put forward detailed proposals as if they were applying for research grants, but with the difference that the criteria for success would include not merely the novelty and the feasibility of the proposals, but some estimate of their economic and strategic promise. The European Commission's recent suggestion that the Euratom treaty provides a framework for channelling common funds into novel enterprises would make sense in this connection.

This week's summit must also give some thought to the infrastructure. One of the reasons why Europe's technology lags behind that of the United States and Japan is that its technological labour-force is ill-provided with first-hand demonstrations that the future can be made to happen here and now. Part of the trouble is that higher education is run on a shoestring (Britain) or is flexible (France and West Germany). Spending more on

Biological manuscripts

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the university education of technical people is not a sufficient answer, although some of that will be needed. More to the point is the need that Europe, in trying to create the foundations for industrial prosperity in the next century, should find some way of enabling the now-young people who will inhabit that world to make their full mark. In many ways, Europe has grown too old.

The third need for the summit is to remember Adam Smith. Inward-looking Europeans see their enterprise as a self-contained imitation of what has already happened elsewhere. Too many Europeans consider that the immediate goal must be to replicate what IBM and AT&T have done in the United States, or what a host of Japanese companies have done in consumer electronics. The fallacy is that success would merely bring the prospect of head-on competition in fields that are certain to seem old-fashioned even a decade from now. But there is no reason why the world should become less interdependent than it is already, for which reason the trick for Europe must be that prosperity should rest on industrial activities complementary to those elsewhere. That is why the objective of those who endorse Eureka should be to build on Europe's present strengths, such as they are, not simply to imitate what others do. The goal must be an economic and economical division of labour, to which end originality is the crying need. Will the summit have the courage to recognize that simple but daunting truth? □

How many (what) miles?

Last week's shuttle flight shows that the Strategic Defense Initiative has a lot to learn.

THE timing of last week's fiasco in which a laser beam from Hawaii was directed at the wrong side of the US shuttle in orbit about the Earth could hardly have been worse. The US Congress is at the stage in the annual budget cycle when critics of the Strategic Defense Initiative (SDI) are energetically — and successfully — chipping away at the huge budget the administration has asked for. While nothing is settled, the chances are that the budget for the financial year beginning on 1 October will be reduced by between a quarter (according to the Senate) and a third (according to the House of Representatives) from the $3,700 million asked for. If the managers of SDI are wise, they will spend at least some of that sum on teaching each other a little about elementary goedesy.

The official explanation of the first failure of the laser test (which worked on later orbits) is quite breathtaking in its innocence. Almost merrily, the spokespeople have been saying that, by some oversight, the "on-board" computer on the shuttle had been programmed "in" statute rather than nautical miles. By their nudges and winks, thay have implied that only some profound perversity can explain the habit that has people who travel in shuttles measuring distance in the maritime measure of nautical miles. No doubt SDI officials will have figured out that nautical implies something to do with the sea, because *Nautilus* is a kind of submarine. In their innocence, they seem not to know that a nautical mile is intended to be the length of a minute of arc along a great circle on the surface of the Earth, although it was prematurely defined as 6,080 feet by the British Admiralty in the eighteenth century.

The SDI people are unlikely to be too downcast by this show of ignorance; they have earned a reputation for being brash. They have a tendency, when they encounter physical problems that would seem insuperable obstacles to others, to make a solution of them a "requirement" of the SDI programme as a whole. The little confusion between statute and nautical miles may, in any case, make them seek safety in unambiguously named kilometres instead (a cause too long dormant in the United States). So perhaps they should be told that the French Revolution had a committee, of which both Laplace and Lagrange were members, which defined the kilometre in much the same way as the nautical miles as one ten-thousandth of the distance between the Equator and the pole. □

Laser proliferation

The US Department of Energy thinks the laser enrichment of uranium feasible; that is bad news.

THE third review conference of the non-proliferation treaty (NPT), due to open at Geneva in August, will be forced to reckon with a late agenda item, the recognition that the simplest way of making nuclear weapons is probably now the enrichment of uranium by means of lasers. That is the implication of what appears to have been a successful demonstration of laser enrichment at the US Lawrence Livermore National Laboratory in California. The laboratory has been able to make five kilograms of uranium-235 in a single purification step. For those who wish to make bombs, the advantages of this route are obvious. Unlike isotope separation by gaseous diffusion or centrifugation, laser separation may be a once-through process. So capital costs are likely to be less and, for potential violators of agreements, separation plants will be inconspicuous.

None of this implies that uranium enrichment has become child's play. At Los Alamos as well as at Livermore, people have been trying for at least a decade to distinguish by means of lasers between the different isotopes of particular elements, with uranium usually at the backs of their minds. The fact that success has eluded them for so long is one comfort, suggesting that governments will not be able to run up material for a few bombs overnight by buying standard equipment from commercial suppliers. Indeed, the technology of copper excimer lasers reported (see p.706) to be the primary energy source for the Livermore process will not easily be replicated elsewhere, at least for the time being. In any case, crucial details such as the identity of the electronic transitions by means of which uranium atoms are excited to near-ionization are sensibly being kept secret. The obvious snag is that uranium atoms are the same the whole world over, and that the investigation of the spectroscopy of uranium required to specify the ingredients of a laser enrichment machine should be little more than few months' work for a couple of competent physicists. It is a fair guess that even governments with no interest in making bombs, but with an interest in what others may be doing, will put this work in hand immediately. Putative bomb-makers will probably be even quicker off the mark.

So does this new development mean that there is no longer any purpose served by attempts to control the spread of nuclear weapons by the physical control of fissile material? Fortunately, not quite. NPT is a compact between non-nuclear governments and the nuclear powers under which the first group undertakes not to make nuclear weapons, and to put up with physical inspection by the International Atomic Energy Agency, while the second undertakes not to help others make bombs but to help with civil nuclear technology, and at the same time to negotiate measures of arms control that will make the world a safer place. The signatory nuclear powers (France and China have not joined NPT) will be given another drubbing at this year's review conference for having done so little on arms control, but it seems unlikely that non-nuclear powers will desert the treaty on that account, or because laser enrichment seems to offer rivals outside the treaty a more convenient route to bomb-making. Keeping safeguards will be more than ever a demonstration of self denial.

The more serious problems are what they have always been, the abstention from NPT of countries which believe they may gain from being thought potential nuclear powers — Israel, Pakistan and so on. If laser enrichment means anything, it is that governments within NPT should have a powerful incentive not merely to control the use made of fissile material but also to see that the international trade in uranium is also well-regulated. For however convenient the new technique may be, it will still take the best part of a tonne of uranium to extract a critical mass of uranium-235, which should be a noticeable amount of such a scarce material. That is the direction in which the interests of the regulators should now be shifted. □

European research

Milan the spur to new collaborations?

Brussels

THE European Commission sees this week's summit meeting at Milan, where the French Eureka proposal is certain to have at least a brief hearing, as an opportunity for persuading the members of the European Communities to take a more serious interest in cooperative research and development But Mr Jacques Delors, president of the Commission, and his technology commissioner, Karl Heinz Narjes, are keeping an open mind on how collaboration might develop.

Delors wants flexibility above all, for which reason he is keeping up his sleeve several different legal frameworks for technological collaboration One possibility is that the existing Euratom treaty might suffice, another that there might be a new treaty along similar lines. But that, everybody agrees, would take too much time. The Commission seems to be saying that it merely wants the member states to agree to collaborate, leaving the details of collaboration to be worked out later.

According to Delors, money is all important. He insists that there should be a "critical mass" of funds for research and development. Part of his ambition is that member states should come to regard common spending on research as the European equivalent of US spending on military developments, where commercial beneficiaries are not expected to repay the public investment.

One mark of members' commitment will be whether they agree to the 14.9 per cent increases of the Communities' research budget marked out for 1986. Narjes believes that research and development will have to take a larger slice of the Communities' budget in future years, perhaps between 6 and 8 per cent of the total by 1990. Whether the member states will happily accept the implication of increased budget contributions to the Community is another matter.

Responses so far from member governments have been mixed, according to Narjes, who sees European initiatives in research and development as essentially collaboration on projects to which industrial companies would contribute, as in existing collaborations. While some governments are concerned at the prospect of Community control of common projects, the Commission notes that British companies have shown more interest than their government in the proposed collaborative programme, Research in Advanced Communications in Europe (RACE).

In its optimism, the Commission does not expect that its plans to expand collaborative research will founder on the expectation of member states of a return proportional to their individual investments. But changes will be needed in what governments expect. And there will also have to be more decisive steps towards the creation of a Europe-wide system of standards and a greater degree of cohesion so that it would be more difficult than at present for competitors elsewhere to "pick off" European states one by one.

Narjes does not expect more from this week's summit than agreement among the member states to settle on two or three projects for further collaboration by the end of 1985. He has a shopping list of areas in which collaboration might be sought, ranging from large computers and superchips to high-speed trains and biomolecular engineering to particle beam physics and optical communication. The Commission would like to see the French Eureka project subsumed in an agreement that Europe's collaborative research programmes should be expanded

Pragmatically, the Commission says that the legal forms of novel collaborations will be decided only after specific programmes have been agreed. One obstacle ahead, according to Delors, is that Europe's technical labour force is ageing more quickly than that of the United States and Japan The Commission's ambitious plans are in part intended to be a spur to the training of more skilled technologists, if only because partners in the new collaborations that may come into being will know that they will benefit only in proportion to the numbers of people able to help exploit the results of research and development programmes.

Anna Lubinska

Chemical weapons

US moratorium to end

Washington

THE US House of Representatives voted last week to end a 16-year moratorium on the production of chemical weapons. Actual production of the new "binary" weapons, which contain two relatively non-lethal chemicals that are mixed together in flight to produce nerve agent, could begin in December 1987.

The Reagan administration, arguing that current stockpiles of nerve-gas shells



are deteriorating and do not represent a "credible" deterrent, had repeatedly sought congressional approval for the new weapons over the past three years. In close votes, the House had repeatedly rebuffed the President. This time, however, heavy White House lobbying paid off. The Republican-controlled Senate had already approved the proposal.

Supporters did make several concessions, however Funds for production would not be released until 1987, and only after the President certifies that the weapons are needed. Supporters also accepted an amendment that would require the NATO (North Atlantic Treaty Organization) allies to declare their willingness to have the US weapons deployed in their countries before production could begin. However, even opponents of the new weapons expect that amendment to be dropped by a House/Senate conference which must meet to reconcile differences between the two houses' bills. Congress will still have to agree to appropriate the funds for production.

The House bill authorizes an initial expenditure of $124 million; the Senate bill, $163 million. Critics have said that the total cost of carrying out the Pentagon's production plan could exceed $4,000 million.

Stephen Budiansky

Nature in Japan

NATURE announces an international conference to be held in Tokyo next year, on 22–24 January 1986, on the theme *Molecular biology becomes biotechnology*. Speakers at the conference will include ten from the United States and Europe and an equal number from Japan. The conference, which will interest molecular biologists in academic and industrial laboratories, is in the series which includes this week's conference in London and that, in conjunction with the University of California, San Francisco, to be held in San Francisco next October. Further details may be had from *Nature*'s offices in London and Tokyo. □

Auto emissions

Europe nears compromise

Brussels

Too tough for some, too lenient for others, the Commission of the European Economic Communities (EEC) was last week required to defend its proposals to limit car exhaust pollution. At the same time, its experts began a last minute search for a compromise to avert an otherwise inevitable deadlock when the ten environment ministers meet in Luxembourg on 28 June. The compromise could involve not only a possible redefinition of the troublesome medium-range category of car (now set at 1.4–2.0 litres) but also different emission standards for cars fitted with three-way catalytic converters and those relying on lean-burn technology.

Reactions to the original proposals (see *Nature* 20 June, p.622) have been sharply divided among the member states. France, Italy and Belgium have joined the United Kingdom in dismissing the emission standards for medium-sized cars as too strict. But West Germany, followed by the Netherlands, Luxembourg and Denmark (which wants to see US-style uniformly strict controls applied to all categories of cars), is set on tougher controls. In addition, the Commission's proposals have reopened the debate on large cars (2 litres and over) that appeared to have been settled in principle on 20 March. The figures put forward by the Commission are unacceptable to Belgium, France and the United Kingdom despite the fact that the use of the three-way converter in the case of large cars had been agreed in March.

The Commission's proposals have also been sharply criticized by an outside expert, Dr Michael P. Walsh, former deputy assistant administrator for the US Environmental Protection Agency's mobile source air pollution control. In two papers made public last week, he maintained that the Commission's proposals ignore a number of important factors. In particular, they do not take account of increased car sales which, given the long timetable for implementing the proposals, could mean "significantly more pollution both in the short and long term".

According to Walsh, by the time the entire fleet is converted to the new standards, nitrogen oxide and hydrocarbon emissions will be almost three times more than equivalent US levels and carbon monoxide almost two and a half times more. "If member states choose to implement more 'permissive' standards, these estimates", he says, "are optimistic."

A major part of the problem is the test cycle used to establish the amount of pollutant emitted. The European test cycle, says Walsh, takes little account of modern driving conditions. A new European test cycle is being run at present, on the basis of which the standards now to be agreed will be modified in a year or two. This, Walsh says, will only serve to complicate the matter of laying down US-equivalent standards now.

The European Commission has always insisted that it is impossible to apply the US cycle to European cars. But Walsh says that European studies have shown that US test procedures are a more faithful reflection of current urban driving conditions. He concedes that the European test is effective in simulating dense congested traffic conditions, but says it will not suffice for assessing limits for NOX and hydrocarbons (which are generated at high speeds and carried over long distances).

Walsh maintains that the Commission's proposals as they stand will cut NOX emissions by only 39 per cent by the year 2010, while HC and CO emissions will fall by 24.4 per cent and 22.8 per cent. On the other hand, if US equivalent standards in the range of 4.6 grams per test HC plus NOX, 16 g/t CO and 2.4 g/t NOX were applied for medium and large cars, the actual reduction in pollution could be 66.7 per cent for CO, 75 per cent for HC and 76.7 per cent for NOX.

In response, the Commission has produced a paper supporting its proposed standards, which it says will reduce all car exhaust emissions by 70 per cent in the case of large cars and 73 per cent for medium cars, compared with 67 per cent reduction reached in the United States. Emissions of NOX, which are a principle concern of EEC, would be reduced to a level only slightly above that in the United States (1.56 million tonnes each year in EEC, compared with 1.48 million tonnes in the United States) with a further reduction possible when new limits (perhaps 5 g/t) are introduced during the planned second phase for small cars under 1.4 litres, bringing EEC emissions down to 1.41 million tonnes.

Commission experts estimate further that if EEC ministers agree to introduce US-type speed limits of 55 m.p.h. (88 k.p.h.) as well, global NOX emissions from Community cars could fall to 15 per cent below US levels. But this suggestion has so far been blocked by West Germany, whose cars incidentally emit the greatest volume of NOX (637,000 tonnes in 1980) and which is under the greatest pressure to adopt tough controls on air pollution. **Anna Lubinska**

Mount Wilson's end

Washington

No rescue plan has materialized to save the Mt Wilson 100-inch telescope, due to be shut down next week by its owner, the Carnegie Institution of Washington (CIW). Although discussions are continuing to find a "suitable operator" to take over the facility, it seems increasingly unlikely that one will be found. The several rescue groups that had been formed by interested astronomers, professional and amateur, have apparently failed to raise the several million dollars necessary to keep the telescope in operation.

Carnegie announced its intention to close the telescope a year ago, citing the increasing financial burden of running both Mt Wilson and its newer Las Campanas observatory in Chile. More recently, CIW revealed plans for shutting down the other astronomical facilities at Mt Wilson. The 60-inch reflector, now used for solar and stellar observations, will continue operating until 1 July 1986. The solar observatories will continue operating only until next October, but CIW is willing, until 1 July 1986, to continue administering salaries and other operational budgets provided by outside grants. In other words, CIW offers a nine-month lease to outside investigators who can find their own money. Stephen Budiansky

Verification in space

Los Alamos, New Mexico

Donald Kerr, director of Los Alamos National Laboratory, is calling for the United States to give more attention to how it would detect and assess new high-technology Soviet anti-missile defences — in other words, a Soviet star wars system. Kerr says that "the possibility should be anticipated" that the United States and Soviet Union might reach agreement to limit star wars defences, and points out that the first requirement of any such agreement would be a means of verifying compliance. Conventional reconnaissance might not be adequate to assess the capabilities of a Soviet star wars system.

Despite the new technical challenges of such a monitoring system, Kerr says he is "moving in that direction". The testing of laser weapons might be detected by looking for atmospherically-scattered repetitive pulses of light, for example, and satellites might be interrogated with particle beams to see if they contained fissile material: the radiation emitted would provide information on the isotopes present.

Kerr plans to argue his case in Stockholm next month at a conference on space weapons and international security hosted by the Stockholm International Peace Research Institute. Los Alamos National Laboratory has long undertaken research into ballistic missile defence and claims to be well placed to develop means of verifying compliance with anti-ballistic missile or other future treaties limiting arms in space. Tim Beardsley

Blood typing

US sale of British reagents

BLOOD-GROUP typing in the United States is likely to be based on monoclonal antibodies, following a deal between Celltech, the British biotechnology company, and Ortho Diagnostic Systems Inc., the Johnson & Johnson subsidiary which is the largest supplier of blood-typing reagents in the United States.

Although the British National Health Service has already switched over to Celltech's monoclonals for ABO blood typing, the technology was not simply transferable to Ortho The US blood-typing test is differently and more rapidly performed than the British test. Moreover, Ortho's blood-typing reagents, based on antibodies from donated blood, are designed to detect blood group antigens at below the threshold at which British tests operate.

These demands have required a two-year period of development by Celltech, with support from Ortho. Because the single monoclonal antibodies against blood groups A and B developed for use in Britain did not perform adequately in the US tests, Celltech embarked on a worldwide hunt for more appropriate antibodies. Despite screening hundreds of candidates, it failed to find sufficiently sensitive single A and B antibodies. So Ortho's reagents are each a cocktail of several monoclonals, some of which Celltech have had to buy in from outside. At least one unnamed US academic laboratory stands to gain.

There have also been regulatory hurdles to cross Because traditional blood-typing reagents are blood products, they are licensed by the Office of Biologics of the US Food and Drug Administration. The monoclonals, although derived from cultured cells rather than blood, have been caught by tradition and subjected to the regulations of the Office of Biologics, although other diagnostic monoclonals have passed through other offices.

Material is at present produced in 100-litre fermenters, but as Ortho's demand builds up, Celltech plans to switch to its 1,000-litre fermenter which can yield 100 grams of antibody in a two-week run. If, as expected, monoclonals are eventually used in half of ABO blood-typing tests worldwide, "a low number of kilograms" of antibody will be needed annually. According to Gerard Fairtlough, chief executive of Celltech, independent estimates put the world market for blood-typing reagents at £7.5 million per year. The initial agreement between Celltech and Ortho is for five years, involving payments of "several million pounds" to Celltech by Ortho.

John Berriman of Celltech sees no obvious competitors One Canadian and one Swedish company have potentially suitable monoclonals, but not Celltech's ability to produce them in quantity. Celltech also hopes to produce a monoclonal antibody for rhesus blood typing, but because mice do not produce antibodies against the human rhesus antigen, it has not been possible to use standard hybridoma technology. Instead, Celltech hopes to develop a system of producing a monoclonal antibody against D antigen, the main rhesus antigen, in human lymphocytes transformed by Epstein–Barr virus. **Peter Newmark**

Searle deals blow to Genex

Washington

GENEX Corporation, which had pinned its hopes for making an early profit on mass production of the chemical L-phenylalanine, the key starting material for the artificial sweetener aspartame, has been dealt a major setback. G. D. Searle, which holds exclusive rights in the United States and most of Europe for the use of aspartame as a sweetener, last week informed Genex that it would be dropping its contract to purchase phenylalanine as from November. Genex is left with an $18 million plant that the company says it built on assurance from Searle that it would continue to be a major supplier. The plant, located in Paducah, Kentucky, was completed only last year and it reached full production three months ago.

Genex's president, J. Leslie Glick, acknowledges that the market for other uses of phenylalanine, mainly the production of pharmaceuticals, is "limited", and could not justify keeping the Paducah plant open.

Genex last year had sales of $20.6 million, almost all of that from phenylalanine. Research and development contracts, which totalled $5.7 million last year, will now once again become the company's major source of revenue. Glick said the company was looking into the possibility of using the Paducah plant for enzyme production or for joint ventures. Only 25 per cent of the facility is now used for phenylalanine production. The company had been reserving the remaining space for expansion in other areas.

Glick says that Genex's loss for 1985 would be less than last year's despite the setback, largely because the high cost of starting up the Paducah plant was included in last year's accounts. Searle refuses to comment on the decision not to renew its contract with Genex. Searle is known to have expanded its own capacity for producing phenylalanine and it also purchases an undisclosed quantity from Ajinomoto in Japan. Genex's process involved an enzyme reaction of cinnamic acid. Ajinomoto (and Searle, which licenses the process from Ajinomoto) uses selected strains of bacteria and fungi that produce large amounts of phenylalanine from sugar.

Searle patents on the use of aspartame expire in the United States in 1992 and in Europe beginning in 1987. Genex's plant is producing about 2,000 metric tons of phenylalanine a year, about two-thirds of Searle's current need, according to independent estimates. It is estimated that by 1987 the demand for phenylalanine will be 5,700 metric tons per year. Searle's sales of aspartame last year were worth a total of $585 million. Stephen Budiansky

AIDS

France to screen blood donors

FRANCE could begin testing all blood donors for antibodies to the virus causing AIDS (acquired immune deficiency syndrome) "within a week", and donors may be told the results. This dramatic action to limit the spread of AIDS in France is presaged by the announcement by the Prime Minister, Laurent Fabius, in the National Assembly last week that, "after much reflection", he had decided that a test must be "obligatory".

The tests will be carried out by the blood transfusion service SNTS (Service Nationale de Transfusion Sanguine), which has been evaluating three different commercially available tests. One is French, prepared by the Institut Pasteur and marketed by the drug company Sanofi, and two US — one from Abbott Industries and the other from Electro-Nucleonics (distributed in Europe by the Dutch company Organon). All three tests are based on the ELISA technique (enzyme-linked immunosorbent assay) using killed virus. Because of the loss of envelope protein during the preparation, all of them are more sensitive to antibody to the virus inner protein (*gag*) than to the envelope protein antibodies.

Exactly which test will be selected is not for SNTS to decide, but Fabius spoke favourably last week of the French product, "which could be exported throughout the whole world". SNTS say that tests could begin within a week, although Fabius himself did not announce a date. He did, however, indicate a cost — FF 200 million (£20 million) a year.

On the question of informing donors if AIDS antibody is detected, Fabius is not yet convinced. He has asked for a "more detailed" analysis, and has set up a committee of experts to report to him as soon as possible. The tests are necessary, but unnecessary fears should be avoided, he said. **Robert Walgate**

Thermonuclear fusion

Inertial confinement in trouble

Washington

ANTARES, the world's most powerful fusion research laser, could face premature retirement later this year after only two years in operation, unless the US Congress takes pity on the $63.5 million instrument. The laser, at Los Alamos National Laboratory, is being used for experiments in inertial confinement fusion, but has proved to be the instrument of its own fate: it has clearly demonstrated that carbon dioxide lasers such as Antares have no potential for economic fusion. Hopes of a reprieve are now pinned on the possible use of Antares in the Strategic Defense Initiative (SDI) programme of research into anti-missile defences.

Antares is capable of producing a 40-kilojoule pulse of light lasting for one nanosecond. The problem is that radiation of wavelength 10.3 micrometres is, with the benefit of hindsight, too long for economic fusion power. When focused onto a glass bead containing fusion fuel, radiation of this wavelength stimulates the emission of hot electrons that pre-heat the fuel and so prevent the high degree of compression and simultaneous high temperature needed to achieve plasma ignition. Carbon dioxide lasers would be useful only if their cost dropped drastically, to about $10 per joule. This is an unlikely prospect; Antares costs $1,500 per joule, and the cheapest imaginable mass-produced carbon dioxide laser would still cost $300 per joule.

The administration's inertial confinement fusion budget request for fiscal year 1986 assumes that Antares will be taken out of service before the end of 1985. The total request at $70 million is about the figure for the current year. Donald Kerr, director at Los Alamos, has testified before Congress that about 100 people may lose their jobs in inertial confinement fusion if the proposed cuts go ahead.

David Cartwright, principal programme manager for inertial confinement fusion at Los Alamos, has recently informally requested an extra $5 million to allow the current series of experiments on Antares to be completed, because recent results suggest that the temperature of the troublesome hot electrons can be reduced by increasing pulse duration from about 1 nanosecond to 5 nanoseconds. This sum would nevertheless keep Antares in operation for only a few months, and would not allow modifications for new experiments. Hence the hopes that the SDI organization might take an interest.

Cartwright says Antares is the only national facility that could be used to test the predicted effects of high intensity laser radiation on nuclear warhead re-entry vehicles. The pulse length and power could without much difficulty be increased to a few microseconds and 0.25 megajoules, a region of importance for laser weapon concepts. Mirrors would have to be repositioned so that, instead of converging in three dimensions on a single point, the laser beams would illuminate a larger spot from one side. A request for $15 million has been made to the SDI organization for this purpose. Antares might also be used to drive X-ray lasers, also of SDI interest.

Congress is sympathetic to the idea of keeping Antares running, and both houses have authorized sums of around $145 million for inertial confinement fusion — twice the administration's request. The appropriations hearings, which will decide how much will actually be spent, have, however, only just started.

Some support for Antares has come from the National Academy of Sciences, which is carrying out for Congress a study of inertial confinement fusion. The committee responsible has made known its opinion that it would be unwise to dismantle a facility while its usefulness is still being evaluated. Congress might therefore take this cue to override the Office of Management and Budget, as it has been known to do before. Congressional aides warn, however, that there may be jurisdictional obstacles to supporting Antares directly through the SDI organization, if only because that would entail a transfer of funds between two subcommittees. On Capitol Hill, subcommittees' territories are guarded jealously, and the political difficulties are seen as far more daunting than the technical modifications that Antares itself would need.

The other major fusion research laser facility in the United States, known as Nova, is close to completion at Lawrence Livermore laboratory. The light produced by the neodymium-glass lasers in Nova has a wavelength of around 1 micrometre, but is then frequency-multiplied by a factor of 3 to give light with 0.35 micrometre wavelength. Even this is not expected to

Uranium enrichment

France also plumps for lasers

THE French nuclear fuel company, COGEMA, announced earlier this month plans to enrich uranium by laser, rather than by the diffusion or centrifugation of uranium hexafluoride gas, and said that this would cut costs by a factor of two. But one French observer says the statement is a "bluff" prompted by competition with the United States for the supply of enriched uranium to Japan and by a wish to pre-empt the announcement by the US Department of Energy of its decision to adopt the laser process.

Research on laser enrichment has been under way at the laboratory of the Commissariat à l'Energie Atomique at Saclay, near Paris, for some years. But the feeling in France appears to have been that the technique was not yet required, so that the project has been poorly supported. According to M. Claude Bearnaud, director of the isotope separation research unit at Saclay, it will be 15 years before full-scale laser separation plants are in operation.

Bearnaud spends some FF 100 million (about £9 million) a year on laser separation experiments with the French SILVA process, which is similar to the AVLIS process, developed by Lawrence Livermore Laboratory in the United States, using uranium metal vapour. But following the COGEMA announcement, he expects his budget to rise next year by some 20–30 per cent, taking SILVA to the "pre-pilot phase". The separation of kilogram quantities of uranium will come only later, Bearnaud says. The Lawrence Livermore Laboratory, after a year's frantic competition with the Oak Ridge advanced gas centrifuge, has now claimed the successful enrichment of some five kilogrammes of uranium.

Even so, says Bearnaud, there is no commercial need for a laser plant as yet. So why the US haste? One British laser physicist believes the spur may be the need to accumulate separated plutonium for the increasing numbers of US multi-warhead (MIRV-ed) missiles. In these missiles space is at a premium, and plutonium, with a lower critical mass than uranium, makes smaller triggers. The United States has plenty of plutonium in spent fuel from its 90-odd pressurized water reactors, but that is contaminated with isotopes which dampen the fast chain reaction which is essential in warhead applications.

AVLIS, or SILVA, could help. The principles could be applied to any isotope system, but Bearnaud says he is not at liberty to say if his group has worked on plutonium.

For commercial uranium separation, on the other hand, French sources are more forthcoming. Estimates are that the optimum size of a laser plant will be some ½–1 million units "separative work" (SWU) a year, enough to supply three to six 1,000-MW pressurized water reactors with fuel. By comparison, the giant Eurodif diffusion plant at Tricastin in France is capable of nearly 11 million SWU a year, but is under-utilized. Laser separation will be cheaper in the long run, according to Bearnaud. Indeed, COGEMA is said to have offered in Japan to halve future enriched uranium prices.

Robert Walgate

be economically viable for fusion; the best hope among lasers is probably the krypton fluoride excimer laser, which has a wavelength of 0.25 micrometres and which has been developed since work on Antares and Nova began. Los Alamos has a small experimental krypton fluoride laser known as Aurora. Many now believe, however, that light ion beams will beat lasers to achieving plasma ignition. What might be the first machine to reach plasma ignition, known as Particle Beam Fusion Accelerator-2, will begin tests early next year at Sandia National Laboratory in New Mexico. **Tim Beardsley**

Proliferation

THE atomic vapour laser isotope separation process (AVLIS), chosen by the US Department of Energy for separating the fissile uranium-235 isotope from natural uranium (see above), relies on selective three-photon ionization in an electric field, which sweeps out the ionized uranium-235.

Success depends on the relatively large (1 GHz) isotope shifts of spectral lines of the actinide elements on the low thermal velocities of such heavy atoms (implying reduced Doppler broadening) and the use of laser beams impinging perpendicularly on a collimated jet of uranium vapour (reducing Doppler effect still further).

In principle, the excitation could be effected in two steps, by a photon carefully tuned to an internal excitation and a photon of almost any wavelength to take the excited electron out to the continuum, leaving the atom ionized. But the ionization energy of uranium-235 is so high that the two photons would have to be at the blue end of the spectrum, where tunable lasers are expensive and where photons tend to degrade laser mirrors. So AVLIS uses instead two low-energy mid-spectrum photons, produced by a dye laser, to effect the finely-tuned selective excitation. A third ultraviolet photon is then used to take the excited electron far out into the continuum, where auto-ionization resonance increases the ionization cross-section tenfold.

The use of two successive excitation photons also has the advantage that each can be fired from different sides of the uranium jet, so that the first-order Doppler effects cancel. The Lawrence Livermore dye lasers are pumped by a high-efficiency high repetition-rate copper-vapour laser which has the advantage over continuous lasers of an extremely high average power.

According to one British laser physicist, such devices "could be run in a university laboratory" to separate kilogram quantities of fissile uranium-235. If a dye-laser system were established in Libya, for example, "it would be invisible", which is "why we're worried about them".

Robert Walgate

Hubble telescope

Space institute's wings clipped

Washington

A STUDY of the Space Telescope Science Institute (STSI) by a National Research Council task group has concluded that the ever-expanding institute should not be allowed to grow much beyond its present size. The study, which was commissioned by the National Aeronautics and Space Administration (NASA), says that new scientific functions required for the Hubble Space Telescope should instead be contracted to outside organizations, with STSI restricted to an advisory role wherever possible.

The task group undertook its study in the light of widespread concern in the



astronomy community about the institute's rapid growth since it was established in 1981. NASA originally planned an institute with a staff of 100 and an annual budget of less than $5 million; STSI's budget for this year, however, is $12.8 million and is expected to reach $21 million by the end of the decade (at 1984 prices). The staff already numbers over 200 and is expected to top 250 before long. Some fear that the institute might squeeze out other NASA astronomy projects.

The task group, chaired by William Gordon of Rice University, agrees that NASA's original idea of having a special institute to manage science for the space telescope was a good one, and says the institute, which is based at Johns Hopkins University, has done a good job of surmounting the many unplanned technical obstacles that have beset the space telescope. But the task group euphemistically suggests that STSI's stated objectives should be modified by adding a requirement that STSI work "within available resources". What those available resources should be is not specified, although the task group notes that STSI was intended to be comparable in size with other ground-based astronomy facilities; that being the case, STSI is already close to the upper limit.

Gordon's task group also has some implied criticism for the Association of Universities for Research in Astronomy (AURA), the organization that is nominally in control of STSI. Gordon says that AURA should play a more active role in mediating in the frequent jurisdictional conflicts that have arisen between NASA and STSI.

Other recommendations include the suggestion that STSI develop remotely accessible data archives and software that can be run on computers at researchers' home institutions, to avoid pressure on computer time at STSI. The task group also says NASA and STSI should plan a joint public information service for when the telescope is operational. In the longer term, however, Gordon says it is up to NASA to make clear its plan for the future of the institute. **Tim Beardsley**

The report, Institutional Arrangements for the Space Telescope: A Mid-Term Review *is by the Committee on Space, Astronomy and Astrophysics, National Research Council, Washington, DC.*

East German physicist

Absence noted

AT a physics conference in Visby, Sweden, this month, Dr John F. Sharpey-Schafer from the University of Liverpool announced that his paper was dedicated to Dr Stefan Frauendorf of the Zentral Institut für Kernforschung at Rossendorf, East Germany. Dr Frauendorf had been invited to read a paper at the conference, but was arrested last November, and is now said to be facing charges of espionage, based on alleged contacts with West German intelligence services.

Dr Frauendorf had for several years been a regular visitor to the Niels Bohr Institute in Copenhagen, where he was working with several other scientists on a joint project on nuclear structure.

At the time of his arrest, he was in the process of reading and correcting a paper, written jointly with the Niels Bohr team, on high spin states. This paper seems to have disappeared at the time of his arrest and, as there was no copy, much work has had to be redone. A letter from the Niels Bohr Institute, enquiring about the paper and deploring the breaking off of collaboration with Dr Frauendorf, brought only the answer that the physicist was under investigation for espionage carried out over a number of years.

The Niels Bohr Institute has been working with Rossendorf for some 20 years, an association which has been especially fruitful over the past five years or so, roughly the period of Dr Frauendorf's visits. Cooperation arrangements have not been entirely broken off by the incident, however; three further visitors have arrived from Rossendorf since the scientist's arrest. **Vera Rich**

Venus

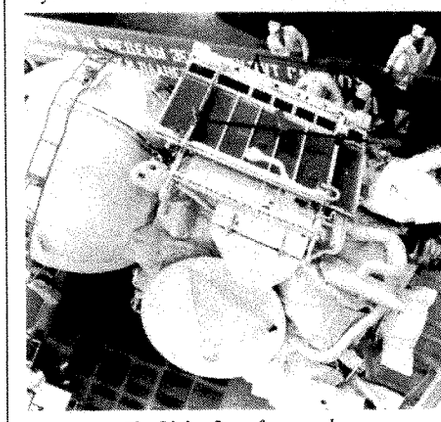
Soviet probes make soft landing

THE first part of the Soviet Veha (Venus-Halley) mission, the descent of atmospheric and surface probes to Venus, has been a success, according to Dr Vladimir Kotel'nikov, first vice-president of the Soviet Academy of Sciences. Indeed, in good Soviet style, the Veha-2 surface probe, which included a rock-gathering minidrill and spectrometer analysis chamber, overfulfilled its norm, working and transmitting data for 22 minutes and 17 seconds rather than for the expected 14 minutes. "Express analysis" of incoming data, moreover, revealed a number of new results, both confirming previous postulates and producing various surprises.

The Veha-2 descent module, which landed in the Rusalka plain (6°27′ S. latitude, 181°5′, longitude), has revealed the presence at that point of soft sandy soil. Previous missions had revealed a more rocky terrain and, although several planetary podologists had postulated that more friable soil might be encountered, this is the first direct evidence of them. Similarly, the two drifting aerostatic probes (carried by helium balloons) directly confirmed the presence of large quantities of sulphuric acid in the venusian clouds. These two probes drifted around the planet's equator for a quarter of its circumference, with a translation velocity of 60 to 70 metres a second. Surprisingly, the balloons encountered updraughts and downdraughts of up to 1 m a second, which hurled them up and down by 200 to 300 m. The drift data from the probes, which were tracked by radio telescopes at Medvezh'i Ozera and Ussriisk in the Soviet Union, Pentikton (Canada), Fort Davis (United States), Madrid, and Parkes (Australia), will be processed in conjunction with the CNES centre at Toulouse. Full results are not expected before the beginning of 1987. At the press conference at the Space Exploration Institute of the Soviet Academy of Sciences last week, speakers stressed that even the initial data "leave no doubt" of the "powerful circulation" in the venusian atmosphere.

This press conference and the approach of the Soviet media in general to this, the first major space event of the Gorbachev era, is instructive. TASS reported the mission without its well-worn formula that this research was carried out in accordance with the directives of the 22nd Party Congress on the application of space to the national economy, which suggests a deliberate effort to take the public into the scientists' confidence.

One radio transmission at 16.30 Moscow time on 15 June took the form of a report from the flight control centre, suggesting that it was happening in real time. An interview with Dr Valerii Barsukov, director of the Vernadskii Institute of Geochemistry and Analytical Chemistry, on volcanism on Venus was "interrupted" by an announcement that Veha-2 had landed. The effect may have been spoiled for discerning listeners by the fact that shorter and less dramatic announcements of the Veha-2 landing earlier that day had also been given out on radio in real time style.



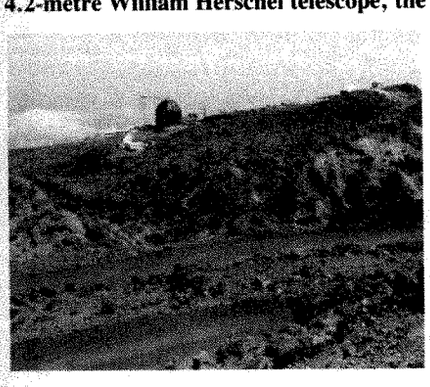
The Veha-2 surface probe.

For the rest, the official spokesmen have stressed the international aspects of the mission. France has participated in providing the apparatus for the aerostatic probes and landing craft, while the Halley's comet part of the mission will involve equipment from "a large number of both socialist and capitalist countries".

Vera Rich

New observatory

THE site of the new international observatory on La Palma, in the Canary Islands, which is to be inaugurated by King Juan Carlos of Spain on 29 June. From left to right the partially constructed dome of the 4.2-metre William Herschel telescope, the



Swedish Solar Tower, the 2.5-metre Isaac Newton telescope, and the 1-metre Jacobus Kapteyn telescopes. Also at the ceremony will be six other heads of state (or their representatives). The participating nations are Spain, the United Kingdom, Denmark, Sweden, the Netherlands and the Irish Republic. (Photograph courtesy of the Science and Engineering Research Council.) □

CERN

Schopper's views on Kendrew

REACTIONS last week to the Kendrew report on the future of British participation at CERN, the European Organization for Nuclear Research (see *Nature* 20 June, p.619), at least from particle physicists in Britain and Europe, are most diplomatically described as a blend of acute scepticism and puzzlement. The scepticism is that a combination of broader international membership and a reduced budget could possibly lead to a 25 per cent reduction in the cost of CERN by the deadline set for 1991, and puzzlement that somebody with Sir John Kendrew's experience of running the European Molecular Biology Laboratory at Heidelberg could ever have thought his panel's recommendations practicable.

Professor Herwig Schopper, the director-general of CERN, while saying he was glad that the report was very positive about the science and management of CERN, emphasized the implications of the recommendations. "Increased membership is not a new idea; some international collaborations have associate members linked to only parts of their programmes, but we have only one major programme [the construction of the electron–positron collider LEP]. Wider membership is a possibility for the future".

A major hindrance to widening CERN's membership in the near future, it seems, is that countries such as Japan and Canada (mentioned by Kendrew last week as possible candidates) would not be attracted by LEP given the advanced state of its development. Looking much further ahead, however, the proposed construction of the Large Hadron Collider in the LEP tunnel would necessitate a significant increase in CERN's budget, and that project could attract a wider membership.

The proposed cut in the cost of CERN of 25 per cent by 1991 would, in the circumstances, have to come from reduced contributions from the member states. Schopper points out that about half of the budget goes on salaries. "CERN's council could decide on lower salaries for new arrivals, but current recruitment rates are low. A decision to reduce existing salaries could result in our being taken to court." So, Schopper says, a 25 per cent cut in CERN's budget would have to come almost entirely from the materials budget, and would effectively emasculate CERN. There is no sign, moreover, that other member states would approve a significant budget cut. "This seems to be a special British problem", said Schopper.

Philip Campbell

British geophysics

Royal Society backs protest

THE management and financial support for geophysics research in Britain are inadequate, while government support is "poorly coordinated, even capricious". This is the theme of the report of a working party on geophysics research set up by the Royal Society under Professor E.R. Oxburgh (Cambridge). The group's report, *Support of Geophysics in the United Kingdom*, published last week, has been endorsed by the Royal Society council.

The starting-point for the working party's argument is what it sees as a mismatch between the rapid growth of reflection seismology surveys for economic prospecting, on which $3,000 million was spent worldwide in 1982, and the scale on which basic geophysics is supported. "If there is one message we wish to convey, it is that geophysics matters to the country", said Oxburgh last week.

The report gives a gloomy account of lost opportunities in British geophysics. The next phase of the Deep-Sea Drilling Programme will "go ahead without British participation" for lack of £2.5 million a year. Except on a "private basis", British scientists will not be involved in the use of the worldwide seismic networks being built by the United States and France for modelling the inner structure of the Earth. It would be "unthinkable . . . at present" to propose British participation in the deep continental drilling programmes being mounted by West Germany or the deep-sea submersible projects of the United States and France. Noting that British geophysicists have in the past done well in elections to the fellowship of the American Geophysical Union, the working party says that the figures also show that "significant numbers of geophysicists have chosen not to work in Britain".

In its analysis of what has gone wrong, the working party says that an estimated 110 academic geophysicists and perhaps as many postdoctoral workers are distributed "in small groups or as isolated individuals" in university departments of physics, geology and even applied mathematics. The report asks for a "greater concentration in fewer centres", offering as models the Mullard Space Science Laboratory of University College, London, and the Robert Hooke Institute for Atmospheric Research at Oxford.

The mechanism of government support for geophysics research is the other chief cause of trouble. The Oxburgh group suspects that the British geophysics community may be too small a constituency of the Science and Engineering Research Council (SERC), which spent £1.2 million on geophysics research grants in 1983–84. This is nearly three times the value of the grants awarded by the Natural Environment Research Council (NERC), most of whose support for geophysics goes on its own institutes and on collaboration ventures.

The working party is stinging in its criticism of NERC (see below), although sympathetic about the council's difficulties. The chief of these is the erosion of commissions for research at NERC institutes from government departments, which is reckoned to have declined from £25 million to £15 million (at present prices) in three years. NERC has suffered "not only its own budget cuts . . . but also enhanced cuts in its contractual income from customer departments".

One result, the report says, is that NERC has been "obliged" to concentrate on work that brings in outside income "with less regard to the scientific content" and often in competition with others. The working party also says that the ability of the institutes to "compete in the marketplace" has been constrained by labour legislation and trades union agreements, citing the way in which the British Geological Survey (the largest institute) has been compelled to take onto its permanent staff a number of people originally hired for short-term projects.

In general, the report says, NERC support for its geophysics institutes ranges from the "marginally satisfactory . . . to the derisory". The Oxburgh group is especially concerned about the danger that geophysical survey work is being neglected. It says that geophysical surveys in the South Atlantic were drawn on by the British government during the Falklands war, contributing to an unquantifiable "saving in lives". The report goes on to say that "strategic national interests are involved, and it is not right that such decisions should depend on the funding priorities of a particular government department at a particular moment".

Noting that the funds made available for new research projects in geophysics have declined by half over the past five years, the working party says that one consequence has been "a pervasive pessimism and loss of morale" among researchers.

The working party is also critical of the lack of coordination between research councils and government departments. Projects falling between NERC and SERC face a "double hazard", where each council has to commit funds before work can go ahead. The report cites the "discouraging history" of a satellite laser-ranger suggested by the Royal Greenwich Observatory, where one council was prepared to meet the high capital cost but the other unable to afford the running costs.

In resignation, the working party turns from British orthodox sources of funds to those that might be found elsewhere. On the basis that much future geophysics will hang on satellite observations, the Oxburgh group asks for greater involvement by the British Department of Trade and Industry, and urges the setting-up of a space research directorate (which has since been agreed). It asks that there should be closer coordination with the military on geophysical problems of mutual interest, and says that British geophysics should take advantage of the interest of the European Commission in the geophysics of energy problems. The report says that industrial companies should spend more on the support of British geophysics, and that problems of commercial secrecy are by the Norwegian experience not the impediments to collaboration they are often said to be. □

NERC pilloried

THE Oxburgh group's criticisms of NERC include the following.

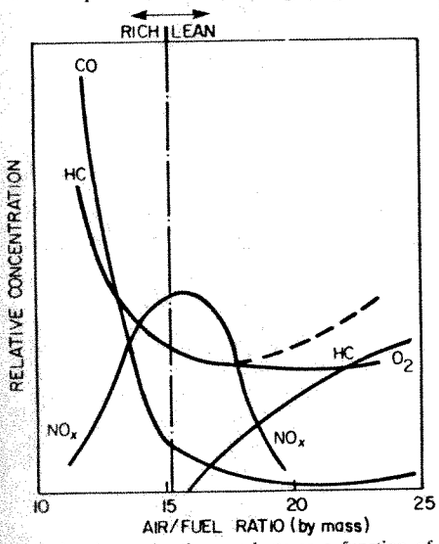
- **"The council, or some of its officers, sees itself as now directing research programmes rather than facilitating research in institutes and universities."**
- **The cost of NERC's central scientific services (mainly a computing network) seems to be too high (at £6 million a year) compared with that on other facilities and decisions about NERC services "are still being made without being approved by appropriate scientific committees".**
- **On the allocation of research vessel time to university scientists, the Oxburgh group complains that people cannot plan ahead. Shortly before the new research ship *Charles Darwin* was delivered last year, NERC laid up most of its other research vessels, forcing academics to put off planned observations. Because allocation of ship-time and running costs are made by different committees, investigators may be blessed with ship-time "but not the much smaller sums" required to make use of it.**
- **The group is concerned about the functioning of NERC headquarters. "We are astonished" at their involvement in minor matters . . . "Directors are not being allowed to direct." Apart from the unregulated growth of the computer network, the group is concerned that active scientists on the council and its committees are not "effectively involved" in the disposition of financial resources. Not surprisingly, the group raises the question whether NERC should continue to be responsible for supporting university research, says that the style of headquarters management makes NERC seem "bureaucratic and insensitive" and asks, as one of its principal recommendations, that the Advisory Board for the Research Councils should consider what should be done.**

In a statement put out earlier this week, Mr Hugh Fish, now confirmed as chairman of NERC, said that he took "great exception" to parts of the report (but a spokesman declined to say what these are). Mr Fish said he plans "to do no more" than seek a discussion of the report with the president of the Royal Society. □

Pollution from automobiles

SIR — A recent article by A. Lubinska[1] discusses the relative merits of catalytic converters versus lean-burn engines. In that comparison she states that "The high air/fuel ratio of the lean-burn engine lowers both carbon monoxide and nitrous oxide emissions, but increases hydrocarbon emissions...."

Nitrous oxide, N_2O, is emitted only in small quantities by internal combustion engines, while nitric oxide, NO, is produced in large quantities[2]. In addition, N_2O is inert in the lower 10 km of the atmosphere while NO plays a major role in both photochemical smog (O_3) formation and acid deposition. She appears to have confused the two gases.



Variation in combustion products as a function of air/fuel ratio[4,5]. NO_x is the sum of NO and NO_2; these two compounds are rapidly interconverted in the atmosphere. The dashed line shows the result of misfiring.

More importantly the article tends to oversimplify a complicated issue. Lean-burn engines have advanced significantly in recent years, but they are technically complex and not immediately available for mass-production. Not all types of vehicles are suitable for lean-burn engines; the increase in gasoline (motor fuel) economy is accompanied by a decrease in power.

As the air/fuel mixture becomes leaner (higher on figure), NO emissions increase before they decrease. Lean-burn engines must run at an air/fuel ratio of 22 to 25 before nitric oxide emissions are significantly reduced. At such lean mixtures, ordinary engines misfire and hydrocarbons rise dramatically. A lean-burn engine must have an efficient fuel-injection system to avoid misfiring[6]. The more advanced fuel injection system and other modifications add to the price of an automobile, expenses neglected in Lubinska's article.

Catalytic converters are more readily available than lean-burn engines but even the implementation of catalysts in European cars is non-trivial. They have been used successfully in the United States but Europe offers more extreme driving conditions. There are more congested cities where the catalyst tends to be too cold and autobahns where routine speeds approach 160 km per hour (100 miles per hour) and the catalyst tends to be too hot.

Lead, even in very small amounts, poisons catalytic converters. In the United States, unleaded gasoline is federally mandated and nearly all driving is within national boundaries. In Europe, where drivers frequently cross national borders, lead-free gasoline may not be uniformly available.

In summary, lean-burn engines are a promising alternative to catalytic converters, but they too have problems. The reduction of air pollution from automobiles is not simple, and a variety of techniques is sure to be needed.

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Research councils

SIR — Whatever the faults of Whitehall, it is never lost for ideas to make research councils more efficient. Last year the Comptroller and Auditor General[1] warned that by appointing the best candidate for the job, the research councils were being flooded with staff of unnecessarily high calibre. This was part of the wider problem of fluid grading. Several research councils, although not the Medical Research Council (MRC), were guilty of basing promotion on the ability of the individual, rather than the name of the job. These suggestions were made in all seriousness and the research councils have duly promised to mend their ways.

It has now been realized that what research councils need, to make them more effective, is flexibility through more short-term and fewer career appointments[2]. It is well known that a civil servant who shows signs of starting to understand a job must be moved on as quickly as possible. This strategy, which has done so much for the British civil service, could well be expected to transform the work of the research councils.

There are one or two difficulties. The Advisory Board for the Research Councils has itself pointed out[2] that scientists on short-term contracts tend to spend the first half of their time learning to do the work and the second half looking for another job. Moreover, the absence of any prospect of employment after the age of 35 could conceivably deter first class candidates from applying. The research councils hope that with some alteration of pension arrangements, those on short-term appointments will move on to jobs in industry though, outside a few narrow specialties, it is far from certain that this is what industry wants.

PhDs with postdoctoral experience are deemed overqualified for the majority of jobs in industry. Most of the remaining jobs require industrial rather than research council experience. As an indication, over the past 3 years, 230 MRC scientists came to the end of short-term appointments; 13 obtained permanent appointments with MRC and 14 obtained jobs in industry. Most of the remainder went on to yet another short-term appointment. It would be no harm if MRC were permitted to let short-term staff benefit from the pension contributions made on their behalf, rather than to use these contributions, as at present, to subsidize the pensions of other staff members, but it is doubtful if this would fully compensate for such appalling career prospects.

Scientists vote with their feet, and short-term appointments tend to produce few applicants, often of indifferent ability. Permanent appointments attract large numbers of applicants, many of outstanding ability. It has never been explained why it is thought that employing less able scientists on disadvantageous terms will produce greater flexibility and effeetiveness than employing more able scientists on advantageous terms. Perhaps the only moral is that the research councils are capable of mismanaging their affairs quite badly enough without the assistance of Whitehall.

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Embryo research

SIR — In the controversy over the status of the human embryo, there exists a state of doubt: some say it is a human being, others say it is not. The views seem fairly equally balanced.

In this impasse, the next step is to examine the consequences should one side or the other eventually be proved to be indisputably correct. On the one side we risk losing a valuable research opportunity — on the other we risk committing murder.

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Observational plate tectonics

The latest report of the international radio-interferometric survey IRIS suggests that the direct measurement of plate movements lies just ahead.

THE exciting prospect that radioastronomers will be able, by the use of long-baseline interferometers, directly to measure the motion of tectonic plates, will be a little delayed, it seems. That is one cautious reading of the latest report on four years of work with the international network of geodetic observing stations operated under the title IRIS (for International Radio Interferometric Surveying), which is itself an outgrowth of the combined operation of the US project POLARIS (for Polar Motion Analysis by Radio Interferometric Surveying) and the West German geodetic radio telescope at Wettzell, Bavaria. In an account of observations to the end of November 1984, W.E.Carter, D.S.Robertson and J.R.MacKay of the US National Geodetic Survey (*J.geophys.Res.* **90**, 4577–4587; 1985) quote estimates of the rate at which two baselines are changing, but with errors that are still too great for comfort.

The idea that radio-interferometry might be used for checking on plate tectonics has been in the air ever since radio telescopes separated by a substantial distance were first used for the accurate measurement of the angular size of distant radio sources, quasars for example. But the technology of very long baseline interferometry (VLBI) is only now good enough, and the network of observatories enough, to bear on plate tectonics.

Schematically, the technique is simple enough. Several telescopes simultaneously observe the same distant radio source, recording their signals on magnetic tape (together with an accurate measure of time, most conveniently provided by a local hydrogen maser). In general, there is a time-lag between the receipt of the same waveform at one station and another which, when multiplied by the velocity of light, will be a simple measure of the separation of the two antennas along the line of sight. But given that the Earth rotates, the time-lag will change systematically with time, and in such a way that it should be possible to infer from the observations not merely the relative geometrical position of the two antennas, their baseline separation included, but also the instantaneous position of the axis about which the Earth is rotating.

Practice is, as always, more difficult. One heartfelt complaint by Carter *et al.* is that the sheer weight of the magnetic tape which has to be assembled at a comparator centre after a day's observation by a handful of stations may amount to several hundred kilograms, "a troublesome constraint limiting the growth of geodetic VLBI". Even so, the planners intend adding to the four core stations (Westford, Massachusetts, the Agassiz station in Texas, Richmond, Florida, and Wettzell) cooperation with the Kashima Observatory in Japan and the Shanghai Observatory on mainland China. Other radio telescopes in the United States and elsewhere join in the network as the opportunity arises; that at Onsala, Sweden, takes part for one day a month.

The most startling result so far seems to be that of the measurement of the Earth's pole position, which has been determined since the beginning of 1984 with an accuracy of 2 milliarc seconds (or 6 cm on the ground). This, at least, is the discrepancy between the VLBI measurements of the pole position and those obtained quite independently from satellite ranging, which confirms the accuracy of both sets of measurements. VLBI measurements every three or five days may be enough to keep track of this polar (Chandler) wobble to within a milliarc second.

For what it is worth, the 1984 observations put the position of the pole on a smooth arc destined to form a near-circle some 16.5 metres in diameter, not very different in amplitude from that measured in the previous year but more than twice as great as the amplitude of the Chandler wobble in the two earlier years. Because the VLBI measurements will be made in any case, and because they can be quickly reduced, they will quickly become the preferred way of tracking the wobble.

The data have also thrown up a wealth of detail about departures of the Earth's rotational speed as given by Universal Time (strictly, UT1). Departures from regularity leading to fluctuations of up to 6 milliseconds occur, with some excursions lasting for several months and others for only a few days. It should be possible to monitor the regularity of UT (or of the Earth's rotational speed) to within 0.1 milliseconds by means of a daily hour-long measurement along a single baseline. That is also a by-product well worth having. Daily monitoring may even make it possible to tell which fluctuations of rotational speed are attributable to what.

Other by-products include some information on the nutation of the Earth's rotation, which reflects displacements between the spinning core of the Earth and the outer solid hollow sphere. The Earth tides responsible for the rhythmic fluctuation of the altitude of the radio telescopes in the network are also evident in the data.

So what is there to be said about the baselines and their variation? It is obviously easier to extract from the measurements of the time-lags and their variation with time the quantities whose time-dependence takes a predictable form. This is one reason why fluctuations of the Earth's rotational speed can be monitored so easily; the time-lag between any pair of stations in the VLBI network will be a sinusoidal function of time whose frequency is that of the Earth's rotation.

By contrast, the baseline lengths appear as factors in the constants by which these variable quantities are multiplied, so that the accuracy with which they can be determined is no greater than that of the observations as a whole. Even so, baseline measurements are said to be repeatable to within about one part in 100 million, a mere centimetre or so of error for the longest baselines, for the time being those based in Europe and the United States.

This is the promise for plate tectonics. Many of the movements expected should be readily verified as further measurements of the baselines accumulate. Indeed, Carter *et al.* tantalize their readers with an estimate of the rate of change of the baseline between Westford (Massachusetts) and Onsala (Sweden) and that between Westford and the Texas station. The conclusion is that the trans-atlantic baseline is lengthening by 14 mm a year (with an estimated uncertainty of 3 mm) and that the baseline between Westford and the Texas station is shrinking by 8 mm a year (with an uncertainty of 1 mm).

The sense of these results, at least, is consistent with what plate tectonics would predict; the contraction of the US baseline, for example, is thought to be a consequence of compressional stress on the American plate at least in the region between the Appalachians and the Rockies (see D. I. Gough *et al. Nature* **305**, 619–621; 1983). The fly in the ointment is the suggestion of seasonal errors in the baseline measurements, implying a need for some refinement of the corrections for transmission through the ionosphere and atmosphere. The general interest now will be to see how quickly the VLBI collaboration will be able to accumulate further data. Another year's observations should make all the difference. **John Maddox**

Meteorites

Earthward bound from chaotic regions of the asteroid belt

from Carl D. Murray

It is easy to accept that meteorites, those occasional stones that fall from the sky, are composed of asteriodal material that has been delivered to the Earth. The problem has always been to demonstrate how, subject to some severe observational constraints, even a single fragment in an asteroid-type orbit can eventually cross the path of the Earth. On page 731 of this issue, Jack Wisdom of the Massachusetts Institute of Technology, guided by known locations of chaotic regions in the phase space at one particular place in the asteroid belt, describes a trajectory that can evolve from a fairly typical low-eccentricity orbit to the high eccentricity necessary to achieve the elusive Earth-crossing orbit.

While there are suggestions of a lunar or even martian origin for some of the meteoritic material that has been examined, photometric analysis of most meteorites strongly supports an asteroidal origin. Meteorites are typically thought to be fragments arising from a minor collision between two asteroids. (The asteroid belt is continually evolving because of collisions, which have average encounter velocities of 5 km s^{-1}). The fragments, which will have slightly different orbital parameters from their parent bodies, eventually enter Earth-crossing orbits.

Although the details of this final process are not known, we have some idea of how long it takes. Any fragment that was initially buried more than a few metres inside its parent body will have been shielded from the effects of cosmic rays up to the moment of impact. From chemical studies of meteoritic samples, it has been possible to calculate for how long they have been exposed to cosmic rays and so when the collision which generated them occurred. The exposure ages are generally around 10^7 years for the common stony meteorites, putting severe time constraints on any delivery mechanism.

Regions of the Solar System that might be expected to act as a source for meteorites fall near the low-order orbital resonances of Jupiter. An asteroid occupying the 2/1 resonance, for example, orbits the sun in half the orbital period of Jupiter. Thus, over successive orbits the asteroid will experience a repeated and cumulative gravitational perturbation from Jupiter that could tend to remove it to a different orbit. Prominent gaps (Kirkwood gaps) in the asteroid belt occur at such resonances and Wisdom has already demonstrated[2] how the 3/1 resonance can be cleared of asteroids. He showed the existence of a large chaotic region of the phase space (defined by positions and velocities) in the vicinity of the resonance. In such a chaotic region an asteroid could experience large and essentially random changes in its orbital parameters, with the final orbit being sensitively dependent on the initial conditions. The key point is that the equations of motion are non-linear and the resulting deterministic chaos is now being recognized as a common feature of non-linear equations for certain sets of starting conditions. The most critical orbital parameter for the asteroid's survival is its eccentricity. A value larger than about 0.3 implies that it will cross Mars. Having shown how such eccentricities could be achieved in the chaotic zone, Wisdom invoked Mars as the final remover of material, whether by impact or scattering. It seemed natural, therefore, to use knowledge of the locations of the chaotic regions as a starting point in a search for regions that could produce Earth-crossing material.

In a paper presented to the Meteoritical Society last summer, George Wetherill of the Carnegie Institution in Washington DC, claimed that the observed influx of the ordinary chrondrite meteorites could be accounted for only by gravitationally accelerated material from a region in the vicinity of the 3/1 resonance[3]. Wisdom has now provided details of such a mechanism using the known locations of the chaotic regions as a basis for finding Earth-crossing trajectories. When he integrated different sets of starting conditions, at the fifth attempt he found a trajectory which became Earth-crossing. The equations he integrated have sufficient degrees of freedom for Arnol'd diffusion to occur — whereby an orbit, given enough time, can eventually 'leak' into different regions of the phase space[4]. (Arnol'd diffusion has yet to be identified in any Solar System integration since the timescales involved are almost certainly greater than the age of the Solar System.) Wisdom's result is that the orbit evolves from low to high eccentricity within a few hundred thousand years, thereby satisfying the cosmic-ray exposure age constraint. The beauty of the mechanism is that it does not involve any complicated procedures that require more than one close approach to another planet.

The 3/1 resonance does not have a monopoly on chaotic regions — the boundaries of all resonances are chaotic, the extent of the chaos depending of such factors as Jupiter's eccentricity and the strength of the resonance. In the past, celestial mechanicians have devoted much effort to the study of the prominent first-order resonances in the asteroid belt, trying to explain such features as the 2/1 Hecuba gap and the 3/2 Hilda group of asteroids. It now seems that an understanding of the dynamics of the innocuous 3/1 resonance will provide answers to some of the most fundamental problems of Solar System dynamics. □

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Avian ecology

Mass mortality in Pacific seabirds

from Jared M. Diamond

What is the evolutionary importance of rare events? Does natural selection proceed constantly, or are its effects concentrated during episodes of widespread mortality? R. and E. A. Schreiber (*Science* **225**, 713; 1984) address this question for some of the world's longest-lived vertebrates, victims of a recent catastrophe.

El Niño Southern Oscillations (ENSOs) are recurrent, widespread climatic events involving major warming of the whole eastern tropical Pacific Ocean. The most recent ENSO, in 1982–83, was the most severe yet recorded. At the site of the Schreibers' study — the low atoll of Christmas Island in the central Pacific —annual rainfall, normally 30 inches, reached 156 inches in the year beginning July 1982, including 16 inches in one day. Among the consequences were extensive flooding of the island, a drop in oceanic primary productivity of phytoplankton dependent on cold surface waters, and hence depleted stocks of fish and squid ultimately dependent on that primary production. Eight million seabirds of the eighteen species that breed on Christmas Island feed on those fish and squid. Although most temperate zone song-birds breed in their second year, lay 3 – 10 eggs and have life expectancies of a few years, tropical seabirds typically have a single large egg with a long incubation time, long periods of juvenile dependency on parents, late onset of breeding and very long lives. Long-term studies of Christmas Island seabirds have revealed many details of their leisurely life cycles. Some

species have lifespans of 50 – 70 years, more than the lifetime of the rings used to tag them. The tropic birds breed only after the age of 5 years, live at least 35 years and form permanent pairs. A young frigate bird is fed for about one and a half years by its mother (the father leaves after 1 month, probably to nest with another female). Fledged sooty terns fly to sea, do not alight for 9 years (presumably sleeping in flight), then infallibly return to their natal island, where they court on the wing, finally alight to copulate, and lay the egg on the next day.

From 1940 to June 1982, a period that included seven ENSOs, there was no recorded instance of total reproductive failure for any seabird species at Christmas Island. During the Schreibers' June 1982 visit, just before the onset of the ENSO, seabird populations were still normal. But by November 1982 there was total reproductive failure for all species. At that time five species (including the two that are usually most abundant) were completely absent, while the remaining species were represented by few adults, with no nests or else nests with dead, underweight or starving chicks. Nests in ground burrows were flooded, many tree nests had been washed out and young in surviving nests starved.

What happened to the millions of adult birds that should have been breeding then on Christmas Island? Did they, too, die, or did they simply go elsewhere until conditions improved? The Schreibers found almost no skeletons of adults on the island, yet bird numbers were still far below normal in October 1983. It is thought that 90 per cent of the breeding seabirds, including those present and attempting to breed in July 1982, died at sea from starvation.

The low reproductive potential of tropical seabirds suggests that it will be years before the breeding colonies of Christmas Island have recovered to their former numbers. Thus, a large-scale natural experiment in fluctuating selection is underway. Intraspecific competition will be relaxed for a long time: how will this affect reproductive effort and individual variation in morphology? Was the mortality in 1982 indiscriminate and total, as presently assumed, or did a few of the fittest survive? These long-lived birds must experience many 'normal' ENSOs in their lifetimes, and a severe one every couple of generations; could there be features of the life histories of the birds that have arisen as adaptations to these rare events? The answers to these questions may emerge from the long-term studies on Christmas Island. □



Frigate bird fishing — one species of seabird that breeds on Christmas Island (photograph courtesy of R. and E. A. Schreiber).

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Meteorology

Clouds and climate regulation

from Richard C. J. Somerville

THE role of clouds in regulating climate is still largely a mystery, and the treatment of clouds in conventional climate models may be seriously inadequate. If cloud effects are as strong as some recent research suggests, considerable uncertainty must characterize the results of even the most advanced present-day models of climate. Predictions of the climate changes resulting from burning fossil fuels, which adds CO_2 to the atmosphere, for example, depend strongly on how clouds are modelled; clouds may tend to amplify changes, or they may act as a giant global thermostat, helping to stabilize the climate.

The potentially large influence of clouds on climate arises from their effects on the transfer through the atmosphere of both incoming solar and outgoing terrestrial radiation. That clouds may dramatically affect surface temperature by both these mechanisms is a matter of everyday experience. Daytime maximum temperatures are substantially lower on cloudy days than on fair ones, and cloudless nights are cooler than overcast ones, other factors being equal. These commonplace examples illustrate the two major influences of clouds on the planetary radiation budget: clouds can alter both the planetary albedo, or reflectivity, and the greenhouse effect, by helping to trap radiation which otherwise would be lost into space.

The average albedo of our planet is about 30 per cent, so only 70 per cent of incident solar radiation is actually available to power the climate system. Clouds cover nearly half the surface of the Earth but account for about two-thirds of the planetary albedo[1]. We do not know whether the albedo or cloud cover were markedly different during, say, the last ice age. In fact, we have no good explanation of the present-day values of these fundamental climatic parameters. It is perhaps even more disquieting that we cannot reliably predict whether or how these parameters are likely to change over the next century, as the concentration of atmospheric CO_2 approaches twice its pre-industrial value. It has recently been estimated, for example, that the temperature increase due to the greenhouse effect produced by doubling CO_2 might be completely offset if the amount of low-altitude cloudiness were to increase simultaneously by 10 per cent[2]. This result emerges from a mathematical model sensitivity study, essentially a calculation of the climatic response to an assumed change in cloudiness.

The predicament of climate theorists is that it is relatively easy to demonstrate that climate is potentially sensitive to clouds — as in the example above — but very difficult to model clouds realistically. Climate models incorporate powerful radiative transfer algorithms that could calculate atmospheric heating and cooling extremely accurately, if only the distribution of all the radiatively active constituents in the atmosphere were known precisely[3]. Two of the most important such constituents, however, are water vapour and cloud, both notoriously changeable and difficult to predict. Idealized mathematical models have been developed to simulate many of the physical mechanisms through which a change in climate might alter clouds, which can then in turn feed back on climate change through their radiative effects[4,5]. Not even the most advanced of climate models, however, simulate all aspects of the present-day global distribution of cloudiness well enough to inspire confidence in cloud forecasts for climates differing significantly from our present one[6].

Many sensitivity studies have shown that climate can be affected strongly by changes in cloud height as well as changes in cloud amount[7]. Recent research has raised the additional possibility that increased atmospheric pollution might alter cloud optical properties enough to influence the global albedo substantially[8]. Another mechanism which might produce a similiar effect is the possible dependence of cloud liquid water content on temperature[9]. In the context of the CO_2 climate problem, both these processes are candidate cooling mechanisms that might at least partially compensate for the warming resulting from the enhanced greenhouse effect. The quantitive importance of these processes is still uncertain, but they illustrate the real possibility that

the effect on climate of feedback from cloud radiation may be significant even in the absence of changes in cloud amount or height.

One important reason for the frustratingly slow progress in this field has been the lack of adequate global cloud observations. Satellite remote sensing has only recently begun to fulfill its considerable promise in this area[9-11]. Parameters that can now be measured with useful precision include cloud amounts, cloud heights, cloud-top temperatures and some optical properties of clouds. Earth radiation budget parameters, such as albedo and outgoing longwave radiation, are now also being monitored by satellites. Although no dramatic breakthroughs seem likely, these data will be invaluable in the gradual development and verification of improved climate models.

For those who are unwilling to wait a century or so to learn whether CO_2 doubling will or will not have its predicted climatic consequences, there may be a quicker payoff from research on cloud-radiation feedbacks. Numerical weather prediction models, on which routine operational meteorological forecasts are based, have become so complex that they are now almost indistinguishable from comprehensive climate models, but the conventional wisdom has long been that short-range atmospheric circulation forecasts, for a day or so in advance, are relatively insensitive to radiative processes. Indeed, such processes were until recently highly simplified or even entirely neglected in the forecast models used by national weather services. As the duration of the predictions increases, however, such cavalier physical simplifications become less justifiable. There is now considerable evidence that a one-week forecast may benefit significantly from more realistic treatments of cloud-radiation interaction[12,13], and improvements in modelling clouds and their effects are likely to increase gradually the competence of an already skilful enterprise. If research on modelling cloud effects can lead to real improvements in one-week forecasts, the benefits to many weather-sensitive sectors of the economy should be substantial.

It is climate theory rather than numerical weather prediction, however, that stands to gain the most from any real advances in our understanding of cloud-radiation feedbacks. It is already clear from sensitivity studies that cloud feedbacks could plausibly either double or halve the typical estimated surface temperature change resulting from increased concentrations of atmospheric CO_2. But our present ignorance of these feedbacks is so great that we cannot even be certain whether the net effect of all the cloud processes is a positive or a negative feedback. □

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Developmental neurobiology

Laminin for axonal guidance?

from Joshua R. Sanes

INTERACTION of neuronal processes with the surfaces on which they grow has been a subject of increasing interest to developmental neurobiologists. *In vitro*, it is clear that adhesion of growing processes, or neurites, to substrates is an important determinant of neurite elongation[1] and that material deposited by non-neural cells can promote and direct neurite extension[2]. *In vivo*, regenerating peripheral axons frequently grow along basal laminae to reach and reinnervate their targets[3]. These two observations have now been brought together in a satisfying way by the demonstration that laminin[4,5], a structural component of adult basal laminae[6], is a major factor promoting neurite outgrowth that is secreted by cultured cells.

In 1978, Collins[7] demonstrated that proteins secreted by heart cells promote neurite outgrowth in a way that distinguishes them from soluble 'trophic' factors such as nerve growth factor (NGF): they bind to the culture dish and seem to act by increasing the adhesion of neurite to substrate. Many cell types have since been shown to secrete such material[7] and efforts are now being made to characterize the responsible factor. These have proceeded along two lines. First, the outgrowth-promoting activities have been purified from medium conditioned by non-neural cells, using neurite outgrowth as an assay. Second, based on the finding that many proteins secreted *in vitro* are components of extracellular matrices *in vivo*, the effects of purified extracellular matrix molecules on neurite outgrowth have been assessed and compared to those of outgrowth-promoting factors. Although several such molecules turn out to be effective substrates for neurite outgrowth, most attention has focussed on laminin, a glycoprotein that is present in virtually all basal laminae and that had already been shown to promote attachment of epithelial cells to collagenous substrates[6]. When tested for neurite outgrowth-promoting activity, laminin seemed to be the most effective of the molecules examined, to affect the widest variety of neuronal types and to be remarkably similar in its effects to the outgrowth-promoting factors secreted by cultured cells[8-13].

As these results accumulated, it became logical to suppose that the secreted outgrowth-promoting activity might actually be laminin, but two observations suggested that it was not. First, antisera to laminin that blocked neurite outgrowth on laminin-coated dishes did not inhibit outgrowth on substrates coated with conditioned medium[4,9,11]. Second, Lander *et al.* found that the factor from conditioned medium contains a heparan sulphate proteoglycan, although they appreciated that the proteoglycan was not necessarily the molecule that the axons recognized[14]. The parallels between laminin and this factor were, however, sufficiently striking to be pursued, and now Lander *et al.*[4] and Davis *et al.*[5] have obtained new evidence indicating that laminin is indeed responsible for the outgrowth-promoting activity in a variety of conditioned media. Laminin co-purifies with the outgrowth-promoting activity through several fractionation steps; it is a major component of the purest factor obtained; antiserum to laminin precipitates outgrowth-promoting activity from conditioned medium; and sufficient laminin is present in conditioned medium to account for outgrowth-promoting activity. The laminin is likely to be complexed with the heparan sulphate proteoglycan previously described[14], because anti-laminin precipitates both molecules from medium. Furthermore, while heparan sulphate proteoglycan alone does not stimulate neurite outgrowth[9], a monoclonal antibody that blocks the outgrowth-promoting activity of conditioned medium[15] recognizes a complex of laminin with proteoglycan (W. Matthew, personal communication).

The failure of anti-laminin to block the activity of the complex remains unexplained and provocative. The laminin molecules in the complex may differ in their primary structure, glycosylation or conformation from the laminin used to generate the serum, or the active site of the complexed laminin might be inaccessible to antibody. It seems odd that the active site of the laminin could be accessible to an axon but inaccessible to an antibody; one interesting possibility is that heparan sulphate on a neuronal mem-

brane could displace heparan sulphate in the complex, giving the axon special access to an active site. In any event, these results argue for caution in interpreting experiments designed to identify active molecules by immunological blockade.

The findings that laminin promotes neurite outgrowth and is a major component of the outgrowth-promoting factor secreted by several types of cells have several implications for research on axonal growth and guidance. First, neurites can be predicted to bear laminin-receptors[6] on their membranes, so it becomes important to identify and characterize these molecules. Second, we will want to learn if and where laminin guides neurite outgrowth *in vivo*. Although the immunochemical difficulties I have already discussed may render interpretation of antibody-blocking experiments difficult, it should be possible to learn how far outgrowth on basal laminae depends on laminin as opposed to other extracellular matrix molecules such as fibronectin and collagen, which also promote neurite outgrowth *in vitro*[8,10,16,17] Since in the embryo central axons grow along basal laminae to reach their targets[18], guidance by laminin may be relevant to the growth of central as well as peripheral axons. Finally, the clinically disastrous failure of mammalian central axons to regenerate following injury is now suspected to be caused, at least in part, by a deficiency in their environment, because they can grow long distances through grafts of peripheral nerve segments rich in basal lamina[19]. Intriguingly, fish optic nerves, which have an unusual ability to regenerate, have very recently been found to accumulate high levels of laminin after injury[11] Could laminin be one of the factors whose absence impedes regeneration in our brains and spinal cords? □

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Astrophysics

Optical bursts – where are they?

from K. Hurley

DESPITE their brevity, and sometimes because of it, transient events are often the most spectacular in astrophysics. Gamma-ray bursters[1], now widely believed to occur on or about neutron stars, are one example; typically they produce a blast of radiation lasting several seconds and with a peak luminosity of at least nine orders of magnitude over the quiescent state, in which they are quite undetectable. Transient phenomena in the optical waveband, apparently associated with some bursters[2,3] have also been detected but, until recently, only on archival photographic plates. Therefore, they were not time-resolved. Now that continuous monitoring of optical bursts is being undertaken, the evidence indicates that they have light curves which strongly resemble their progenitor gamma bursts, and a clever argument has been advanced which uses this fact to constrain the still unknown distances to bursters.

Our present understanding of the gamma-burst phenomenon is still quite incomplete. It is much easier to list the objects with which gamma bursters are not associated, such as steady X-ray and γ-ray sources, than it is to identify convincingly any quiescent counterpart to them. This has proven to be perhaps the major obstacle to progress, as it leaves the distance to the source uncertain by four orders of magnitude. So, although a wealth of circumstantial evidence points to strongly magnetized neutron stars as burst sources in at least some cases, the question of the type of stellar system — if any — in which the neutron star resides has still not been answered.

Perhaps nowhere has the problem been so acute as with the fascinating burster of 5 March 1979[4]: not only was its peak intensity (at Earth) the largest recorded for a gamma burst, but also its localization coincides with the N49 supernova remnant in the Large Magellanic Cloud (LMC), at a distance of 55 kiloparsecs. A straightforward interpretation would be that the burst originated on the neutron star which produced the remnant. But a number of objections can be raised to this idea, not least that, if the source radiated isotropically from the LMC, then for a brief instant it outshone (in γ-rays alone) the entire luminous output of our Galaxy. A tantalizing new piece of evidence was announced last summer at a meeting at Stanford University and subsequently in *Nature*[5] During a five-month period of optical monitoring of the 5 March source, three optical bursts were detected and clearly time-resolved. Their light curves generally resembled the time histories of gamma bursts, although no simultaneous γ-ray activity was noted at the times of the optical bursts (the instruments in orbit at the time were probably not sensitive enough to record it). Thus, the relation between the 5 March gamma bursts and the optical bursts remains tentative. However, as R. London of Lawrence Livermore Laboratory was quick to point out, if the two bursts are associated, the distance to the source is strongly constrained, an idea later confirmed by J.-M. Hameury, J. P. Lasota and S. Bonnazola of Meudon Observatory.

The argument is that if the optical radiation is due to a thermal mechanism (for example, reprocessing of gamma radiation[6] in an accretion disk or in the atmosphere of a stellar companion), the luminosity of the mechanism cannot exceed that of black-body radiation. Then, since the dimension of the optical source cannot exceed ct, where c is the speed of light and t is the rise time of the optical flash, a relation is obtained between the maximum distance to the source and its temperature. That temperature turns out to be at least several million electron volts for a source in the LMC. In general, it is difficult to construct thermal models with these temperatures and furthermore, the 5 March burster has never been observed to emit at these energies. The conclusion is that its source is much nearer than the LMC. In arriving at this conclusion, a number of special models for the optical radiation have to be excluded: coherent emission, sources in relativistic expansion, beaming and special geometries that appear to violate causality.

In addition to providing distance constraints, time-resolved optical flashes may do for γ-ray bursters what they did for X-ray bursters[7], that is, allow an estimate of the dimension of the reprocessing region (via both the rise time and the duration of the flash). In the 5 March case, for example, a reprocessing region with a typical dimension of 30,000 km is indicated, ruling out reprocessing in the atmosphere of a non-degenerate stellar companion and suggesting the possibility of an accretion disk.

But what of the tentative nature of the association between optical flashes and gamma bursts? There has never been any simultaneous observation of the two, and since optical instruments tend to be many orders of magnitude more sensitive (as energy-flux detectors) than γ-ray burst instruments, for the time being only optical flashes associated with the largest (relatively infrequent) gamma bursts can be expected to give rise to coincident detections. Simultaneous direct imaging and photometry of an optical flash should

soon be possible with a unique system now being deployed at the European Southern Observatory. The experiment, being conducted by H. Pedersen, consists of a battery of small automated telescopes each trained on the position of a particular γ-ray burst source.

Much of the uncertainty surrounding optical flashes comes from the possibility that they are generated rather closer to home, for example by meteors in the upper atmosphere. The use of two detection systems separated by even one kilometre eliminates these effects by parallax. Such a study was recently undertaken on an intercontinental baseline[8] and another two-telescope proposal is under consideration by the European Southern Observatory. On top of this, a novel electronic camera array being installed at Kitt Peak National Observatory[9,10], will eventually provide permanent night-time mon-itoring of much of the sky for optical transients down to magnitude 11, with source localization at the arc-second level. It is expected that some twenty optical flashes associated with gamma bursts will be detected per year (not to mention a host of other short-timescale astronomical events), dramatically increasing the probability of a simultaneous optical and gamma-ray detection. This set up will also greatly increase accuracy of localization, in the wake of which may come both the long-awaited identification of the counterpart and the answer to the question of the distance scale. □

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Laser physics

Trapping cooled sodium atoms

from B. W. Petley

SPECTROSCOPISTS have long dreamed of being able to make measurements on atoms or ions which are essentially at rest, and even on single atoms in that condition. For if they could do so, the study of fine detail of atomic transistions would no longer be impeded by the broadening of spectral lines as a consequence of the Doppler effect, or by the fact that, in work with beams of atoms and ions, the accuracy of observations is limited by the time spent by individual atoms and molecules within the action region of the apparatus. One practical consequence could be the further refinement of the accuracy of atomic frequency standards.

With the recent publication of techniques for using lasers to 'cool' atoms, or to rob them of translational velocity, and with the availability of electromagnetic traps for small numbers of atoms, the dream seemed about to come true. Now, Alan L. Migdall, John V. Prodan and Bill D. Phillips of the Gaithersburg laboratories of the National Bureau of Standards, together with Thomas H. Bergeman and Harold J. Metcalf of the State University of New York, Stony Brook, have reported the trapping of laser-cooled sodium atoms. The results were presented at the spring meeting of the American Physical Society on 24 April 1985 and have just been published (*Phys. Rev. Lett.* **54**, 2596; 1985). Other groups are rumoured to be close to success as well. Thus, Metcalf (see opposite) reports that A. Ashkin and his colleagues have extended their laser trapping techniques from 10 mm oil droplets to sodium atoms. We can expect a lively conference season in the months ahead.

The first-order Doppler effect, which is proportional to v/c for individual atoms and for a source in thermal equilibrium, usually results in a broadening of spectral lines by a few GHz (or about 0.1 cm^{-1}). The use of tuneable dye lasers in conjunction with atomic and ion beams has for some time made it possible to observe only those atoms that are travelling perpendicular to the line of sight, which for practical purposes removes the first-order Doppler broadening. For many purposes, however, the second-order (relativistic) effect, proportional to $(v/c)^2$, remains an impediment to accurate measurements.

The laser cooling techniques recently reported follow earlier suggestions by Letokhov and others in Moscow, by Schawlow and Hansch at Stanford and by Wineland and Dehmelt. A beam of atoms is decelerated by absorbing a counter-propagating beam of laser light. The objective is to cool such beams to milli-Kelvin (mK) temperatures. The trade-off with laser cooling is that, as atoms are slowed by absorbing N counter-propagating photons in the beam direction, they are heated in the transverse direction by the isotropically re-emitted photons as $N^{1/2}$, which may amount to 50 mK for 20,000 photons. As the atoms have an energy spread, it is convenient to express this as a 'temperature'.

Although laser cooling had already been demonstrated by groups in Moscow, Washington and elsewhere, optical pumping effects, which deplete the proportion of the atoms in the state absorbing the counter-propagating radiation, had hindered the achievement of the desired mK temperatures. Each time, for example, that a sodium atom absorbs a 589 nm photon its velocity is slowed by about 3 cm s^{-1}, but optical pumping effects and Doppler shifts that occur after the absorption and isotropic re-emission of about a hundred photons, limit the velocity reduction to about 3 m s^{-1}, which is a long way short of the reduction by about 600 m s^{-1} required to bring the atoms to rest.

By overcoming these optical pumping effects, two groups have successfully cooled atoms to mK temperatures. Both used sodium atoms, whose 589-nm transition is so conveniently accessible with the rhodamine-6G laser dye, and both used the same transition (Fig. 1) using the indicated σ^+ line to slow the atoms. Diverging beams of atoms were cooled by a counter-propagating laser beam which converged at the same angle (3 mrad) onto the source. For this transition, the optical pumping effect results from the decay from an upper level into the F=1 ground state. In addition to avoiding populating such levels, the frequency of the incident radiation must also track the changing Doppler shift during the absorption of the 20,000 or so photons required to bring the atom to rest. Where the two groups differ is that one changed the frequency of the laser and the other the frequency of the atomic transition as the atoms are slowed.

W. Ertmer *et al.* (*Phys. Rev. Lett.* **54**, 996; 1985) swept the laser frequency using an acousto-electro-optic travelling wave modulator and added a further weak modulation 1,772 mHz side-band to pump any atoms which reach the 3S(F=1) state back up to the 3S(F=2) state. This produces a pulse of cooled atoms. On the other hand, J. Prodan *et al.* (*Phys. Rev. Lett.* **54**, 992; 1985) tuned the transition frequency by passing the atomic beam through the magnetic field produced by a tapered solenoid. The magnetic field and light polarization exploited the Zeeman effect to avoid the optical pumping. Deceleration was a two-stage process; the atoms were slowed to a low velocity in the solenoid and then drifted a further 0.4 m into the observation region where they

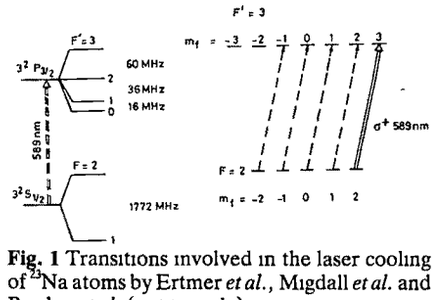


Fig. 1 Transitions involved in the laser cooling of ^{23}Na atoms by Ertmer *et al.*, Migdall *et al.* and Prodan *et al.* (not to scale).

received a further 0.1 to 0.4 ms pulse of cooling laser radiation. The method produces position-dependent cooling. With a strongly saturated transition, the atoms will spend about a half of their time in the upper energy state, radiating at a rate governed by their natural lifetime of about 16 ns. Thus their maximum deceleration would be about 10^6 m s^{-2}, which means that a sodium beam could be stopped in about 1 ms, after travelling a distance of about 0.5 m. The deceleration achieved by Prodan *et al.* was about half maximum.

The temperatures of the cooled atoms were monitored by the fluorescence produced by a weak laser beam crossing the atomic beam at a convenient angle. Ertmer *et al.* estimate that some 10^6 atoms per ml could be cooled to a velocity distribution whose width corresponds to a temperature of 50 mK (25 MHz half-width). The corresponding velocity spread obtained by Prodan *et al.* was equivalent to about 100 mK with a density of about 10^5 atoms per ml. For their subsequent successful trapping of some of their cooled atoms, Prodan and his colleagues used a trap comprised of two opposed coils (reminiscent of an opposed Helmholtz pair); the downstream coil is energized once the atoms have drifted into the trap. The coils produce a field gradient of about 1 T m^{-1}, which reflects and traps atoms with an axial temperature of below 17 mK. The upstream coil provides a gradient field which is used in the final stage of laser coding once the atoms have entered the trapping region. The atoms then spread out to fill the 20 ml volume of the trap and some 10^4 atoms are trapped for about 0.83 s (the decay time). The next stage will be to attempt to use optical trappings to cool the atoms even further so that they are sufficiently at rest to dispense with the trap. Meantime many exciting experiments are in prospect.

Atomic frequency standards

To appreciate the possibilities of the new technique one has only to consider a paper of J. J. Bollinger *et al.* (*Phys. Rev. Lett.* **54**, 1000; 1985) in which the successful operation of an atomic frequency standard based on laser cooled ions is reported. Bollinger *et al.* trapped $^9Be^+$ ions in a Penning trap, whereby the ions were confined axially by a hyperbolic electrostatic potential distribution, and radially by the combined action of the electrostatic and magnetic fields. The ion motion comprised the cyclotron motion about the magnetic flux axis and the circular ($E \times B$) drift (or magnetron motion) each in the radial plane, together with the axial motion. Each may have a temperature associated with it. In the experiment described, the cyclotron and axial temperatures were less than 100 mK and the magnetron temperature less than 2 K (with the cooling laser on continuously). Some 2,000 ions were confined for many hours in the trap, with ion cloud diameters of 0.3 to 0.5 mm. The laser radiation was directed along the axis of the field and tuned to be slightly on the low frequency side of the absorption. The radiation not only cooled the axial motion but also coupled with the magnetron motion, and so could be used to compress (cool) the ion orbit.

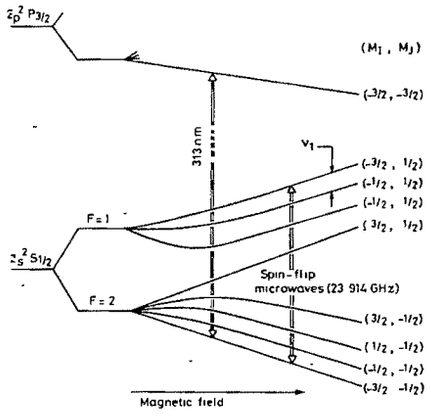


Fig. 2 The Zeeman splitting in a magnetic field, and the hyperfine structure of $^9Be^+$ showing the transition used for laser cooling by Bollinger *et al* (not to scale)

Bollinger *et al.* report a measurement of the hyperfine ground-state transition frequency ν_1, at its magnetic-field independent point (0.8149 T), of 303 MHz with an estimated standard deviation uncertainty of only 57 μHz (see Fig. 2). The experiment involved triple resonance, requiring first, 20 μW of 313 nm radiation to excite the 2S-2P optical transition; next 23.9 GHz microwaves to excite the indicated electron spin-flip transition; and finally, radio-frequency radiation to excite the transition ν_1. The last was provided by a frequency synthesizer which was referenced to a hydrogen maser. The microwave radiation served to make the rate of excitation of the optical transition (and hence the observed fluorescence) sensitive to the upper level of the state pumped by the ν_1 line.

To excite and interrogate the ν_1 transition, a 0.5–2 s pulse of 303 MHz radiation was followed by a second 303 MHz pulse, 10 or 19 s later. This leads to a resonance some 25 mHz wide. It was only necessary to change the radiofrequency by millihertz steps over a 0.1 Hz range in order to scan the resonance. The optical and microwave signals were applied for 3 s before the first

Optically trapped oil droplets

In a recent article in *Physical Review Letters*[1], A. Ashkin and J. Dziedzic of AT&T Bell Laboratories report the first stable trapping and manipulation of single minuscule oil droplets using only the forces derived from the pressure of laser light. Although the force exerted by light on any object is normally so tiny that it is not of importance, the special circumstances contrived by the experimenters make it the dominant force. The techniques have a wide range of potential applications.

The new aspect of the work derives from the fact that the light beams are alternating rather than constant. Recent interest in optical trapping of free neutral atoms has produced an optical Earnshaw theorem[2] showing that steady radiation pressure traps cannot produce stable confinement, just as in the case of steady electrical fields and charged particles. However, just as ion traps can work very well with alternating fields, optical traps can work very well with alternating beams. Using an arrangement of mirrors and shutters to produce the appropriate beams, Ashkin and Dziedzic have confined and manipulated oil droplets of only 0.001 cm diameter for up to five hours. This was accomplished in the air, at atmospheric pressure, with no physical contact with the drop and no observable deterioration by heating.

Arthur Ashkin has been thinking about particle and atom trapping for a long time. In 1970, in his first paper on the subject[3], he laid out the fundamental principles and suggested a number of applications. The attractive features of his technique for manipulating micron sized particles particles are the lack of physical contact, complete control over motion and the lack of heating or disturbance by radiometric forces. The earliest experiments suggested the possibility of separation of particles by size or composition, as well as showing how to transport, levitate and handle them. Later in that same year, Ashkin described the possible application of these ideas to free neutral atoms[4], a subject of much interest in recent years (reviewed in ref. 5).

Ashkin and colleagues have also succeeded in detaining free sodium atoms in a configuration of laser beams that produces viscous optical forces (S. Chu, personal communication). Our own group has recently accomplished the first real trapping of neutral atoms by confining laser-cooled sodium in a magnetic trap for more than 1 s (see accompanying article[6]).

Whereas it is clear that a new field of applied physics, involving the optical manipulation of free neutral atoms, micron size particles and other tiny objects, is being born, we cannot yet foresee the complete range of its applications. They will include materials processing, information storage, precision atomic spectroscopy, studies in classical mechanics and chaos, isotope separation, control of chemical reactions and manipulation of single cells or even macromolecules. Surely there will be many others.

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pulse and after the second pulse, serving as state-preparation and state-sensing pulses, respectively.

Despite the cooling, the second-order Doppler-shift correction −107 μHz was still important. It arose from the heating of the ion cyclotron and axial motions to about 3 K during the 10 to 19 s that the laser was switched off and is probably due to small imperfections in the construction of the trap. It was also necessary to apply a correction of about −6.1 μHz to allow for the rotation of the earth (and ion trap) around the ion cloud.

The same team has gone on to use their apparatus to set 300-fold lower limits on the spatial anisotrophy of inertia by performing a Hughes–Drever type of measurement (Prestage, J. D. *et al. Phys. Rev. Lett.* **54,** 2387; 1985). Their sensitivity was better than 50 μHz to changes in the orientation of their $^{9}Be^{+}$ ions with respect to the Sun and Galaxy (see Gallop, J C. & Petley, B. W. *Nature* **303,** 53; 1983). Various groups have explored certain clock transitions in $^{137}Ba^{+}$, $^{171}Yb^{+}$, $^{199}Hg^{+}$, $^{201}Hg^{+}$ and other ions, to the limits set by commercial caesium beam standards, by using different methods to cool the ions.

Other spectacular measurements with ion traps have been reported. Thus, the fluorescence signal from a single trapped ion has been seen (Neuhauser, R. *et al. Phys Rev* **22,** 1137; 1980) and, recently, Gabrielese, G. *et al.* (*Phys. Rev. Lett.* **54,** 537; 1985) reported an investigation of relativistic hysteresis effects in the cyclotron motion of a single electron that was trapped continuously over a period of ten months. In addition, the Dehmelt and Van Dycke group at Seattle, and others at Mainz and elsewhere, have made high precision measurements of the electron magnetic moment anomaly and the ratio of proton mass to electron mass.

There is no lack of narrow optical lines that could be studied with trapped ions, but all have drawbacks and there are formidable experimental difficulties to be overcome. Thus, Q values in excess of 10^{14} are anticipated for the $^{1}P_{0}-{}^{1}S_{0}$ transition in Tl^{+}, Al^{+} or Ga^{+} as well as in the D←P←S Raman transition in Ba^{+}, the S←D two-photon transition in Hg^{+} or the $^{2}P_{3/2}\leftarrow{}^{2}P_{1/2}$ transition in Pb^{+}. Similarly, many high-Q transitions will be explored with trapped atoms — the 1S→2S transition in atomic hydrogen will be of particular interest. By contrast, the Q value of the current caesium frequency standards is in the region of 10^{9}.

In the shorter term, the highest accuracies will be achieved on the measurements of fine and hyperfine structure transitions that lies at convenient radio and microwave frequencies. For the higher frequencies, the dye-laser technology must be improved to give wider frequency coverage and to use narrower line-widths. Also frequency multiplication into the visible region and beyond will have to be simplified and increased in accuracy.

As the techniques advance, we can expect to gain improved knowledge of atomic and ionic phenomena. One can even contemplate experiments involving new levels of sophistication and accuracy, such as parity conservation, gravitational interactions with atomic energy levels, Hughes–Drever type measurements, measurements of the properties of fundamental particles and, some day, the 1S→2S transition in anti-hydrogen. Moreover, we can expect greatly improved devices that will benefit technology as well as science — better frequency standards will almost certainly be one of the first gains. □

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Archaeology

Man as part of the ecosystem

from Henry Cleere

Sir Mortimer Wheeler once described archaeology as a "humanistic science", and in that phrase he neatly encapsulated the confused, almost schizophrenic, view the discipline has of itself — a view, moreover, that is shared by those outside the field. Officially, the matter was resolved more than thirty years ago: government support to the Council for British Archaeology is paid through the British Academy rather than the Royal Society or one of the science research councils.

Whether or not archaeology can lay claim to be a science by virtue of its methodology remains a subject for debate, although it is undeniable that science now has an expanding role to play in archaeology. Of course, this is no recent phenomenon: physical and biological sciences have contributed to the interpretation of archaeological data for many years. Accurate dating of archaeological material since 1945 has used techniques such as radiocarbon analysis, thermoluminescence and dendrochronology. The provenancing of stone, ceramic and metal artefacts has been improved immeasurably by advances such as neutron activation analysis, atomic absorption spectroscopy, electron-probe microanalysis and scanning electron microscopy, recently reviewed in ref. 1.

This, however, is science coming to the aid of archaeology in its more traditional guise — the classification and interpretation of human artefacts. Over the past three decades there has been a significant change in archaeologists' perceptions of the range of materials available to them in their work. In modern excavations it is no longer enough to retrieve and examine artefacts and structures that are the result of direct human intervention: man is now seen as a component of an ecosystem, reacting with that ecosystem as it evolves over time and often profoundly and permanently modifying it to his own advantage. For the excavator it is essential to retrieve a wide spectrum of non-artefactual material from ancient contexts to recreate the environment of a site or of a region. This can help us to clarify the extent to which the environment has influenced the socio-economic development of a human community and how far man has wrought changes on that environment.

The new sub-discipline of 'environmental archaeology' is eclectic in the materials that form its basis. Plant remains may be macroscopic (from tree trunks to seeds) or microscopic (fossil pollen, phytoliths and diatoms). The presence or absence of pollens from the weeds of cultivation in the pollen diagram from a site can provide socio-economic information about a human community that artefactual assemblages may merely hint at or not represent at all. Palaeozoology embraces mammalian (and human) remains, fish and bird bones, insects of all kinds and molluscs, particularly land snails. Certain species of insects and land snails are highly stenotopic (adapted to a narrow range of habitats) and so their identification in an assemblage from an excavation can supply much specific information about a site and its environment. The correct identification of soils and sediments on and around early settlements provides important climatic and geomorphological date that can be crucial to the interpretation of a site and its community.

Several general studies on environmental archaeology have recently been published[2-7]. Some excavations have been carried out with the recovery of environmental data as a primary research objective: for example, half of the report[8] on excavations at Farmoor, near Oxford, is concerned with the analysis and interpretation of environmental material. New techniques, such as froth flotation, have been developed to allow recovery of more complete and representative samples of biological material; the wealth of material has necessitated new analytical methods[9]

The potential of environmental archaeology in enhancing the understanding of past societies is immense. However, that potential in Britain could not have been realized without the encouragement and financial assistance of the Ancient Monuments Laboratory of the Historic Buildings and Monuments Commission for England (formerly of the Department of the Environment), as part of its 'rescue' archaeology programme. An indication of the importance of that support is provided

by a recent government report[10] which contains a "retrospective/prospective review" of the environmental archaeology of three regions (East Anglia, south-west England and northern England) and one historic town (York) that has been revealed by work sponsored by the laboratory over the past decade. These are the first regional overviews of past environments (as opposed to reports on the environments of specific sites) have been prepared. Although the reports stress problems resulting from lack of data, the information that has been assembled has produced several syntheses with profound implications for archaeology For example, precise information on forest clearance and farming systems is emerging which, together with the results of aerial and ground surveys, radically changes the accepted view of the extent and nature of prehistoric and Roman settlements Analysis of animal bone assemblages is throwing new light on dietary habits, animal husbandry and butchery practices. Analysis of large assemblages of human skeletal material, especially from urban cemeteries, is producing much invaluable — and often unexpected — palaeodemographic and palaeopathological data, which can be related directly to conventional archaeological data

Whereas the enormous potential of environmental archaeology is clear, the government report also emphasizes the inadequacy of funds to more than nibble at the edges of the subject, the material for which is becoming more voluminous with every excavation. Nevertheless, the report is a well-documented exposition of the value of treating human communities as components of a complex ecosystem rather than as isolated phenomena that do not interact with their environments This is where the future lies for archaeology: let us hope that this will be realized by those who can make that future a reality. □

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Aggregation

Models of the sol – gel transition

from George Hentschel

ANYBODY who has seen a cold soup turn into jelly has witnessed a sol–gel transition. This is caused by the branching reactions that occur in the polymerization of polyfunctional monomers in a solvent. The gel point separates a sol phase, consisting of a distribution of growing but finite clusters in the liquid medium, from a gel phase where a macroscopically large branched network spans the system with fluid in its interstices. Yet, even today, it is not known whether monomer diffusion allows some reaction sites on the surface of a growing cluster, or whether one can treat all possible growth sites on an equal basis. A new kinetic-growth model, introduced by P. Meakin and Y. Termonia (*Phys Rev. Lett.* **54**, 1083; 1985), now tackles possible diffusion-limited selection by combining aspects of both diffusion-limited aggregation (DLA) and the phenomenon of percolation. This is also critical because the dynamics of a fluid moving through a porous medium will cease to be determined by hydrodynamics when porosity is sufficiently ramified.

The model is a modification of the kinetic growth model of Z. Alexandrowicz for percolation clusters (*Phys. Lett* **80A**, 284; 1980). Place a monomer at the centre S_0 of a hypercubic lattice with coordination number f. Choose a probability P which represents the average fraction of new monomers joining the cluster per unit time At time $t=1$, random variables z_i equally distributed in the interval [0,1] are chosen for each nearest-neighbour site of S_0. If $z_i<P$, a new monomer is placed at the site, whereas if $z_i>P$, the site is blocked for all times. This creates a set of new monomers S_1. In the original model of Alexandrowicz, for all times $t\geq 2$ the algorithm described above is repeated for each new set of branch tips created at the previous step and not blocked. This identifies the sets $S_2, S_3, \ldots, S_t$ of new monomers which join the growing cluster at each unit interval until for $P<P_c$ a point is reached where no new branch tips are created, as all possible growth sites are blocked. For $P>P_c$, the growth does not stop and a macroscopic cluster will span the lattice.

The novelty in the model of Meakin and Termonia is their contention that growth sites are not all created equal As in DLA, the probability P_i for a branch tip on the surface to react with a monomer is larger than for one embedded in the cluster: monomers have to diffuse into the bulk to reach such a growth site and are likely to have reacted before they can do so. To estimate the P_i for each new branch tip in this model, a large number of random walks from each vacant site is simulated and the fraction that escapes from the aggregate gives the relative probability of a monomer reaching that site. To find the absolute probabilities P_i, these relative probabilities have to be normalized so that an average fraction P is likely to be incorporated into the cluster at each step of the growth Once the P_i have been estimated, each possible growth site is occupied or blocked by a new monomer according to whether a random number z_i chosen from the equal distribution [0,1] is less than or greater than P_i. As in the case of percolation, a gel-like transition takes place at some critical rate of incorporation of monomers, P_c. The clusters are fractals with a fractal dimension which is that neither of DLA nor of percolation but, rather, more tenuous.

To see this model in the context of previous attempts to understand the gel point, note that diffusion has usually been neglected completely, and the rate-determining step assumed to be the reaction. All unreacted groups on a polyfunctional monomer were treated as equally likely to react, so there were no geometric constraints in the model. A Smoluchowski equation could be set up with reaction rates proportional to the product of unreacted species in each cluster, and solved for the cluster distribution. This model is soluble and exhibits a sol–gel transition. Though the model is satisfactory away from the gel point, the classical exponents do not agree with experimental results close to P_c.

One geometric constraint that can be introduced easily into the reaction process is to argue that some unreacted sites in the cluster may not be able to react because they are blocked, with some probability P by their neighbours This would allow one to compare percolation and gelation to see whether they lie in the same universality class, as they both show transitions from finite to macroscopic spanning clusters. Unfortunately, percolation exponents are also in disagreement with experimental findings.

In the new model, the ease or difficulty of finding a partner in a unreacted group depends on the ability of a monomer to reach it, as well as blockage by its neighbours. The critical exponents of this model lie between those of classical theory and percolation. Meakin and Termonia compare their model with experimental tests and conclude that it agrees better than do either the percolation or the classical models, although discrepancies still exist. Clearly, monomer diffusion is important in clustering close to the sol–gel transition □

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Extracted leaf protein in British agriculture

SIR — Extracted leaf protein (LP) is not made commercially in Britain and there is little research on it here. It is made commercially in Denmark, France, Italy, Japan, New Zealand, Poland, Spain, the United States and the Soviet Union. Because it is possible that it is we who are out of step, it seems timely to reconsider the arguments for LP production.

No one has questioned the argument, advanced more than 40 years ago and amply confirmed experimentally since, that the annual yield of protein in the form of LP can be greater than the yield of a protein concentrate by any other type of farming. Similarly, the demonstrations that LP can replace fishmeal or soy in pig and poultry diets, made 20 years ago in the National Institute for Research in Dairying and the Rowett Research Institute, have been confirmed in many other institutes. The residue remaining after protein extraction contains less water than any crop normally dried for conservation as winter feed for ruminants. Logically therefore, drying should be economical. There is ample evidence that the residue, because non-nitrogenous material is extracted as well as protein, still contains 1.5 to 2.0%N (in the dry matter) and that it makes good silage from which there is no wasteful and polluting effluent. This residue, whether fresh, dried, or ensiled is readily eaten by ruminants. Many experiments show that its feeding value is, weight for weight, as great as that of the original crop: this is presumably because more of the N in it is true protein and because comminution makes it more readily digestible.

One reason for the relative lack of interest in Britain in the use of LP as an animal feed, is the emphasis that has been put here on its value as a human food in countries with a humid climate and protein deficiency. As a result of successful human feeding trials, there are in these countries several production units managed by local enterprise rather than by sponsorship from outside. Its value and acceptability are now established. Even vegetarians in Britain usually eat diets containing an adequate amount of protein. There is therefore little need here for this application of LP. However, we feed pigs and poultry on imported fish meal, ground nut meal and soya: these could be largely replaced by home-produced LP. That substitution would not exacerbate the widely condemned overproduction of agricultural products. On the contrary, while economizing on imports, it would offer farmers an alternative to those crops which are now over-produced.

Another reason for lack of interest is that insufficient attention has been paid to the number of potential sources of LP In early work at Rothamsted, a dozen suitable sources were described: the list could be extended, and it has been further extended in tropical countries. But in most countries where there is commercial production, attention is confined to grass mixtures and lucerne. The latter is agronomically attractive, but its juice froths inconveniently and, unlike some other species, it yields LP with an unappealing flavour. LP can also be extracted from by-products such as potato haulm, sugar beet tops and vegetable discards If effort were put into collection, by-products could supply 100,000 tons of protein anually in Britain as well as a residue suitable for cattle feed.

Yet another reason for lack of interest is the use of unsuitable equipment and techniques in such large-scale trials as have been made. High-speed pulping equipment was used in early work at Rothamsted because we were sure it would work: it is still the most satisfactory equipment in agronomic studies. But pulping by slow-speed rubbing, or extrusion through a die, uses less power and is nearly as effective. If the pulp is less than 1 cm thick after pressing, and if pressure is maintained for a few seconds, juice expression is satisfactory with 1 to 2 kg force per cm^2 (100 to 200 kPa). The vast screw expellers, which have been used in some unsuccessful trials, are extravagant and perform poorly because tightly packed fibre impedes the outflow of protein-rich juice. There are simpler and cheaper methods for expressing juice: more should be devised, for this is the least satisfactory part of the process.

Lack of success in feeding trials with unfractionated juice was predictable. Its protein content is small and variable, its cation content is often excessive and, with some species of leaf, it contains unappealing or slightly toxic components When a production routine has been established, there is no need to separate coagulated LP completely from the juice. If the coagulum is allowed to settle, the sediment has a relatively constant composition, and the supernatant carries away most of the deleterious material. Furthermore, if conservation is necessary because of an uneven supply of leaf, the compact sediment needs less of the preservative. Heating is preferable to any other method of coagulation. It is an illusion to regard it as expensive. Much of the necessary heat can, with a counter-current arrangement, be supplied by juice which has already been heated. If the pulper is driven by a diesel or steam engine on site, exhaust heat will be more than adequate for coagulation.

Without an estimate of costs, the place of LP in British agriculture cannot be assessed. Costs are obviously inflated when the equipment used is more elaborate and expensive than necessary, and when the value of the residual fibre as cattle feed, and the possible value of the fluid which is separated from the coagulum, are not included in the calculation. So many factors tend to invalidate the gloomy conclusions drawn from trials which made inadequate use of existing knowledge and experience, that it was premature to decide that LP production would not be advantageous in Britain. No one has rebutted the basic propositions on which advocacy of LP production depends. The objective is sound, but execution has hitherto been faulty.

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Calmodulin and eukaryote evolution

SIR—Whilst reading the recent report by Sudhaker Babu *et al.*[1] on the three-dimensional structure of calmodulin, I was again impressed by this protein's ubiquitous role in eukaryotes. From cotton seed to rat testis it shows itself in a remarkably constant condition and appears to show that a similar ancestor must have been present at the dawn of eukaryote evolution.

Rightly or wrongly the next step is to review the prokaryotes for 'ancestral types' and *Escherichia coli*[2] has a contender in the form of the acyl/carrier protein which has a section resembling that of the E-F hand[3] structure. Also, the antibiotic amphyomycin has been shown[4] to have a calcium dependent action upon Gram-positive bacteria. Its structure[5] shows it not to contain an E-F hand (it only contains 11 residues) but at least a second finger, that is, the loop of the helix–loop–helix described by Kretsinger and Barry[3].

If the evolutionary link between *Streptomyces* antibiotic production and eukaryote calmodulin seems far-fetched, does this mean that the second finger of the E-F hand is the only decent way to employ amino acids in the capture of calcium ions?

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

A new approach to an absolute timescale from measurements of orbital cycles and sedimentary microrhythms

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When the sedimentary microrhythms of two groups of Jurassic zones forming part of the chronostratigraphical scale are analysed against recent radiometric age scales there is good agreement with present-day cycles resulting from the obliquity of the ecliptic. Sedimentary microrhythms resulting from orbital forcing can be used to establish either the relative or the true length of chronostratigraphical zones and thus provide a new approach towards an absolute timescale. Such a scale will be powerful in the analysis of other cycles and perturbations in the geological record.

THE inadequacies of the radiometric timescale for practical use in much of the Phanerozoic sedimentary record is well known. Reliance has always been placed on the relative timescale provided by evolutionary changes for local and international correlation and age determination. It is the biostratigraphical and chronostratigraphical scales[1] which form the standard for geological work on sedimentary rocks on land an in the oceans. But a numerical scale is obviously required for any useful analysis of sedimentary, tectonic and evolutionary processes. I show here how recognition of a causation for some sedimentary microrhythms in terms of the influence of orbital forcing cycles can provide a new approach to the establishment of an absolute timescale, using the evidence of the effect of such cycles in comparatively recent deep-sea cores[2-4] and the recognition that sedimentary microrhythms common in the stratigraphical record[5,6] may result from Milankovitch-type cycles[7].

I will use two examples from the Jurassic to illustrate the technique. These examples enable integration with the chronostratigraphical scale. The Jurassic also demonstrates the essential problems with the current radiometric timescale. Table 1 lists several recent attempts to give the duration of the Jurassic and shows the uncertainty of each method. As to the finer divisions of the Jurassic used on radiometric scales, three approaches have been used recently. The first is to consider the 10 or 11 Jurassic chronostratigraphical stages to be of equal duration and to divide the number into the accepted duration for the system[8]. The second is to take the number of ammonite zones within a stage as the more reasonable estimate of its duration and to adjust stage length accordingly[10]. The third proposal[11,12] is a weighted scheme using zonal and subzonal divisions. All three methods are based on the assumption that the evolutionary changes, which form the basis for stage, zonal and subzonal division, proceed at a uniform rate. Yet that is an hypothesis which a palaeontologist would wish to test, and the evidence presented here shows that is false.

Sedimentary microrhythms

There has been a wide recognition of rhythmic sedimentary events[5,6,13] at a scale of ~0.2–3.0 m throughout the Phanerozoic and parts of the Precambrian. However, these rhythms are usually asymmetrical cycles of the ABCABC or ABCDABCD type, and there seemed little immediate relation to the regular symmetrical cycles (such as ABCDCBA) which one would expect from orbitally controlled patterns. Perhaps that is a reason why such interpretations have been regarded as speculations. Figure 1 illustrates how rhythmical or asymmetrical cycles may rise from symmetrical forcing oscillations when set against a regular trend in a changing parameter, for example, sea-level

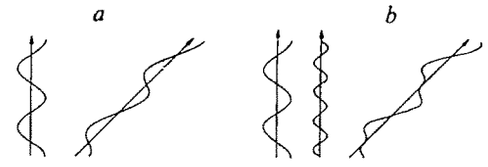


Fig. 1 Net effect of symmetrical oscillations and long-term trend giving a stepped effect of stasis and more sudden change. This model can be interpreted, for example, in temperature, sea-level, salinity, faunal abundance or climatic terms. *a*, Effect of one cycle; *b*, effect of two cycles, such as the tilt and precession cycles.

change against a subsiding sea floor, temperature oscillations against a progressive temperature change or climatic oscillations against a climatic trend. The simple patterns illustrated in Fig. 1 show a period of rapid change followed by relative stasis of the type needed to explain many microrhythms. Such a situation may be provided by a combination of the Milankovitch-type orbital oscillations[7]. More complex interpenetration of several cycles and combinations of cycles with different control could give very elaborate effects. In such cases, different amplitudes and wavelengths can give harmonics and varieties of pattern which might, at first glance, seem bewildering, but which would have simple primary cause[9]. This is illustrated in Figs 1 and 5 which show how the effects of simple cycles which can be resolved using Fourier transforms.

Many microrhythms in the geological record result from quite different causes, for example, turbidite flows resulting from continental shelf and slope instability, nearshore storm events, periodic fault movements or spasmodic volcanicity. Orbitally related phenomena may be best studied in deeper sedimentary basins away from such phenomena. To proceed with the construction of the new timescale only a few sequences well-related to the chronostratigraphical scale are needed, then normal biostratigraphical techniques can be used.

Table 1 Estimates of the duration of the Jurassic period based on recent radiometric timescales

Ref.	Date	Duration of Jurassic (Myr)
Van Hinte[35]	1976	55–60
Harland *et al*[36]	1982	69
Odin[37]	1982	74
Kent & Gradstein (in Palmer[34])	1982	64
Snelling[11]	1985	70

Jurassic examples

To demonstrate a possible relation between sedimentary microrhythms and orbital patterns two cases will be considered from English Jurassic sequences which form part of the chronostratigraphical scale, one from the Lower and one from the Upper Jurassic. Figure 2 illustrates the typical sequence for the south of England along the Dorset coast. The large-scale rhythmicity shown is not of concern here but was appreciated as early as 1822 (ref. 14) and has been described in detail elsewhere[15-17]. Each of these major rhythms usually show finer-scale microrhythms, the immediate cause of which have been investigated extensively in terms of small sea-level changes and anoxic events[16]. Two examples will be treated here, part of the Lower Lias and part of the Kimmeridge Clay, chosen because their unambiguous relation to the chronostratigraphical scale. The initial postulate for this study is that the observed regular small-scale rhythmicity indicates the overprinting of a periodic enrivonmental change.

The careful measurements of the Lower Lias microrhythms of Lyme Regis, Dorset by Lang form the basis for the calculations given here[18,19]. The microrhythms typically comprise a thin laminated, dark, organic-rich shale followed by lighter coloured calcareous shale with bioturbated limestone beds at the top[20-22]. Not all units are developed and higher in the succession the limestone units are often lost. The succession of these microrhythms and their correlation with the standard chronostratographical scale[23] is illustrated on Fig. 3*a* using Lang's data and terminology for bed numbers. The Lower Lias sequence is nearshore and could be expected to be incomplete[23]; it represents a deepening sequence from possible supratidal stromatolitic Upper Triassic beds below. Some data from Somerset[24] are also considered.

The broad features of the Upper Jurassic Kimmeridge Clay have been described[25-27]. The typical microrhythms comprise alternations of well-laminated oil shale and/or laminated organic-rich shale with more calcareous, lighter coloured shale or mudrock. This sequence is interrupted by dolomitic or coccolith limestone bands, termed stone bands, which form convenient markers. Immediately above these markers microrhythms are often not clearly seen. The Blackstone, or Kimmeridge Oil Shale, is the best developed and thickest oil shale. This and associated oil shales are thought to be the main source of oil in the North Sea fields and for the nearby Wytch Farm field (Fig. 3*b*)[26]. An illustration of a cliff section showing some of these microrhythms is given on Fig. 4*b*.

Analysis of microrhythm data

Only Arkell[28] in this century has had sufficient detailed biostratigraphical command of the whole Jurassic to give judgement on zones which might have reasonable balance throughout the system. He used 58 zones, excluding the non-marine Lulworth Beds to which we may assign two zones for calculation, giving 60 in all. Arkell's subdivision of the Kimmeridge Clay was provisional and if revisions of the sequence between the Mutabilis and Pectinatis Zones[25,26] are accepted, the total of zones is not altered but all the Kimmeridge Clay data on Fig. 3*b* can be used. More recent lists give up to 74 zones[23]; if this value is preferred other calculations may be done, but I feel that this introduces too much relative subdivision at certain levels.

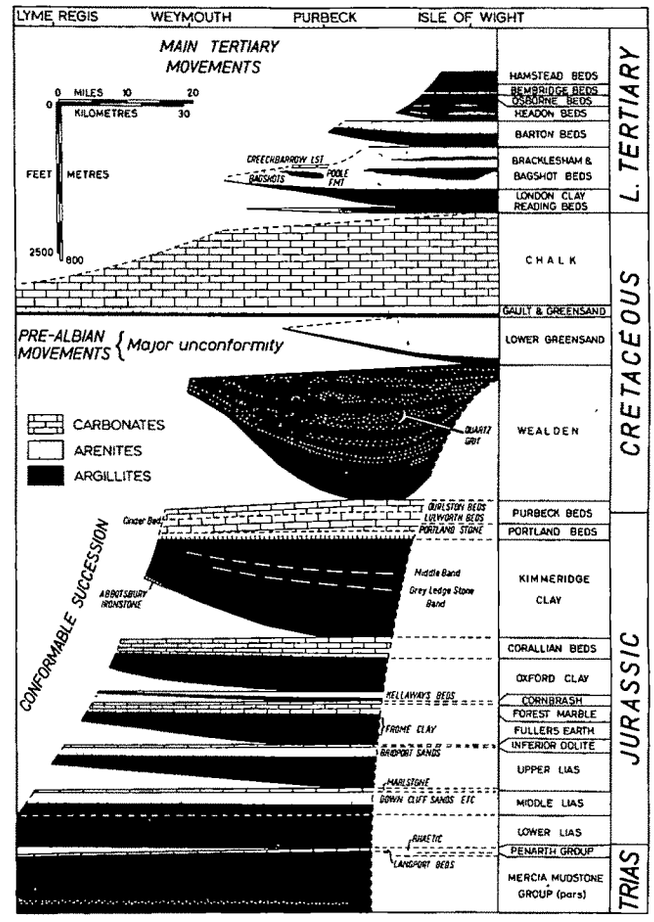


Fig. 2 Mesozoic and Tertiary sequence of the Dorset coast drawn to emphasize the large-scale rhythmicity of the succession and to indicate the levels of the sequences discussed in greater detail (after ref. 14).

The four earliest Jurassic zones used here[23] compare with the first three of Arkell's scheme[28] to estimate zone duration. Using Lang's limestone units for the first approximation (Fig. 3*a*) these give the following crude numbers of microrhythms for each zone. (Shown in parentheses are interpolated rhythms which have been added for use in the calculations.) Planorbis Zone (beds H25–H56), 16(6); Liasicus Zone (beds H57–H83), 13(6, assuming six rhythms when complete between each of refs 2–4), Angulata Zone (beds H84–20), 19(5); Bucklandi Zone (beds 21–46), 13 (14). This gives a total of 91 for the four zones (three in Arkell's scheme[28]). No estimate is made for the Semicostatum Zone, but rhythmicity clearly continues (Fig. 3*a*).

Data from Somerset[24] suggest that at Doniford Bay the following values hold: Planorbis Zone (beds 14–42), 10; Liasicus Zone (beds 43–69), at least 18; Angulata Zone (beds 80–145), 39 (or 40 at Blue Ben); Bucklandi Zone (beds 146–203), 27. This gives a total of 94 microrhythms. But there is considerable subjectivity in the estimation of these numbers and some discrepancy between them and those of Dorset. The Lias evidence is used in the absence of better data, but a more thorough regional and international study should provide more reliable values.

The Kimmeridge Clay microrhythms used for the calulations are shown in Fig. 3*b* where interpolated levels are the numbers

Table 2 Estimates of the time duration of microrhythms of the Lower Lias (Planorbis to Bucklandi Zones) and Kimmeridge Clay (Autissioderensis to Wheatleyensis Zones)

Duration of Jurassic (Myr)	Duration of each zone (Myr)	Duration of Lias rhythms (kyr)	Duration of Kimmeridge rhythms (kyr)	Average (kyr)
60	1.00	33.0	40.8	36 9
65	1.08	35.6	44.1	39.8
70	1.17	38.6	47.8	43 2
75	1.25	41 2	54 9	48.1

Several possible duration times are used for the Jurassic, and it is assumed that the zones are typical in length for the Jurassic using a slight modification of Arkell's zonal scheme[28] (see text for details and the effect of other assumptions).

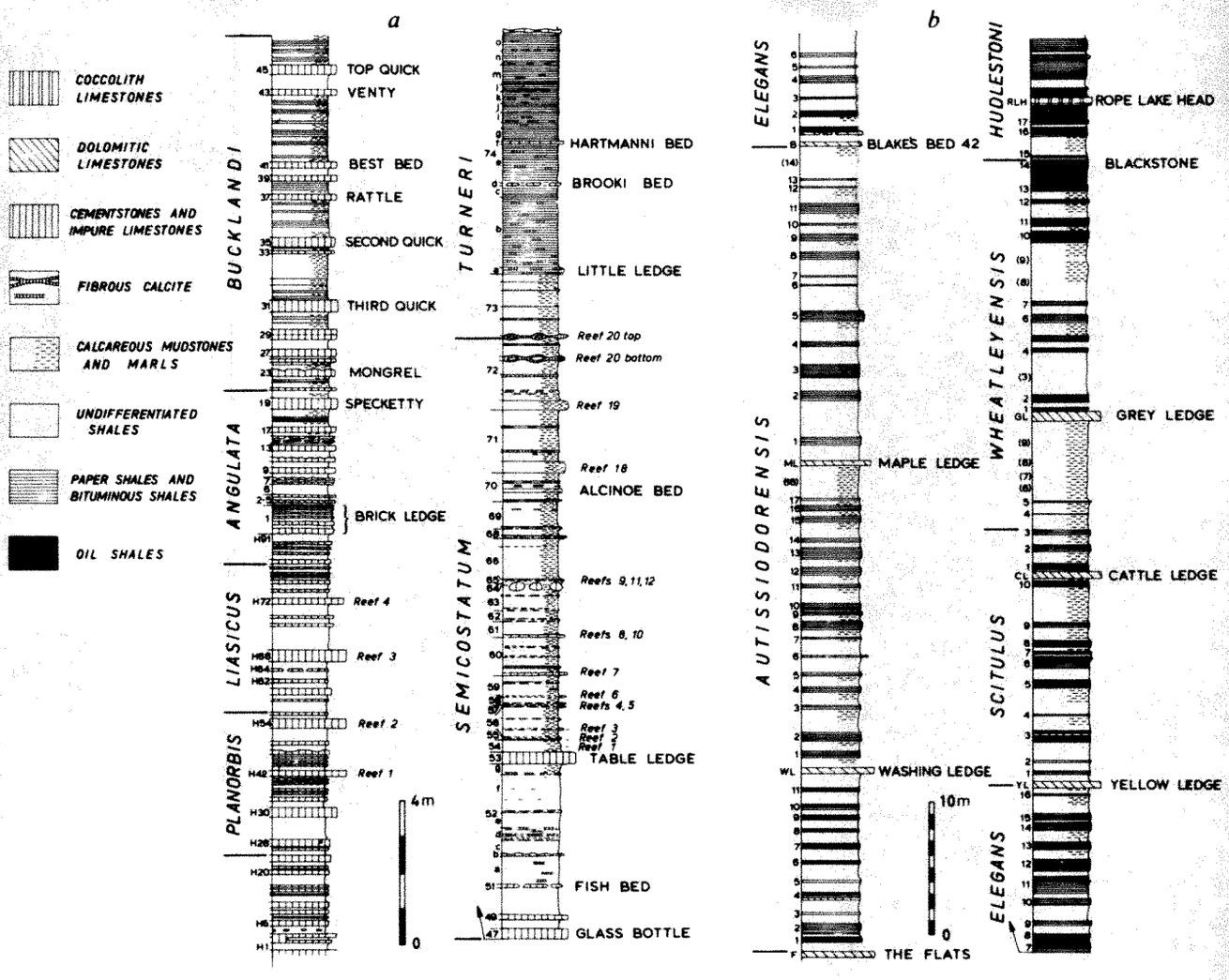


Fig. 3 Detailed sequences from the Jurassic of the Dorset coast illustrating sedimentary microrhythms. *a*, Part of the Lower Lias of the coast near Lyme Regis based on detailed descriptions and bed numbers of Lang[18,19]. The base of the Jurassic and Hettangian Stage is taken where *Psiloceras* enters in bed H25 and the base of the Sinemurian is taken at Bed 21. Numbers with prefix H refer to the section west of Seveh Rock Point, those without prefix extend from the Point eastward to the foreshore below Black Ven. *b*, The section near Kimmeridge between Kimmeridge Bay and Clavell's Hard based on published data of Cox and Gallois[25]. The bed numbering is new and the marker beds are abbreviated so that they form a prefix for the bed numbers given above them up to the next marker bed. A numbering system is given for reference which refers to units above particular marker beds. Thus, ML5 refers to fifth organic-rich shale above the Maple Ledge Stone Band. The boundary between the Lower and Upper Kimmeridgian (*sensu anglico*), and between the Kimmeridgian (*sensu gallico*) and Tithonian stages is usually drawn at Blake's Bed 42 (B).

in parentheses. Zonal assignments are those of a recent revision[26]. The numbers used are as follows: Autissiodorensis Zone (F1 to MP14), 45; Elegans Zone (B to B16), 17; Scitulus Zone (YL to CL3), 15; Wheatleyensis Zone (CL4 to GL14), 21. This gives a total of 98 microrhythms. Table 2 gives calculations of the time for each microrhythm using only the Dorset data.

Interpretation

There are several uncertainties in the values used, of the period taken for the length of the Jurassic, of the number of Jurassic zones and of the assumption that the eight zones used are a fair sample for the Jurassic. Nevertheless, the agreement of an average of the microrhythm period and an average of current estimates of the duration of the Jurassic with the value of 41 kyr (ref. 4) which is the oscillation resulting from the obliquity of the ecliptic at present is very close (Table 2), perhaps fortuitously so. The differences between the Lias and Kimmeridge results could be due to different weighting in the true zonal periods involved. I suggest that the obliquity cycle may be the primary cause of the dominant rhythmicity. If the obliquity periodicity has changed little through time, then estimates of the duration of the zones, using the 41-kyr value give the following results (in Myr): Planorbis Zone, 0.86; Liasicus Zone, 0.78; Angulata Zone, 0.94; Bucklandi Zone, 1.11; Autissiodorensis Zone, 1.84; Elegans Zone, 0.7; Scitulus Zone, 0.62; Wheatleyensis Zone, 0.86. For the present, these values should only be taken, in their blocks of four, as relative times which may be assigned to the zones. Note that if the 41-kyr cycle did hold in the Jurassic these values indicate (within the limit of sampling) which duration is the more probable for the Jurassic. The present evidence would favour the Cambridge scale[36], but other time and zonal scales give different results because of unbalanced zonal weighting. Thus, Hallam[29] gives the Kimmeridgian (*sensu gallico*) and Tithonian 22 zones and a span of 11 Myr, which would give a period of 20.4 kyr for the Kimmeridge microrhythms. Data from the same review would give an average of 62.5 kyr for the Hettangian microrhythms (giving, as expected, an average of ~41 kyr). The true value will only be approached when relative lengths for zones, using the technique proposed here, are assembled throughout the Jurassic. But do such errors explain the diversity of estimates of microrhythm duration in other parts of the stratigraphical column[7]?

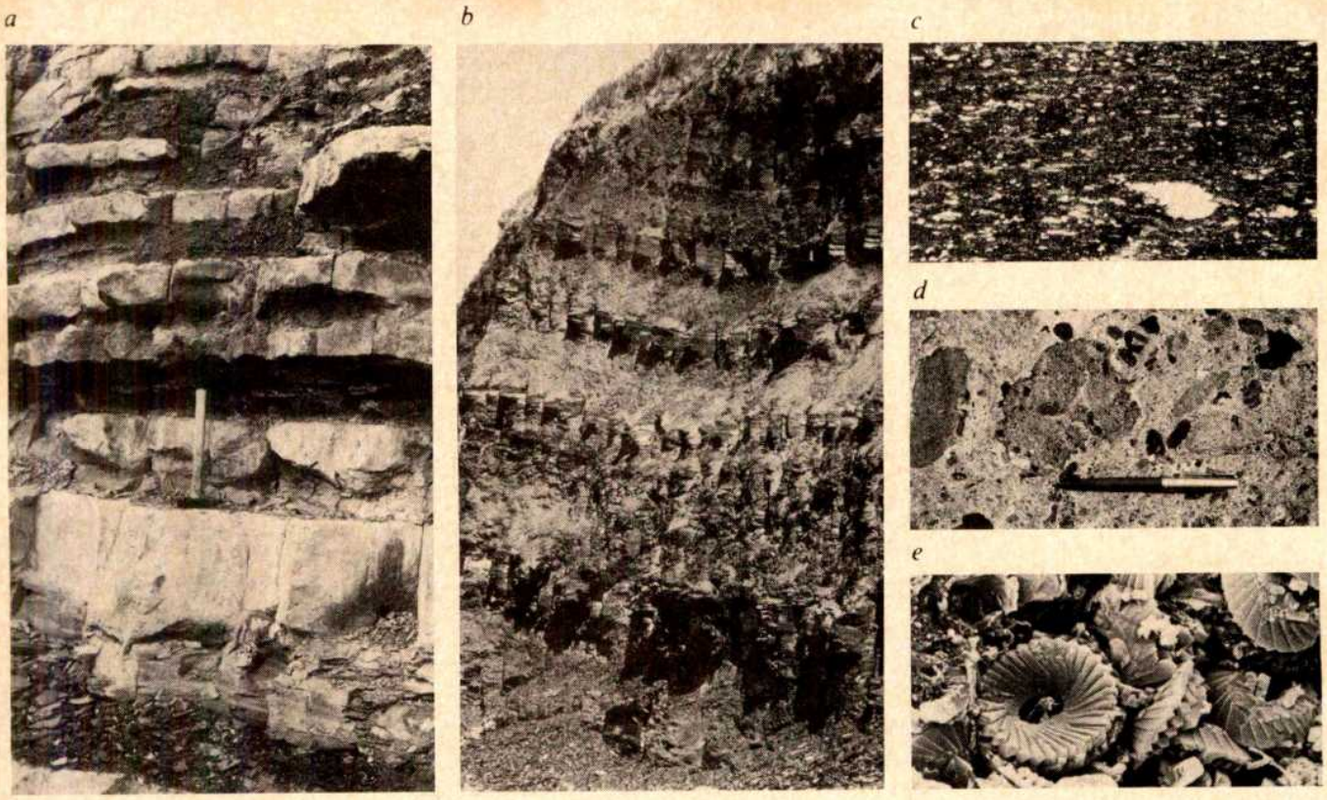


Fig. 4 Features of Dorset Jurassic sedimentary microrhythms. *a*, Part of the Lower Lias succession in Chippel Bay, west of Lyme Regis. The hammer is on the unit called Specketty (Bed 19). *b*, A cliff profile in Kimmeridge Bay, just west of Gaulter Gap, showing the weathering profile of microrhythms above The Washing Ledge Stone Band (Bed WL2 is at the base of the cliff). Four zones are represented (Autissiodorensis to Wheatleyensis). *c*, Section of The Blackstone (Bed GL14) illustrating clasts within the spore-rich oil shale. *d*, Debris deposits in the White Stone Band west of Freshwater Steps, Kimmeridge. Pen, 13 cm long. *e*, Stereoscan photograph of a specimen from the Rope Lake Head Stone Band showing well-preserved coccoliths without a trace of biodegradation. Width of photograph, 15 μm.

The obliquity cycle operates by the shifting of latitude and the microrhythms may be explained, for example, by small climatic shift[29,30]. Thus, oscillations in and out of the tropical forest belt might explain the organic-rich, anoxic phase and the hitherto anomalous evidence for turbid flow and coarse debris within parts of oil shales and organic-rich shales in the Dorset Jurassic (Fig. 4*c*, *d*). This provides a better model than reliance on sea-level changes, although these may be involved in some cases. Perhaps the most promising model is that invoking cold-water benthic versus warm-water planktonic regimes[31]. Although the amount of variation in tilt is likely to be less in the past because of the greater number of days in the year, it seems reasonable to suppose, as a working hypothesis, that the period of the obliquity cycle is probably constant.

Clearly, there are other oscillations that may affect the system, both at greater and smaller frequencies. Thus, the Lias Beds H30 to H66 show three major units forming the reefs, each separated by five minor limestones, which may indicate six oscillations between the maxima of a larger cycle. The Lias sequence discussed here has been the subject of Walsh power-spectra analysis, suggesting orbital-precession (19 and 23 kyr) and a 31-kyr obliquity cycle in addition to those mentioned[30]. These values depend on the assumption of 1-Myr zones and of sedimentation rates. The effect of such cycles seems limited to the introduction of irregularities in the rhythmic pattern. In the group of zones considered here, limestones comprise ~25% and argillites the remainder. A single, probably varved, limestone from above the Brick Ledge gives a 'varve' average of 850 μm. Estimates for varves in organic-rich levels have been given[20] as 9–17 μm and the average, 14 μm, may be used for calculation. Given a total thickness of 25.6 m, these values suggest a depositional time of 1.5 Myr when the expected duration for average zones would be ~4 Myr giving a total of time hiatus of 62.5% comparable with another recent estimate[30] of 70%. But I suggest here that the major rhythmicity signature, estimated as 41 kyr, is probably nearly complete and the loss occurs in individual rhythms.

The Kimmeridge data suggest a repetition of coccolith limestones or dolomitic limestones with a cycle, perhaps a harmonic of about twice or three times that shown in Lias Beds H30 to H66 referred to above. Such signatures of oscillation interaction may prove to be time definitive. A computer model, from a program by R. Middleton, is shown in Fig. 5, and will be used to illustrate an interpretation of the Kimmeridge Clay data. The dominant periodicity (41 kyr) is that obtained by present calculations. The expected 100-kyr cycle is added weakly as it shows no very obvious visual effect. The largest period (taken at 451 kyr) is suggested by the stone band position (F to F11, YL to YL10, CL to CL(9) taken as about 11 times the supposed obliquity cycle) and is used to illustrate the fact that the different controlling oscillations may operate differently. Thus, the points marked *a* on Fig. 5 show times when climatic shift crosses the environmental threshold, enabling the production of organic-rich or oil shales, the threshold being indicated by the straight line given an arbitrary trend. This threshold will be expected to vary from place to place and, in particular, anoxia will reduce in shallower and more agitated waters. At the points marked *b* in Fig. 5, however, the threshold is not crossed and the rhythms may not be obvious in the sediments and they will not show as organic-rich shales. This is the justification for the interpolations used in the calculations (Fig. 3*a*, *b*). A cycle of larger wavelength affecting the shape of the orbit, similar to the present-day 100-kyr cycle (but given on the model a 451-kyr periodicity), could result in periods of much higher global insolation and hence, for example, in more extreme temperatures. This might explain periods of coccolith blooms (Fig. 4*e*) and the periodic formation

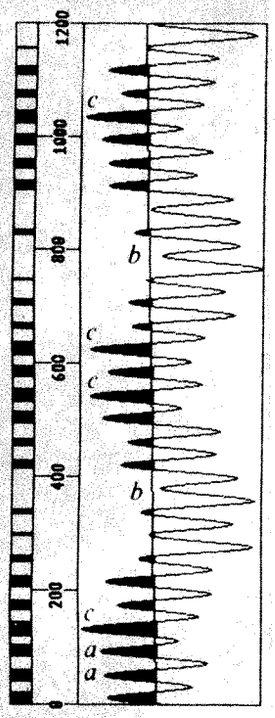


Fig. 5 Computer simulation of microrhythm patterns using 41 kyr as the main obliquity periodicity with amplitude taken as unity. Additional oscillations arbitrarily introduced at wavelengths of 451 and 100 kyr with amplitudes of 0.6 and 0.3, respectively. The vertical column indicates conditions when organic-rich shales may be produced if the trend line represents an environmental threshold for their formation. Scale in kyr.

of the marker stone bands, which would be under quite a different control from the obliquity latitudinal shift. Two such cycles could interpenetrate in their magnitude and in their effect. In this interpretation, it is assumed that the carbonate marls necessary for the production of the diagenetic dolomitic stone bands[32] arise from pelagic carbonates formed on occasions similar to those producing coccolith blooms. A small portion of this sequence, including the Blackstone, has been the basis of Fourier analysis using detailed chemical data[33] and assumptions of sedimentation rate has given evidence of smaller-scale cycles. When the chronostratigraphical scale for the Jurassic is calibrated numerically by the summation of relative duration data, Walsh power spectral analysis and Fourier analysis should enable the provision of very precise astronomical data on planetary perturbations.

At a larger scale are the rhythms producing widespread anoxia in the Jurassic in the Toarcian, Callovian and mid-Kimmeridgian (*sensu anglico*) but whether they have a regular period has still to be demonstrated.

The many examples of rhythmicity described in other parts of the Jurassic and in other stratigraphical systems need to be studied to contribute to the calibration of the timescale and the establishment of a numerical scale linked to the chronostratigraphical scale along the lines suggested here for the Jurassic. It should be emphasized, as is illustrated by Table 2, that even in the relatively well-known Jurassic, bizarre results may follow the choice of an inaccurate radiometric time scale. Subject to the monitoring suggested here, orbital forcing patterns may become the more reliable tool.

Conclusions

If the microrhythms discussed are periodic then, whatever their cause, they provide a better means of assessing the relative lengths of zones for purposes of subdividing the radiometric timescale than the present assumption that stages, zones or subzones are of equal duration.

Assuming values for the average duration of blocks of Jurassic zones based on current radiometric scales, the period for the microrhythms discussed accords well with the present value (41 kyr) for the period resulting from the obliquity of the ecliptic. If this is the primary cause of the sedimentary rhythmicity then it can be used to estimate the absolute length of zones. But other values result from different premises.

It is argued that either relative methods or, eventually, orbital cycle methods can be used to build up a precise date in years for zonal sequences so that the chronostratigraphical scale can become a numerical means of estimating geological processes quantitatively.

I suggest that such a quantitative scale is the only convincing way in which larger-scale stratigraphical and palaeontological perturbations can be dated and interpreted.

I conclude that the assumption made in subdividing the existing radiometric timescale that zones or subzones are of equal duration is false.

If the primary cause of the main rhythms examined is periodicity in the angle of the Earth's tilt then the interpretation of them in terms of slight shifts of climatic belts becomes reasonable. Other rhythms may have quite different orbital forcing controls, such as global insolation changes. The examples given are intended to illustrate a technique rather than to provide final values or interpretations for the phenomena discussed.

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Amino-acid substitutions at codon 13 of the N-*ras* oncogene in human acute myeloid leukaemia

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DNAs from four out of five patients with acute myeloid leukaemia (AML) tested by an in vivo *selection assay in nude mice using transfected mouse NIH 3T3 cells were found to contain an activated N-*ras *oncogene. Using a set of synthetic oligonucleotide probes, we have detected a mutation at codon 13 in all four genes. The same codon is mutated in an additional AML DNA that is positive in the focus-formation assay on 3T3 cells. DNA from the peripheral blood of one patient in remission does not contain a codon 13 mutation.*

THE use of focus assays to detect morphological transformation of NIH 3T3 cells in DNA transfection experiments has shown that between 10 and 30% of human tumours contain altered forms of either the c-Ha-*ras*-1 gene, the c-Ki-*ras*-2 gene or the N-*ras* gene. These genes encode GTP-binding proteins of 188 and 189 amino acids that are located at the inner surface of the cell membrane and which are evolutionarily highly conserved[1,2]. In the 15 or so cases of altered *ras* genes in human tumours that have been analysed so far, activation has been shown to result in a single base pair mutation which leads to the substitution of either a glycine at amino acid 12 or glutamine at amino acid 61 (refs 3–17). However, *in vitro* mutagenesis experiments have shown that mutations at amino acids 13, 59 and 63 can also lead to transforming activity[18].

We have studied the presence of activated *ras* genes in acute myeloid leukaemia (AML), a disease characterized by abnormal proliferation and differentiation of cells of the myeloid, monocytic and erythroid lineage. This disease is subdivided into six groups (FAB M1–M6; ref. 19) according to which immature cell is dominant in the population. Using a direct *in vivo* selection assay in nude mice of transfected 3T3 cells[20], we find that four out of five AML DNAs tested contain an activated N-*ras* gene. An altered N-*ras* oncogene was also detected in another AML sample using the focus-formation assay. We analysed these activated N-*ras* genes for the presence of mutations using a set of synthetic oligonucleotide probes and report here that all five activated N-*ras* genes are found to contain a mutation at codon 13.

Transfection of NIH 3T3 cells

In initial experiments using focus formation on NIH 3T3 cells as an assay for the transfer of transforming genes, DNAs prepared from five samples of AML cells were found to have low, if any, transforming activities (D.T. and C.J.M., unpublished results). However, in a different laboratory one out of three AML DNA samples was found to have transforming activity[38]. Because focus formation may not detect all transforming genes (for example, some alterations to *ras* genes lead to only a minimal change in morphology of the transformed cells[21]), we sought to increase the sensitivity of the transformation assay. Previously, Blair *et al.*[20] have shown that direct *in vivo* selection of transformed cells can be used as an assay for the transforming genes in transfected cells. As an additional step to ensure that all the injected cells had been transfected with donor DNA, DNA samples were co-transfected with the plasmid pSV2Neo (ref. 22) and the transfected cells selected in antibiotic G418. A similar approach has been described by Fasano *et al.*[23], but we injected a smaller number of cells than these authors. We found that tumours arose 30–55 days after inoculations of NIH 3T3 cells transfected with four out of five preparations of DNA from AML samples (AML 33, 49, 73, 77). Of 15 other human tumour DNA samples tested in the same way, 10 failed to give any tumours, even within 90 days, whereas 5 other samples gave tumours 32–80 days after inoculation of transfected cells (Table 1). Normal DNA prepared from either mouse livers or human lymphocytes gave a very low incidence of tumours—0/3

Table 1 Co-transfection and nude mouse tumorigenicity of AML DNAs

DNA + pSV2Neo	FAB classification of AML	Primary tumour incidence per co-transfection of 20 μg DNA	Latent period (days)	Secondary tumour incidence per co-transfection	Latent period (days)
Normal mouse DNA		3/28	94	NT	
Normal human		0/3			
HT1080		3/3, 1/3	30–70	NT	
AML 33	M1	1/3	50	3/4	39
AML 49	M5	1/3, 2/3, 1/3	37	3/3	27
AML 73	M2	1/3	30	1/4	34
AML 77	M4	1/3	55	NT	
AML 50	M5	0/3	90	NT	
15 Human tumours (non-AML)		10×0/3, 4×1/3, 1×2/3	32–80	NT	

20 μg Cellular DNA and 300 ng pSV2Neo were precipitated with calcium phosphate into each of three 60-mm plates seeded 1 day previously with 2×10^5 NIH 3T3 cells; 22–24 h later, the co-precipitate was washed off and the cells incubated in normal growth medium (Dulbecco's modified Eagle's medium + 10% calf serum) for 20 h. Each dish was then trypsinized and seeded to a 150-mm dish containing selective medium with 1 mg ml^{-1} G418. After 7–10 days of growth in selective medium, each dish contained $1–1.5\times10^3$ G418^R colonies and a total of $3–4\times10^6$ cells. 1.5×10^6 cells were then injected into each of two inguinal subcutaneous sites of *nu/nu* mice. NT, not tested.

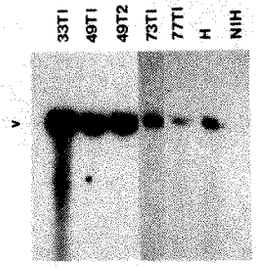


Fig. 1 Southern blot analysis to show the presence of human N-*ras* in NIH 3T3 transfectants from AML DNAs. 33T1, tumour in nude mice derived from co-transfection of NIH 3T3 cells with pSV2Neo and AML 33 DNA; 49T1 and 49T2, two tumours that arose from separate experiments with AML 49 DNA; 73T1 and 77T1, tumours that arose from experiments with AML 73 and 77 DNA, respectively. Control samples are DNAs from normal human fetal lung and NIH 3T3 cells. In each lane 5 μg *Eco*RI-digested DNA was separated. Arrow, 8.8 kb.
Methods. DNAs were digested with *Eco*RI, electrophoresed in a horizontal (0.7%) agarose gel and blotted to nitrocellulose. The filter was hybridized (5×SSC, 20 mM sodium phosphate, 5× Denhardts' solution, 10% dextran sulphate, 50 μg ml^{-1} denatured salmon sperm DNA, 50% formamide) at 42 °C for 16 h with 10^8 c.p.m. ml^{-1} of a ^{32}P-labelled probe obtained by nick-translation of the 800-bp *Eco*RI/*Sst*I fragment of N-*ras* contained in pAT8.8 (ref. 24). This fragment does not hybridize to mouse DNA and detects a single 8.8-kilobase (kb) fragment in *Eco*RI digests of human DNA. After hybridization the filters were washed twice in 0.1×SSC, 0.1% SDS at 60 °C and then exposed to Kodak XAR-5 film at −70 °C with intensifying screens.

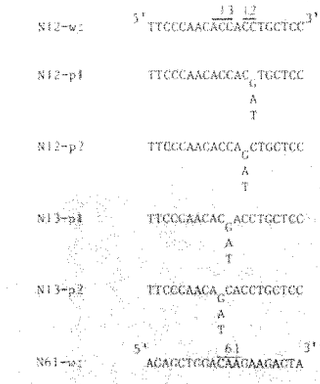


Fig. 2 Sequence of the 20-mer probes used to detect mutations in codon 12, codon 13 and codon 61 of the N-*ras* gene. Sequences are derived from Taparowski *et al.*[12].
Methods. The N12 and N13 probes were synthesized using as template chemically synthesized 20-mer and an 8-mer primer complementary to the 3' end of the 20-mer. This primer-template complex was incubated with [α-^{32}P]dCTP (3,000 Ci mmol^{-1}; Amersham), cold dGTP, dATP and TTP, and DNA polymerase I as described by Bos *et al.*[17]. The 8-mer primer carried a 5'-terminal phosphate group which enabled us to separate the labelled oligomer from the unphosphorylated template on a polyacrylamide urea gel. The N61 probe was synthesized similarly but now using [α-^{32}P]dATP (see ref. 17). The oligonucleotides were prepared by a phosphotriester approach[37] using a fully automatic synthesizer (Biosearch SAM-I).

(human) and 3/28 (mouse)—and any tumours which did appear only arose 90 days after inoculation. DNA prepared from HT1080 cells, known to contain an activated N-*ras* oncogene[24], produced tumours 30–70 days after inoculation in 4/6 co-transfections. The transforming activity detected in the first transfection with AML DNAs could be passaged serially with similar or slightly greater efficiencies (Table 1).

To examine whether any of the tumours that arose in nude mice resulted from the acquisition of a human *ras* oncogene, DNA prepared from the tumours was analysed by Southern blotting with probes for N-*ras*, Ha-*ras* and Ki-*ras*. It was found that DNA prepared from each of the primary transfectants from the four AML DNAs detected by *in vivo* selection (Fig. 1) and the one detected by the focus assay contained the human N-*ras* gene. In some, but not all, transfectants the human N-*ras* gene was amplified 20–30-fold. There was no evidence for transfer of any of the other *ras* oncogenes, and tumours that arose after co-transfection of non-AML DNA did not contain human *ras* genes (data not shown).

Mutations at codon 13

To analyse the transfected N-*ras* genes in the NIH 3T3 transfectants derived from AML DNAs, we used synthetic 20-mer oligonucleotide probes. Using these probes we are able to detect single base pair (bp) substitutions in the N-*ras* gene based on the fact that a fully matched hybrid between the oligomers and genomic DNA is more stable than a 1-bp mismatched hybrid[25]. For the positive detection of mutations, we have synthesized groups of oligomers varying at one position in a codon (see Fig. 2). One oligomer of each group will then hybridize to form a fully matched hybrid with each of the possible mutations in a base pair. For example, the oligomer group N12-p2 consists of three oligomers, one of which will form a fully matched hybrid with an N-*ras* gene mutated at the second base of codon 12.

When *Pst*I-digested DNA from four AML transfectants (33T, 49T, 77T and 1T) was hybridized to the probe corresponding to the sequence of the normal N-*ras* gene around codon 61 (N61-wt) and washed under conditions in which only a fully matched hybrid is stable, a strongly hybridizing signal was seen (Fig. 3*A*). In two transfectants (49T and 33T) the signal was very intense, showing that the N-*ras* genes were highly amplified (see also Fig. 1). No hybridization was seen with the oligomer groups corresponding to mutations at codon 61, indicating that none of the N-*ras* genes in the transfectants contained mutations at codon 61 (data not shown). In contrast, the oligomer corresponding to the normal sequence of the N-*ras* gene at codon 12 (N12-wt) failed to form a fully matched hybrid with any of the AML transfectant DNAs (Fig. 3*B*), although it did hybridize to human cellular DNAs containing single-copy representations of the wild-type sequences at position 12 (lanes *a*, *b*). However, when the transfectant DNAs were hybridized with the groups of probes corresponding to mutations at each of the base pairs of codon 12 (N12-p1, N12-p2), fully matched hybrids were not detected (Fig. 3*C*, *D*).

The absence of fully matched hybrids with either normal or mutant probes led us to investigate whether any mutations occurred in the sequence surrounding codon 12. Recently, Fasano *et al.*[18] have shown by *in vitro* mutagenesis that mutations in codon 13 (Gly) can lead to transforming activity of the c-Ha-*ras*-1 gene. Therefore, we analysed the transfectant DNAs by hybridization with probes (N13-p1 and N13-p2) that could recognize mutations at the first or second position of codon 13. The oligomer group N13-p2 hybridizes with each of the AML transfectant DNAs (Fig. 3*F*). Those transfectants with the highest copy number of the N-*ras* gene showed the greatest signal with the N13-p2 probe, but hybridization was also seen in a transfectant (77T1) that only contains one or a few copies of N-*ras* (compare Figs 1 and 3*F*), whereas no hybridization was detected with control DNAs having the wild-type sequence (Fig. 3, lanes *a*, *b*). Thus, we conclude that the N13-p2 probe forms a fully matched hybrid with the AML transfectant DNAs. No hybridization was detected with the N13-p1 probe to the transfectant or control DNAs (Fig. 3*E*). Therefore, our results show that the transfectant DNAs contain N-*ras* genes with a mutation at the second position of codon 13. Recently, we have found that the N-*ras* gene in transfectant DNA of AML 73 also

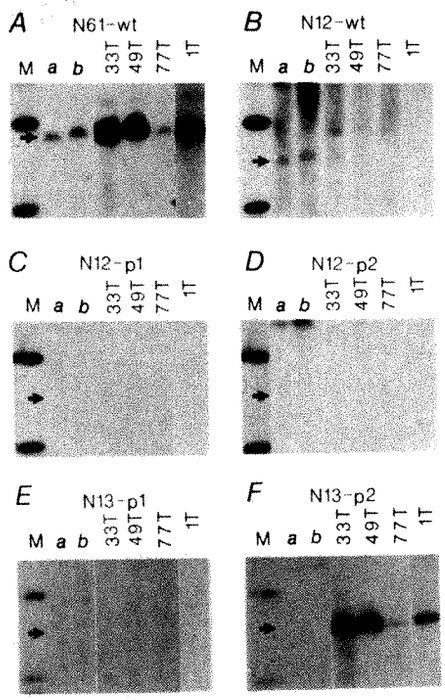


Fig. 3 Hybridization of synthetic oligomer probes to genomic DNA of 3T3 cell transfectants of four different human AMLs. The panels are replicates of each other with DNAs of HL60 (*a*) and HT1080 (*b*) as control and the DNAs of the AML transfectants 33T, 49T, 77T and 1T. The panels were hybridized to different oligomer probes: *A*, N61-wt; *B*, N12-wt; *C*, N12-p1; *D*, N12-p2; *E*, N13-p1; *F*, N13-p2; M, fragments of phage λ DNA digested with *Hin*dIII (4.3 and 2.3 kb). Arrows indicate the position of the N-*ras* fragment.

Methods. Hybridization was essentially as described by Bos *et al.*[17]. Genomic DNA (10 μg) was digested with *Pst*I and electrophoresed on a 0.5% agarose gel. The gels were denatured in 0.4 M NaOH, 0.8 M NaCl, neutralized in 0.5 M Tris-HCl (*p*H 7.2), 1.5 M NaCl and dried. The dried gel membranes were hybridized at 50 °C with N61-wt and at 53 °C with the other probes in 5 × SSPE (SSPE = 10 mM sodium phosphate *p*H 7.0, 0.18 M NaCl, 1 mM EDTA), 0.3% SDS and 10 μg ml^{-1} sonicated *Escherichia coli* DNA. Hybridized gels were washed in 2 × SSPE, 0.1% SDS at room temperature, in 5 × SSPE, 0.1% SDS at 53 °C for 15 min and finally in the same solution at either 59 °C (for N61-wt) or 63 °C (for the N12/13 probes) for 5 min. Gel membranes were autoradiographed for 2–4 days using intensifying screens.

hybridizes with the N13-p2 probe (data not shown) and thus contains a mutation at the same position.

Mutation to valine or aspartic acid

The use of mixed oligonucleotide probes allows the detection of the position of a mutation in a codon rather than establishing the precise mutant sequence. To analyse the exact nature of the mutations present in the N-*ras* gene of the AML transfectants, we hybridized DNAs to the individual oligonucleotide probes corresponding to the three possible mutations at the second position of codon 13. To prepare these individual probes, the labelled oligonucleotides of group N13-p2 were electrophoresed on a polyacrylamide gel (Fig. 4, insert 1), the individual bands excised and the separated oligomers further purified by electrophoresis on cellulose acetate strips (Fig. 4, insert 2).

To identify the separated oligomers, they were sequenced as described in Fig. 4 legend. The slowest-running oligomer (N13-p2/a) on the polyacrylamide gel had the sequence TTCCCAACATCACCTGCTCC and thus identifies a GAT codon 13 (Asp) in a fully matched hybrid. The second oligomer (N13-p2/b) identifies a GTT codon (Val), whereas the third and fastest-running oligomer (N13-p2/c) identifies a GCT codon (Ala). The separated oligonucleotide probes were then hybridized to *Pst*I-digested DNA from four AML transfectants. As shown in Fig. 4*A*, oligomer N13-p2/a forms a fully matched hybrid with DNA of transfectants from AML 1 and 33, whereas oligomer N13-p2/b forms a fully matched hybrid with DNAs from transfectants of AML 49 and 77 (Fig. 4*B*). The probe N13-p2/c did not hybridize with any of the transfectants (Fig. 4*C*) or with any of the separated oligomers, to control MOLT-4 DNA or to 10 genomic copies of the plasmid pAT8.8 (refs 13, 24), which contains the wild-type sequence at codons 12 and 13 (lane p). From these results we conclude that the N-*ras* gene in transfectants of AML 1 and 33 DNA contains a GAT codon 13, substituting Asp for Gly, whereas the transfectants from AML 49 and 77 contain a GTT codon 13, encoding Val. Recently, we found that AML 73 also hybridized specifically with N13-p2/b and thus contains a Val at position 13 (data not shown). The fact that the transfectant DNAs form fully matched hybrids with different members of the N13-p2 group is further evidence for the specificity of the hybridization, as each DNA now serves as a control for the other. There is no apparent correlation between the classification of the AMLs and the amino-acid substitution: AMLs 1 and 33 (Asp) were classified on the FAB scheme as M4 and M1 respectively, whereas AMLs 49, 73 and 77 (Val) were classified as M5, M2 and M4 respectively.

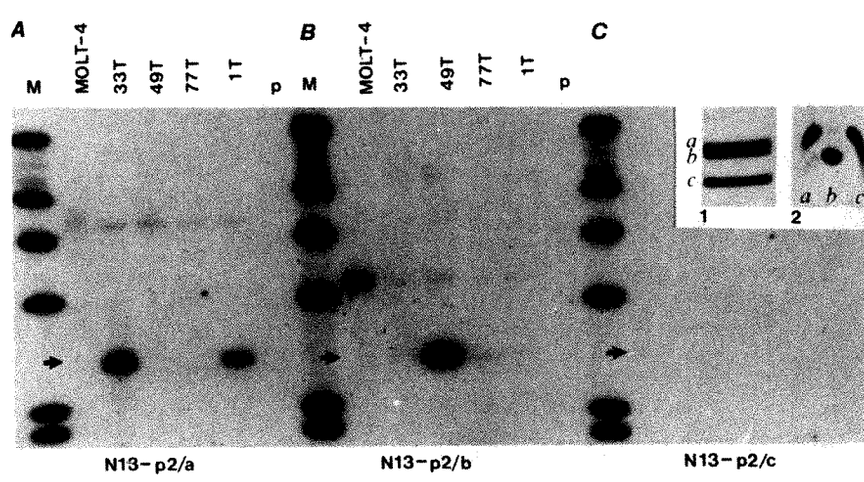


Fig. 4 Hybridization of separated N13-p2 oligomer probes to genomic DNAs of the AML transfectants. The panels are replicates of each other, with *Pst*I-digested DNAs of MOLT-4 as control and the DNAs of AML 33T, 49T, 77T and 1T. The panels were hybridized to N13-p2/a (TTCCCAACATCACCTGCTCC; panel *A*), to N13-p2/b (TTCCCAACAACACCTGCTCC; panel *B*) and to N13-p2/c (TTCCCAACAGCACCTGCTCC; panel *C*). Arrows indicate the position of the N-*ras* fragment; M, phage λ DNA digested with *Hin*dIII; p, 10 genomic copies of plasmid pAT8.8. This plasmid contains an 8.8-kb fragment of the activated N-*ras* gene HT1080 (CAA_{61}-AAA; ref. 24).

Methods. The three members of oligomers N13-p2 (*a*, *b* and *c* in inserts 1 and 2) were separated on a 20% polyacrylamide gel (mono/bis = 62.5 : 1) in 0.5 × TBE (TBE = 90 mM Tris, 90 mM boric acid, 2.5 mM EDTA) (see insert 1). To eliminate contamination, the isolated oligomers were further purified by electrophoresis on cellulose acetate strips in 7 M urea, 5% acetic acid, 5 mM EDTA *p*H 3.5 at 5,000 V (see insert 2). The fragments were eluted in 1 mM EDTA and used directly for hybridization. The sequence of each oligomer was determined by synthesizing N13-p2 with an end-labelled primer and cold deoxynucleoside triphosphates. This end-labelled probe was separated into the three members of N13-p2 and sequenced according to Maxam and Gilbert[26]. Gel separation and hybridization were as described in Fig. 2 legend.

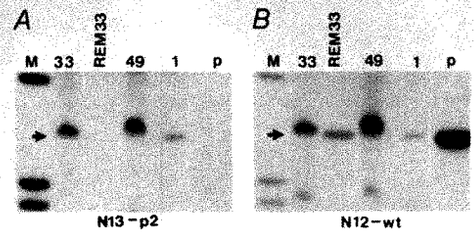


Fig. 5 Hybridization of N13-p2 (*A*) and N12-wt (*B*) oligomer probes to genomic DNA of isolated blast cells of AML patients 33, 49 and 1, and to genomic DNA of blood cells from AML patient 33 after remission (REM33). The differences in hybridization signal between the lanes result from loading differences and not from amplification of the N-*ras* gene. Arrows indicate the position of the N-*ras* band. Lane p, 20 genomic copies of the plasmid pAT8.8 containing the wild-type sequence at codons 12 and 13 (refs 13, 24). Gel separation and hybridization were as described in Fig. 3 legend.

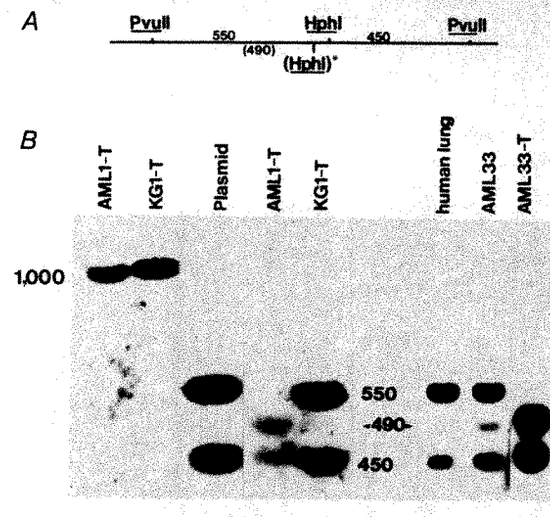


Fig. 6 *A*, Map of *Pvu*II and *Hph*I restriction enzyme sites in and around the first exon of N-*ras* to show the sizes of fragments generated after digestion of the normal N-*ras* gene and N-*ras* with Asp at codon 13. Restriction enzyme sites are taken from Brown *et al.*[13], Hall *et al.*[24] and A. Hall (personal communication). *B*, Southern blot analysis to show the altered *Pvu*II/*Hph*I fragments resulting from the presence of a new *Hph*I site generated by the Asp 13 mutation in the original leukaemic DNA from patient AML 33 and in transfectant DNAs of AML 1 and AML 33. This new *Hph*I site results in a *Pvu*II/*Hph*I fragment of 490 bp compared with 550 bp in wild-type DNA (see map).
Methods. DNA was digested either with *Pvu*II or *Pvu*II and *Hph*I, electrophoresed on a 1.4% low gelling temperature agarose gel and transferred to a nitrocellulose filter. The filter was hybridized with the nick-translated 1.0-kb *Pvu*II/*Pvu*II fragment of plasmid pAT7.8 (refs 13, 24) (see map). DNAs: pAT7.8 is a plasmid containing the *Bam*HI/*Eco*RI fragment with the first two exons of N-*ras* cloned from normal DNA[13], AML 33 is the original leukaemic sample, AML 33T a tumour derived after co-transfection into NIH 3T3 cells, AML 1T is a tumour derived from a focus isolated after transfection of NIH 3T3 cells with AML 1 DNA (note that this is a different transfectant from that used in Figs 3, 4 and 5 and contains fewer copies of N-*ras*), KG1-T (a transfectant from KG1 DNA that contains a position 12 mutation; J.W.G.J. and J.L.B., unpublished).

Somatic mutation in leukaemic cells

To exclude the possibility that the mutations at codon 13 were acquired during transfection and selection for transformed cells, the DNAs from three leukaemias were analysed. DNA was digested with *Pst*I, electrophoresed on an agarose gel and hybridized *in situ* to the oligonucleotide probes N12-wt and N13-p2. Hybridization of the gel to the mutant-specific N13-p2 oligomer group showed that all three AML DNAs hybridized, but no hybridization was detected to a sample containing 20 copies of a plasmid carrying the wild-type 12 and 13 sequences (Fig. 5*A*). Thus, the DNAs from these original leukaemic cells also contain the mutation at codon 13, eliminating the possibility that the mutations had arisen as a result of experimental manipulation.

The availability of DNA prepared from the peripheral blood of one patient (no. 33) in remission (REM33) allowed us to investigate whether the codon 13 change was present in normal cells. Figure 5*A* shows that REM33 DNA did not hybridize with the N13-p2 mutant-specific probes. As a control, we rehybridized the gel with the N12-wt probe and obtained a signal with the normal-sequence probe hybridized to REM33 DNA (Fig. 5*B*). Therefore, we conclude that this DNA (and thus the normal tissues of the patient) does not contain an altered N-*ras* gene hybridizing with N13-p2, and that the altered N-*ras* gene has arisen by somatic mutation. Hybridization with the normal-sequence probe was also obtained with the three DNAs from leukaemias, which could result from the presence of normal cells in the leukaemic sample. However, the high white blood cell counts of the leukaemic samples ($54–140 \times 10^9$ cells per l), coupled with the high proportion of cells with blast morphology (95–100%), makes it likely that the hybridization signal with the N12-wt probe is a result of the leukaemic cells containing both a normal and a mutant N-*ras* gene. The presence of amplified copies of the N-*ras* gene in some transfectants suggested that the position 13 mutations might act in concert with an increased copy number of the N-*ras* gene, but examination of the AML DNAs by Southern blotting shows no evidence of N-*ras* gene amplification (data not shown).

Restriction site polymorphism

The N-*ras* 13 mutation in AML 33 and AML 1 (Gly–Asp) changes the sequence GGTGG to GGTGA, which is the recognition site for the restriction enzyme *Hph*I. This new *Hph*I site will shorten a 550-bp *Pvu*II/*Hph*I fragment by 60 bp (see Fig. 6). We have analysed the original leukaemic DNA of AML 33 and transfectant DNAs from AMLs 1 and 33 for the presence of this extra *Hph*I site by Southern blot analysis. To that end we have compared the length of the *Pvu*II/*Hph*I fragments in these DNAs with the lengths of fragments in N-*ras* genes containing the wild-type sequence at and around codon 12 and in a codon 12 mutant (for details see Fig. 6 legend). The digestion of a plasmid, pAT7.8, containing the first and second exons of a normal N-*ras* gene[13] and of a transfectant, KG-1T, mutated at codon 12 (J.W.G.J. and J.L.B., unpublished results) generates a 550-bp *Pvu*II/*Hph*I fragment and a 450-bp *Hph*I/*Pvu*II fragment when hybridized with a probe of the *Pvu*II/*Pvu*II fragment of pAT7.8 (Fig. 6). However, when DNA from AML 33 is digested with *Pvu*II and *Hph*I, a novel 490-bp fragment, as well as the 450- and 550-bp *Hph*I/*Pvu*II fragments, is detected. The presence of both the 550-bp and the novel 490-bp fragment confirms our observation with oligonucleotide probes that this DNA contains N-*ras* genes with both a wild-type codon 13 and an Asp 13 mutation (compare Fig. 5). Southern blotting of a transfectant from AML 33 (AML33-T) and a transfectant of AML 1 (AML1-T) shows that these contain only the novel 490-bp and the 450-bp *Hph*I/*Pvu*II fragments as expected, because transfectants will contain only the mutated transforming N-*ras* allele. As a new *Hph*I site could not have been generated by a mutation in codon 12, the restriction enzyme analysis confirms the observations with oligonucleotide probes that AML 1 and AML 33 contain a codon 13 mutation of Gly to Asp.

Discussion

We have found that DNAs isolated from AML contain altered N-*ras* genes. These activated oncogenes have also been detected in three other samples of AML cells from patients[27–29]; in three AML cell lines, HL60 (ref. 28), Rc2a and KG-1 (ref. 38); and

recently in three more AML samples (D.T. and C.J.M., unpublished results). Why there should be a bias towards activation of N-*ras* genes in these leukaemias is not clear, but it is noteworthy that also in cell lines of acute lymphoblastic leukaemia, N-*ras* normally seems to be the activated *ras* oncogene[27,30]. N-*ras*, rather than the other *ras* genes, may be critically involved in the control of proliferation and differentiation of cells in the haematopoietic lineage. Alteration of this gene may then lead to the malignant state of the cells. The activation of the same *ras* oncogene in multiple isolates of similar tumours is reminiscent of findings that there is a reproducible pattern of *ras* gene activation in tumours induced by the same carcinogenic agent[31-33]. Therefore, a specific carcinogenic agent may be responsible for the N-*ras* activations in AML.

Unlike previously described examples of *ras* gene activation[3-17], we have not found mutations in codons 12 or 61. Instead, we have observed that mutations occur in codon 13, resulting in a replacement of Gly by either Val or Asp. One example of a codon 12 and a codon 61 mutation in AML cells has been noted previously[17,34] but, as only ~10–20% of malignancies seem to contain *ras* genes with mutations at codon 12 or 61 (for example, see ref. 16), the absence of codon 12 or 61 mutations in the samples we have analysed may result from the comparatively small sample size. The detection of codon 13 mutations in our samples of AML raises the question of why they have not been seen before. If codon 13 changes are restricted to AML, then they may not have been reported previously because only two mutant N-*ras* genes have been characterized from AML[17,34]. Furthermore, most of our samples were of FAB subtypes M1, M4 and M5, which have not been studied before. A restriction of codon 13 mutations to AML would imply that there is some constraint on the type of N-*ras* mutation in AML. Such a constraint could arise through the mechanism of mutation or through selection for the type of alteration in p21 protein. If codon 13 mutations are found in other malignancies, it is surprising that they have not been reported previously.

Our experience indicates that N-*ras* codon 13 mutants can be relatively inefficient in inducing foci. Although foci were detected in one laboratory with a DNA, AML 1, containing Asp 13 (ref. 38), they were not detected in another laboratory with DNA containing the same mutation, AML 33 (D.T. and C.J.M., unpublished results). This discrepancy has now been resolved by the observation that when the focus assays were kept for longer, foci induced by DNA derived from AML 33 were apparent 19–20 days after transfection but not at the usual time of examination at 15–16 days. However, foci from DNAs containing Val 13 were still not apparent even after leaving the assays for 20 days. Cells cultured from tumours resulting from co-transfection with DNAs containing the Asp 13 mutation also look more transformed than those that contain Val 13 mutants (D.T. and C.J.M., unpublished). These results are consistent with the idea that Val 13, which is an activating mutation not identified previously[18], and perhaps Asp 13 in N-*ras*, are relatively weak transforming alleles for NIH 3T3 and may be more difficult to detect than codon 12 or 61 mutations.

Our observation of multiple cases of mutations at codon 13 suggests that the activation of the N-*ras* gene at this site is an essential step in causing these AMLs. However, it is unlikely that the activation of the N-*ras* oncogene is the only step involved in the genesis of AML, because much evidence favours the argument that changes to more than one gene are involved in neoplastic transformation (see ref. 1 for review). Furthermore, specific chromosome translocations and other aberrations are associated with AML[35]. These chromosome aberrations occur at sites distinct from the 1cen–1p21 chromosomal site of N-*ras*[36] and separate aberrations are found in the different subgroups of AML. For example, a translocation, t(8;21), is found in AML M2 and an inversion of chromosome 16, inv16, in AML M4 with increased eosinophils (see ref. 35 for review). In contrast, we have found activation of N-*ras* in samples of AML from four different subgroups (M1, M2, M4 and M5); thus, the activation of N-*ras* seems to be affecting a process that is essential to all cells in the myeloid lineage, whereas the chromosome aberrations affect genes whose activities are more limited.

For the analysis of the N-*ras* mutations, we have used a set of specific oligonucleotide probes. This assay system is not only powerful in the detection of mutations in transfectant DNAs but also in the detection of specific mutations in tumour DNA. Using this assay system and the *Hph*I polymorphism generated by the Asp 13 mutation, we are currently analysing a large number of AML DNAs to gain more precise insight into the occurrence of different *ras* gene mutations in this leukaemia.

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Meteorites may follow a chaotic route to Earth

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It is widely believed that meteorites originate in the asteroid belt, but the precise dynamical mechanism whereby material is transported to Earth has eluded discovery. The observational data for the ordinary chondrites, the most common meteorites, impose severe constraints on any proposed mechanism. The ordinary chondrites are not strongly shocked, their cosmic ray exposure ages are typically <20 Myr, their radiants are concentrated near the antapex of Earth's motion and they show a pronounced 'afternoon excess' (for every meteorite which falls in the morning two fall in the afternoon). Wetherill[1] concluded that these data could only be explained by an "unobserved source" of material with perihelia near 1.0 AU and aphelia near Jupiter. His subsequent, more sophisticated investigations have not changed this basic conclusion. Recently I have shown[2,3] that there is a large chaotic zone in the phase space near the 3/1 mean motion commensurability with Jupiter and that the chaotic trajectories within this zone have particularly large variations in orbital eccentricity. Since asteroidal debris is quite easily injected into this chaotic zone, it could provide Wetherill's 'unobserved source' if chaotic trajectories which begin at asteroidal eccentricities ($e < 0.2$) reach such large eccentricities that Earth's orbit is crossed ($e > 0.57$)[4]. In this report I present a numerical integration which demonstrates that at least some of these chaotic trajectories do have the properties required to transport meteoritic material from the asteroid belt to Earth. Combined with the Monte Carlo calculations which show that the resulting meteorites are consistent with all the observational constraints, the case for this chaotic route to Earth is fairly strong.

Wetherill[5,6] has completed an extensive new Monte Carlo study to determine the meteoritic yield from this chaotic zone, with some reasonable assumptions concerning the actual dynamics. He assumes that on a timescale of 10^6 years, chaotic trajectories evolve to become Earth-crossing. During much of this time the trajectories are Mars-crossing. He estimates that there is a 50% chance that the material will become Earth-crossing before Mars removes it from the chaotic zone. (It has been suggested previously[7] that meteorites could be brought to Earth from the 3/1 resonance with the assistance of Mars. The timescale for this process is, however, longer than the time for collisional disruption of meteorite-sized bodies, and fragments from any larger bodies which have taken this route will not satisfy the orbital constraints for the ordinary chondrites. It is essential, therefore, that trajectories can become Earth-crossing without the assistance of Mars.) Once the fragment becomes Earth-crossing, however, perturbations from Earth remove it from the chaotic zone in 10^4 years, the approximate duration of the Earth-crossing spikes in eccentricity. With this assumed dynamical input, the subsequent evolution is followed with the Monte Carlo method[8,9]. Wetherill's model includes planetary close encounters, secular resonances, collisional destruction in space and atmospheric ablation. He concludes that major quantities of asteroidal fragments from bodies adjacent to the 3/1 gap can be transferred to Earth. His computed flux is comparable to the observed flux of ordinary chondrites. The afternoon excess of meteorite-sized fragments obtained directly from the chaotic zone is too large, but when the fall-times of these fragments are combined with the fall-times of fragments obtained from larger objects derived earlier from the 3/1 gap, the predicted fall-time ratio agrees with the ordinary chondrite value of 2/3. Similar agreement is found for the other observable parameters.

Greenberg and Chapman[10] have also considered the consequences of this transport mechanism; while they also compute a flux of chondrites consistent with the observed flux, their physical assumptions and methods are quite different from those of Wetherill. The purpose of this letter is not to enter that debate, but to make firm the dynamics on which it is based. If trajectories in the 3/1 chaotic zone do not have the required properties the discussion is moot.

In this article 'chaotic behaviour' refers to deterministic solutions of hamiltonian systems of equations that behave erratically and show sensitive dependence on initial conditions (positive Lyapunov characteristic exponent). On theoretical grounds[11], a chaotic zone is generally expected to be associated with each commensurability in a dynamical system, though these chaotic zones are often microscopically small. Using Schubart's numerical averaging procedure[12], Giffen[13] showed that the 2/1 commensurability in the planar-elliptic restricted three-body problem is accompanied by a sizeable chaotic zone. Scholl and Froeschle[14,15] extended Giffen's work to the other principal commensurabilities; while they found chaotic behaviour near the 3/1 commensurability, they concluded that the chaotic zone was small and unimportant. In fact, there is a large chaotic zone near the 3/1 commensurability, and trajectories within this zone have the peculiar property that their orbital eccentricity can remain low ($e < 0.1$) for as long as a million years and then suddenly assume very large values ($e > 0.3$)[2,3]. The long-term evolution of a typical chaotic trajectory has bursts of low eccentricity behaviour followed by bursts of high eccentricity behaviour, each of apparently random duration. Furthermore, when the effects of phase mixing are removed, the boundaries of the 3/1 Kirkwood gap correspond, within the errors of the asteroid orbital elements, to the boundary in the phase space of the chaotic zone. The 3/1 Kirkwood gap can be explained entirely by removal of the highly eccentric chaotic orbits and the also highly eccentric quasiperiodic librators by collisions with Mars and, as reported here, Earth.

These discoveries depended on the development of a new 'mapping' technique for studying the long-term evolution of trajectories near commensurabilities that is approximately 1,000 times faster than all previous methods. This method is approximate (as are the methods used in earlier studies), but the essential features of the picture presented above have been verified by conventional numerical integrations of the exact equations of motion for the planar-elliptic restricted three-body problem[3]. In particular, the width of the chaotic zone is in perfect agreement with that found using the mapping method. Even at eccentricities as high as 0.4, the trajectories using the mapping and the exact solutions are in good qualitative agreement, even though fourth-order terms in eccentricity are neglected in the disturbing function. Independent investigation has confirmed this qualitative agreement[16].

In the planar-elliptic problem, the eccentricity of chaotic trajectories which begin at asteroidal eccentricities ($e < 0.2$) seems to be limited to values <0.5; this is large enough for the trajectories to be Mars-crossing but not Earth-crossing. However, in the three-dimensional elliptic problem, chaotic trajectories computed with a mapping reach much larger eccentricities, large enough to be Earth-crossing ($e > 0.57$). Now the approximations used to derive the mapping are certainly invalid at such large eccentricities, but the success of the planar mapping at eccentricities as large as 0.4 encourages one to take these high eccentricities seriously. The actual eccentricity limit can be determined only by numerical integration of the exact equations of motion. Such integrations are time-consuming, but the answer is vital to our understanding of the origin of meteorites.

One approach to this problem is simply to integrate a chaotic trajectory until it reaches Earth-crossing eccentricities. Experience with the mapping, however, indicates that this may take several million years. Furthermore, not all chaotic trajectories are equivalent; there is an integral which divides the chaotic zone into a one-parameter family of chaotic zones. Some

of these may have Earth-crossing trajectories and others not; we do not accurately know the extent of the eccentricity variations for any chaotic trajectory. It might then be necessary to integrate several chaotic trajectories for several million years to find the Earth-crossing route. This approach is not practical without a dedicated computer. Instead, I have sought, by trial and error, trajectories which begin near that point in the phase space where transitions from low eccentricity behaviour to high eccentricity behaviour occur, with as much guidance from the mapping as I have been able to glean. (While the mapping does give the correct qualitative behaviour, the correspondence is not precise enough to directly determine initial conditions.)

The numerical integrations include the Sun, the four major planets and a test body. Pluto is clearly not important because of its small mass. The inner planets are included with the Sun. I am attempting to show that the long-term evolution of a chaotic trajectory without close planetary encounters will bring material within the influence of Earth's gravitational field. It seems fair to ignore Earth until this is the case. The neglect of Mars perturbations is clearly more serious. I argue that Mars substantially affects the solution only during close encounters and that these close encounters may be taken into account after the fact in a statistical manner, much as Wetherill[6] has done. (His approach may not be entirely satisfactory. As mentioned previously, there is a family of chaotic zones, not just one. Minor encounters with Mars will be sufficient to cause a shift from one member of this family to another without removing the trajectory entirely from the chaotic zone. Several such shifts may occur before Earth is reached. Nevertheless, such trajectories would essentially be injected directly into Earth's control. Thus this synergism between Mars perturbations and chaotic dynamics is qualitatively different from previously proposed mechanisms[7] that rely on Mars perturbations to assist in the transfer of material from the Kirkwood gaps.)

The integrations were performed in equatorial rectangular coordinates referred to the mean equinox and equator 1950.0. The origin was the baricentre of the Sun and four inner planets. The unit of time was 40 mean solar days; the origin of time was taken to be 1982 August 19.0 (JD 2,445,200.5). The extrapolation algorithm of Bulirsch and Stoer[17] was used to perform the integrations. The relative accuracy per integration step was chosen to be 10^{-11}; the variable integration step was of the order of 1 yr. The twelfth-order Cowell method used by Cohen *et al.*[18] in their 1-Myr integration is less satisfactory when the eccentricities are large as in the integrations reported here. The initial conditions for the major planets were obtained from those used by Cohen *et al.* by numerical integration from their origin of time (JD 2,430,000.5) to mine. Their initial conditions for the major planets were in turn taken from the work of Eckert *et al.*[19], where the equations of motion can be found. No effort has been made to correct the initial conditions to properly account for my removal of Pluto. Because of the large Pluto mass used by Eckert *et al.* this introduces a relative discrepancy

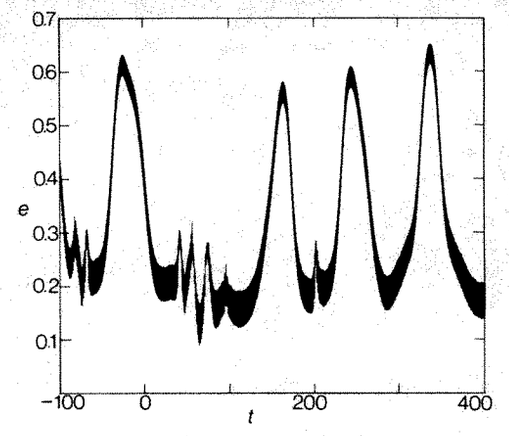


Fig. 1 The eccentricity (e) as a function of time (t, in millennia) plotted for the chaotic trajectory, which at times is deep in the asteroid belt (when $e < 0.15$) and at other times is strongly Earth-crossing ($e > 0.6$). The time origin is 1982 August 19.0.

of one part in a million in the Cartesian coordinates just in the integration from their origin of time to mine. (I have verified, however, that when Pluto is included the program satisfactorily reproduces the tables of Eckert *et al.*[19].) The accuracy of these numerical integrations was tested directly by integrating the planets forward with one test meteoroid 170,000 years, and then integrating them back to the time origin with a different meteroid on an independent trajectory. Since the time step chosen by the integrator is mainly determined by the eccentricity of the meteoroid, these two integrations are completely independent. The initial conditions of the major planets were reproduced to one part in 10^4. The skeleton of the integration is thus adequately represented.

It is well known that chaotic trajectories are difficult to follow precisely. In a chaotic zone neighbouring trajectories separate exponentially with time, and consequently so do round-off errors. Fortunately, the rate of this exponential separation is rather small for the 3/1 chaotic zone[3]; nevertheless, even a small exponential growth of errors is unbeatable. Now it has been argued[20] that even though any particular trajectory cannot be followed precisely, the qualitative behaviour of the trajectory will be correct if reasonable care is taken and furthermore that a true trajectory can be found which shadows the computed trajectory. This is not as wild a claim as it may seem, for it has been proven for the special class of Anosov dynamical systems. It is probably not strictly true for Hamiltonian systems in general, but most people who study chaotic behaviour in dynamical systems believe that it is practically true. The actual rate of separation of nearby trajectories in the 3/1 chaotic zone allows strict confidence to be placed in segments of 80,000 years[3]. Over longer times we may only hope that some true trajectory shadows

Table 1 Equatorial frame coordinates and velocities at JD 62043200.5

	x (AU)	y (AU)	z (AU)
Meteoroid	$0.262137541577 \times 10^{1}$	$-0.949476934922 \times 10^{0}$	$-0.179096284774 \times 10^{0}$
Jupiter	$-0.351023142831 \times 10^{1}$	$0.339348310152 \times 10^{1}$	$0.151726945011 \times 10^{1}$
Saturn	$0.777797463489 \times 10^{0}$	$-0.824734443595 \times 10^{1}$	$-0.369212909944 \times 10^{1}$
Uranus	$-0.748876946495 \times 10^{1}$	$-0.165985490544 \times 10^{2}$	$-0.644552105739 \times 10^{1}$
Neptune	$0.287412144601 \times 10^{2}$	$0.842938531215 \times 10^{1}$	$0.260621846977 \times 10^{1}$
	$\dot{x}$ (AU/40 days)	$\dot{y}$ (AU/40 days)	$\dot{z}$ (AU/40 days)
Meteoroid	$0.311286716224 \times 10^{0}$	$0.216759866523 \times 10^{0}$	$0.772871903870 \times 10^{-1}$
Jupiter	$-0.212645617591 \times 10^{0}$	$-0.206291875136 \times 10^{0}$	$-0.807809459066 \times 10^{-1}$
Saturn	$0.232711603323 \times 10^{0}$	$0.234902479708 \times 10^{-1}$	$0.286504328835 \times 10^{-2}$
Uranus	$0.145063206654 \times 10^{0}$	$-0.519858201645 \times 10^{-1}$	$-0.249322339022 \times 10^{-1}$
Neptune	$-0.382851184016 \times 10^{-1}$	$0.109983656044 \times 10^{0}$	$0.465724813196 \times 10^{-1}$

the computed trajectory. In any case 80,000 years is long enough to cover the most interesting behaviour.

I have made five numerical integrations, each spanning ~500,000 years. The initial conditions were chosen in the chaotic zone as determined by the three-dimensional mapping. The initial eccentricity for the first four was 0.15, the mean eccentricity of the asteroids; for three of these the resulting peak in eccentricity was between 0.5 and 0.54, insufficient for Earth-crossing. Earth's maximum aphelion occurs at 1.07 AU (ref. 21); for semimajor axes near the 3/1 commensurability ($a \approx 2.5$ AU) an eccentricity of 0.57 is required to just graze this distance. The results of the first three integrations were encouraging but not sufficient. The fourth integration, however, reached an eccentricity of 0.564 after 170,000 years. Its initial conditions (JD 2,445,200.5) were: semimajor axis $a = 0.4816 a_J$, eccentricity $e = 0.15$, inclination = 5°, mean anomaly $M = 180° + 3M_J$, longitude of perihelion $\varpi = \varpi_J$ and longitude of ascending node $\Omega = \varpi_J$. These elements are heliocentric osculating elements relative to the equinox and ecliptic 1950.0, and the subscript J refers to Jupiter. These initial conditions are on the representative plane[3]. This trajectory came so close to Earth-crossing (0.01 AU) that it appeared possible to change the coordinates enough to make it Earth-crossing and still remain in the chaotic zone. The first attempt to do this was, however, unsuccessful. The resulting trajectory was clearly quasiperiodic in nature. With a somewhat smaller increase in eccentricity, however, the route to Earth was found.

The initial conditions for this chaotic trajectory are given in Table 1. The trajectory was integrated both forward and backward in time from that point. The eccentricity as a function of time (in millennia) is shown in Fig. 1; the peak in eccentricity occurs near $t = 340{,}000$ yr with the value $e_{max} = 0.652$; the minimum perihelion distance is $q_{min} = 0.861$ AU, which is strongly Earth-crossing. The peak near $t = -20{,}000$ yr has $e_{max} = 0.632$ and $q_{min} = 0.910$ AU. The minimum in eccentricity occurs near $t = 60{,}000$ yr during a short burst of low eccentricity behaviour with the value $e_{min} = 0.089$; this value is lower than the mean eccentricity of the asteroids. The semimajor axis remains between 2.47 AU and 2.53 AU as expected. The inclination to the ecliptic varies between 1° and 8° in a way that is not obviously correlated with the eccentricity. The behaviour of the resonant argument $\sigma = l + 2\varpi - 3l_J$, where l is the mean longitude, is very complicated. During the eccentricity spikes it librates about a slowly varying centre which makes one revolution during the spike; during the low eccentricity bursts it circulates. The behaviour of the semimajor axis is correlated with the resonant argument: during the eccentricity spikes the semimajor axis oscillates across its full range of variation, but during the low eccentricity bursts it alternately oscillates on one side or the other of the centre of that range. This behaviour is familiar from the mapping studies, and consistent with the qualitative picture of these bursts presented previously[3]. The maximum Lyapunov exponent for this chaotic trajectory is $\lambda \approx 10^{-3.8}\ \mathrm{yr}^{-1}$.

The peak in eccentricity that occurs near 170,000 years, near our starting point, has $e_{max} = 0.583$ and $q_{min} = 1.034$ AU. Since q_{min} is less than Earth's maximum aphelion of 1.07 AU I was initially satisfied that this trajectory was indeed Earth-crossing; on closer examination this turned out not to be the case. I first checked that the argument of perihelion (the angle between the longitude of the ascending node and the longitude of perihelion was satisfactory. It turns out to be correlated with the peaks in eccentricity, and to be ideally placed for Earth-crossing (near zero). If it had been near 90° when the eccentricity was large, then Earth's orbit would not intersect the meteoroid's orbit—the meteoroid's orbit would have crossed the ecliptic near Mars. However, a more subtle avoidance mechanism was operative. During the peaks in eccentricity the longitude of perihelion aligns with Jupiter's longitude of perihelion. Although it is not commonly known, Earth's perihelion is also correlated with Jupiter's perihelion. Earth's maximum aphelion distance always occurs when the perihelia of Earth and Jupiter are aligned. Thus, if the meteoroid and Earth are both near their maximum eccentricities, their orbits are aligned so that no crossing occurs. It is a simple matter to calculate from Brouwer and van Woerkom's solution[22] of the secular motions of the planets that when Earth's perihelion is anti-aligned with Jupiter's perihelion, Earth's maximum aphelion is only 1.031 AU. During this peak the meteoroid came quite close to Earth, but "An inch is good as a mile". Nevertheless, the other peaks in eccentricity are strongly Earth-crossing and no avoidance mechanism is operative.

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Poorly graphitized carbon as a new cosmothermometer for primitive extraterrestrial materials

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The presence of carbon in primitive extraterrestrial materials has long been considered a useful indicator of prevailing geochemical conditions early in the formation of the Solar System. A recent addition to the suite of primitive materials available for study by cosmochemists includes particles collected from the stratosphere called chondritic porous (CP) aggregates[1]. Carbon-rich CP aggregates are less abundant in stratospheric collections and contain many low-temperature phases (such as layer silicates) as minor components[2,3]. We describe here the nature of the most abundant carbon phase in a carbon-rich CP aggregate (sample no. W7029*A) collected from the stratosphere as part of the Johnson Space Center (JSC) Cosmic Dust Program[4]. By comparison with experimental and terrestrial studies of poorly graphitized carbon (PGC), we show that the graphitization temperature, or the degree of ordering in the PGC, may provide a useful cosmothermometer for primitive extraterrestrial materials.

A detailed analytical electron microscope (AEM) investigation of CP aggregate W7029*A has established that parts of this aggregate experienced nominal flash-heating no higher than

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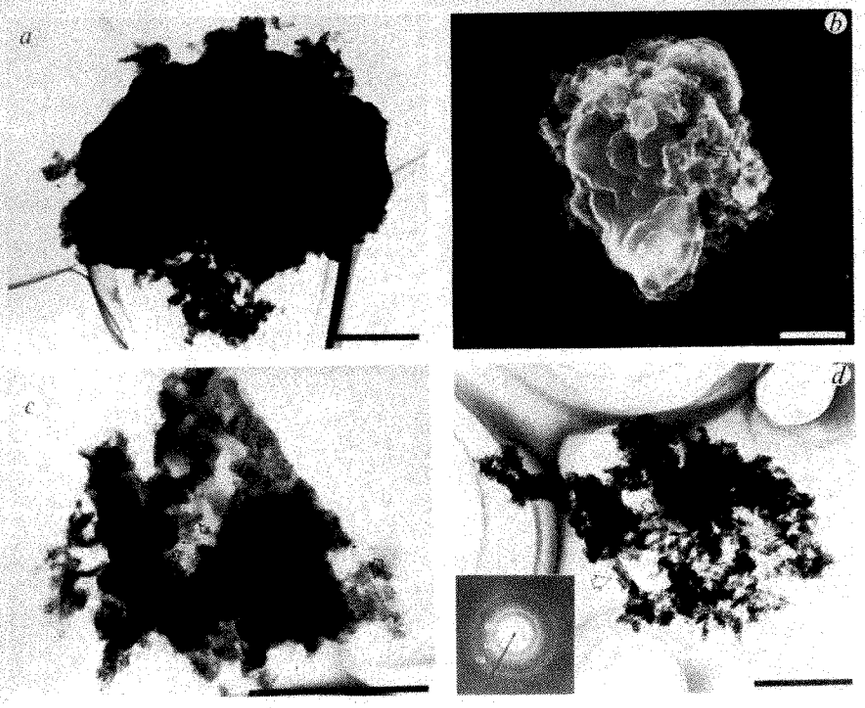


Fig. 1 Examples of carbon particles in CP aggregate W7029*A. *a*, TEM image of clumped carbon particle with granular areas along the margin. *b*, SEM image of the same carbon particle as *a*, but tilted to show individual plates and granular strings (arrowed). *c*, TEM image of granular particle showing two-dimensional fibrous domains (arrowed). *d*, TEM image of an open, fluffy granular cluster with three small sheets (arrowed) and its typical SAED pattern of diffuse rings (inset). Scale bars, 0.5 μm.

300–400 °C during atmospheric entry and that terrestrial contamination of the aggregate was not significant[3]. The fluffy morphology and chondritic bulk composition of CP aggregate W7029*A, as well as unique mineralogy, are reliable indicators of its extraterrestrial origin[2,3]. A modal analysis of 224 grains from this CP aggregate shows that ~45% are carbon-rich phases and ~30% are low-temperature phases (such as layer silicates, geothite and sulphides). Further details on the mineralogy of minor phases in CP aggegate W7029*A, as well as collection, curation and processing procedures at the JSC Curatorial Facility are given elsewhere (refs 3, 5 and refs therein). Data on CP aggregate W7029*A were obtained with a JEOL 100CX AEM operating at 100 kV and equipped with a Princeton Gamma Tech System IV energy dispersive spectrometer (EDS). Electron energy loss spectra (EELS) were collected with a Gatan spectrometer attached to a Philips EM 420 AEM operating at 120 kV. The interlayer spacings obtained from calibrated electron diffraction patterns and listed in Table 1 for CP aggregate W7029*A have a relative error of 1%.

Carbon-rich particles in CP aggregate W7029*A form separate clump-like masses. (EDS analyses of 65 clumped masses are consistent with materials in which elements with atomic number >10 are absent. In addition, electron energy loss spectroscopy of selected clumped masses confirmed the presence of carbon only.) Carbonaceous material with similar morphologies have been described in two CP aggregates containing silicate and magnetite platelet grains[6]. Figure 1*a*, *b* shows transmission and scanning electron microscope (TEM and SEM) images of a typical carbon particle in CP aggregate W7029*A. The clumps of carbonaceous material range in size from 0.2×0.3 μm to 1.5×2.0 μm. These clumped masses appear to consist of stacked sheets with lobate circumferences and may have a ragged topography (Fig. 1*b*). Individual sheets may be decorated by fine-scale (0.01–0.05-μm diameter) granular carbonaceous material. These granules may coalesce to form decorative strings around and up on individual sheets, or may form larger granular clusters with an open, fluffy texture (Fig. 1*c*, *d*). Within these granular clusters, small sheets are occasionally observed (see Fig. 1*d*). TEM images of the clumped masses show two-dimensional fibrous domains (100–1,400 Å long and 45–60 Å wide), open, randomly oriented loops and subcircular structures ~150–500 Å in diameter. Selected area electron diffraction (SAED) patterns of the granular clusters from CP aggregate W7029*A show broad, diffuse rings (inset, Fig. 1*d*) and have 002, 10*l* and 11*l* interlayer spacings consistent with PGC[7]. In addition, the range and type of morphologies observed for the carbon phases in CP aggegate W7029*A are similar to those observed for PGC formed by carbonization, that is the removal of oxygen and nitrogen, and subsequent graphitization of hydrocarbons[8,9].

Other primitive extraterrestrial materials, particularly the carbonaceous chondrites, contain variable amounts of carbon

Table 1 Interlayer spacing, dimensions of the graphite component and fraction of ordered layers in PGC in three carbonaceous chondrites and CP aggregate W7029*A

		Carbonaceous chondrites		
	CP aggregate W7029*A	Orgueil (CI)	Cold Bokkeveld (CM)	Allende (CV)
d_{002} spacing (range) (Å)	3.50 (3.47–3.53)	3.8 (3.5–4.1)	3.6 (3.4–3.9)	3.47 (3.39–3.5)
Fibres L_a (Å)	<100–1,400	60–200	100–420	190–270 150–1,000*
Fibres L_c (Å)	45–60	20–40	35–140	60–100
Polygonal and subcircular structures (Å)	150–500	2,000–3,500	1,500–2,000	100–150*
Fraction of ordered layers (p)	0	0	26	35

Meteorite data are from refs 7, 10* and CP aggregate W7029*A data from the present study. The number of samples for Orgueil, Cold Bokkeveld meteorites and CP aggregate W7029*A is one; for the Allende meteorite five samples are used. In each sample, a larger number of individual particles have been studied (for example, 65 in the aggregate). Note that the true length of fibrous crystallites (L_a) may be greater because lattice fringes can only be observed if the graphite basal reflection (d_{002}) is parallel to the incident electron beam. L_c, the width (= thickness) of fibrous crystallites.

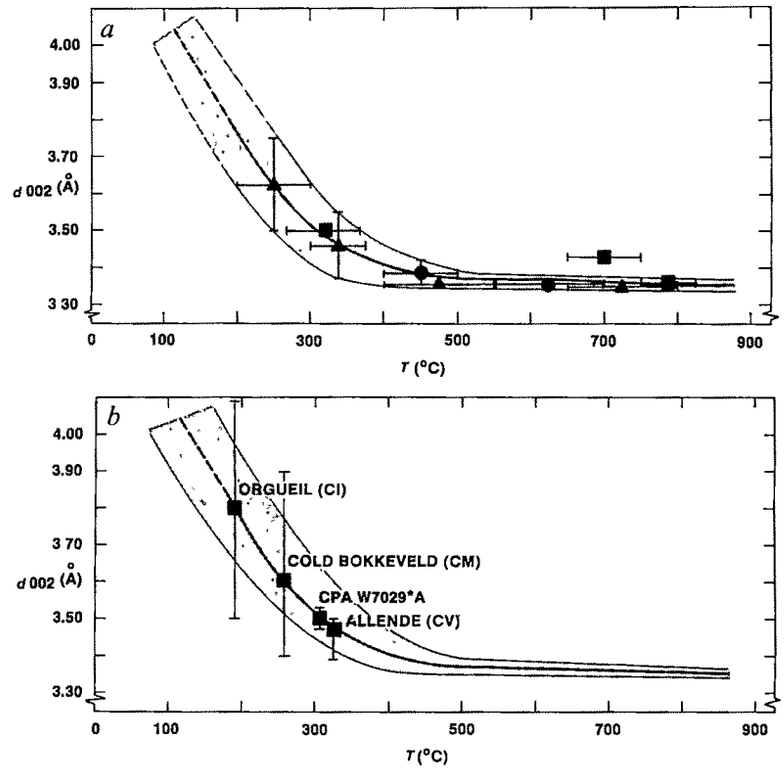


Fig. 2 *a*, Average d_{002} interlayer spacing (Å) in naturally occurring poorly graphitized carbon in terrestrial metamorphic rocks plotted against temperature (°C). The shaded area denotes uncertainty in the data Sources. ●, ref. 11; ■, ref. 13; ▲, ref. 14. *b*, d_{002} interlayer spacing in PGC from carbonaceous chondrites and from CP aggregate W7029*A plotted on the curve for PGC in terrestrial metamorphic rocks.

phases. AEM studies of acid residues of the Allende CV meteorite show that carbon phases form as tangled, fibrous (graphitic) crystallites, or aggregates of crystallites, in a featureless (possibly amorphous) matrix[10]. Subcircular to polygonal concentric structures with the graphite basal plane spacing were also observed[10]. SAED patterns from carbonaceous material in the Orgueil (CI), Cold Bokkeveld (CM) and Allende (CV) meteorites have been obtained by Lumpkin[7]. These SAED patterns typically show broad rings corresponding to the 002, 101 and 111 reflections of turbostratic carbon, or PGC.

The average d_{002} interlayer spacings for each of these primitive materials and for CP aggregate W7029*A are listed in Table 1. For the Orgueil, Cold Bokkeveld and Allende meteorites the size of fibrous crystallites increases uniformly with decrease in average d_{002} interlayer spacing (Table 1). In addition, the diameter of subcircular to polygonal concentric structures decreases from the Orgueil to Cold Bokkeveld to Allende meteorite. In Table 1, all four classes of primitive material show distinct signatures for the PGC. Our compilation of data suggests that a parameter related to the environment of formation may be responsible for the variation in d_{002} spacing, size and morphology of PGC in all four primitive extraterrestrial materials.

There exists a large body of data on the processes of carbonization and graphitization[8,9], in both experimental conditions and within natural systems[11]. These data provide further insight into the probable formation conditions for PGC in CP aggregate W7029*A and selected carbonaceous chondritic meteorites. The d_{002} interlayer spacing is a characteristic structural feature of poorly graphitized carbons which can range from values of 4.8 to 3.44 Å (the average interlayer spacing of extensive, randomly stacked graphitic layers) to 3.354 Å, the value typical of fully ordered hexagonal graphite[8]. Experimental work has shown that this variation in d_{002} interlayer spacing is temperature-dependent. Thus, with heat-treatment temperature the d_{002} interlayer spacing steadily decreases from 4.8 to 3.44 Å (ref. 8). The sizes of tangled graphitic fibres are directly related to the formation temperature of poorly graphitized carbon[9] and increase with increased heat-treatment temperature and/or time. These experiments[8,9] are of short duration and use pure starting materials. In all experiments, the extent of graphitization of carbon compounds is strongly influenced by the presence of a catalyst[12].

A similar temperature-dependency for d_{002} interlayer spacing PGC can be observed in natural terrestrial environments. Graphitization in terrestrial rocks may occur over long periods of time (millions of years) (long duration of heat-treatment) and may also be influenced by an active fluid phase, lithostatic pressure or the presence of catalytic minerals (such as silicates and carbonates)[11]. Owing to these influences, in general, the temperature recorded by graphitization of PGC in a metamorphic rock[11] will be lower than that determined by experiments with pure starting materials[8].

Both X-ray diffraction and AEM have been used to study graphitization in terrestrial metamorphic rocks[11,13-15]. Using data collected with both techniques, we have plotted (Fig. 2*a*) the average d_{002} interlayer spacings for PGC from a suite of metamorphic rocks against their peak metamorphic temperatures. In all cases, peak metamorphic temperatures used in Fig. 2*a* are those given by the original authors[11,14] except for the sequence described by French[13]. For these data points, we estimated peak metamorphic temperatures using recently published petrological data for these contact-metamorphosed Precambrian iron formations[16-19]. The ranges of d_{002} interlayer spacings and peak metamorphic temperatures for each data point are represented by vertical and horizontal error bars, respectively, in Fig. 2*a*. As we show, the average d_{002} interlayer spacing for PGCs in terrestrial metamorphic rocks decreases with increased peak metamorphic temperature up to ~450 °C. Above this temperature the carbon phase may become fully ordered graphite.

The mean apparent interlayer spacing (d_{002}) can be related to the degree of order (degree of graphitization), or the fraction of sp^2 carbon layers in PGC. For synthesized PGCs, an expression relating the mean apparent interlayer spacing and the fraction, p, of ordered layers may be written as $d(002) = 3.44\text{-}0.086(1-p^2)$ (ref. 20).) Using this equation, we have listed in Table 2 the calculated degree of order for each natural occurrence used to construct Fig. 2*a*. This tabulation indicates that the degree of order determined from average d_{002} values does not vary uniformly with estimated peak metamorphic temperatures. However, we can understand this variation in degree of order for terrestrial rocks if we consider the duration of peak metamorphic heating as well as the peak metamorphic

Table 2 Interlayer spacing and estimated peak metamorphic temperature for PGC from terrestrial metamorphic rocks

d_{002} spacing (Å)	Peak metamorphic temperature (°C)	No. of observations	Degree of order
Poorly graphitized carbon			
3.75–3.50	200–300 (14)		0
3.55–3.37	300–375 (14, 23)	27 (14)	0–57
3.36–3.35	400–500 (14, 23)		74–80
3.50	320±50 (16–19)	8 (13)	0
3.43	650–750 (16–19)		6
3.42–3.36	400–500 (11)	8 (11)	12–74
Fully ordered graphite			
3.35	600–800 (14, 23)	8 (14)	100
3.36	750–825 (16–19)	8 (13)	100
3.36	500–700 (11)	8 (11)	100

The numbers in parentheses refer to the source of data. Data points from refs 11, 14 are regionally metamorphosed rocks; data points from ref. 13 are for contact metamorphic rocks. References 16–19 and 23 are used to estimate peak metamorphic temperatures for contact metamorphic rocks in ref. 13 and for regionally metamorphic rocks in ref. 14.

temperature. For example, graphitization takes place in contact metamorphic rocks at ~1,000 °C. However, for regionally metamorphosed rocks a comparable degree of graphite order occurs at ~600 °C if the duration of metamorphic heating is at least 1,000 yr (ref. 11). For metamorphic rocks at lower temperatures, kinetic factors tend to dominate the graphitization process and the range of values for the d_{002} interlayer spacing may reflect a random statistical effect of non-uniform degrees of order within a given sample. Thus, it is appropriate to use the average d_{002} interlayer spacing for individual samples to derive graphitization temperature, or degree of order.

The curve in Fig. 2*a* shows the relationship between the average d_{002} interlayer spacing and peak metamorphic temperature for PGC in terrestrial metamorphic rocks. In Fig. 2*b*, the values of the d_{002} interlayer spacing for selected primitive extraterrestrial materials are superimposed on this curve for terrestrial occurrences. We then estimate graphitization temperatures for the carbonaceous chondrites at ~190 °C (Orgueil), ~260 °C (Cold Bokkeveld) and ~335 °C (Allende) and for CP aggregate W7029*A at ~315 °C. The inverse relationship between sizes of fibrous crystallites and diameters of subcircular to polygonal concentric structures[9] in the carbonaceous chondrites (Table 1) is also compatible with higher temperatures for Allende compared with the Orgueil meteorite.

Graphitization temperatures derived from Fig. 2*b* are higher than the calculated accretion temperatures for carbonaceous chondrites: <42–87 °C for C1 and CM chondrites, <127 °C for CV chondrites[21]. Graphitization in these meteorites may be reasonably related to post-accretion processes, as graphitization is an irreversible process[8] and the temperatures obtained from Fig. 2*b* are probably minimum estimates. The curve in Fig. 2*a* probably averages the effects of all parameters which influence graphitization of poorly graphitized carbons on a geological timescale. The presence of active catalytic phases will lower the graphitization temperature[11]. However, unless catalytic activity in these primitive extraterrestrial materials was more pronounced than in the terrestrial occurrences used for Fig. 2*a*, the curve in Fig. 2*b* represents graphitization temperatures for PGCs in these extraterrestrial materials. If catalytic activity in these meteorites and CP aggregate W7029*A was indeed more dominant than is assumed for the curve in Fig. 2*b* all data points would shift towards lower temperatures.

We have shown that graphitization temperatures in terrestrial rocks may be a useful guide to the interpretation of graphitized carbon occurrences in extraterrestrial materials. The degree of graphitization in a suite of primitive extraterrestrial materials follows a simple trend which may be related to the duration and temperature of peak metamorphic heating during formation. Graphitization temperatures recorded in this suite of extraterrestrial materials and derived from terrestrial trends are consistent with metamorphic temperatures experieneced after condensation of the solar nebula but early in the history of the Solar System. Further investigation of graphitization in extraterrestrial materials may provide useful constraints on the formation of parent bodies for primitive chondrites and CP aggregates[22].

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Radial distribution studies of non-framework aluminium species formed during dehydroxylation of zeolite HY

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The use of stabilized zeolite Y as a cracking catalyst for converting petroleum into gasoline, fuel oil and petrochemical feeds is widespread[1]. During the stabilization process, when the ammonium-exchanged form of zeolite Y is heated to high temperatures, aluminium is extracted from the zeolite framework[1–3]. The nature of this non-framework aluminium has been of considerable interest because of its possible influence on the zeolite acidity and thermal stability. Indirect information on the nature of the non-framework Al (NFA) has been obtained from infrared spectroscopy, X-ray diffraction, absorption and nuclear magnetic resonance (NMR) studies. To obtain further knowledge of the NFA species, we have now performed radial electron distribution studies of H–Y and H–Y dehydroxylated in vacuum and steam. These studies suggest that the non-framework Al species probably contain edge-shared octahedral $Al_m(O,OH)_n$ chains similar to those found in $NaAlSi_2O_6$ (jadeite), $CaAl_2(OH)_4SiO_4$ (chantalite) and $Al_2(OH)_2TeO_3SO_4$.

The loss of Al from the framework and the development of octahedral Al is evidenced by (1) infrared spectroscopy from

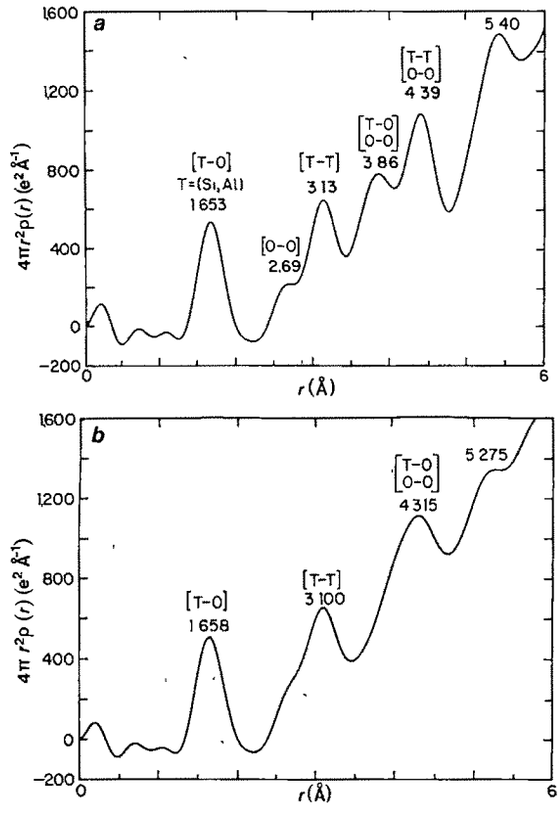


Fig. 1 Experimental RDFs for HY (*a*) and HY-DH (*b*) Both in vacuum; temperatures 300 °C (*a*) and 650 °C (*b*).

shifts in the infrared (Si, Al)-oxygen (T-O) stretching frequencies[4-9]; (2) X-ray fluorescence spectroscopy from shifts of the SiKβ and AlKβ lines[10-13]; (3) X-ray diffraction from the decrease in cell dimensions[5,7,8,14]; (4) X-ray structure analyses of dehydroxylated Y[14]; (5) ^{29}Si-NMR from increases in the Si/Al ratios[15-17]; and (6) ^{27}Al-NMR from observation of spectra arising from octahedral Al species (refs 18-20 and P. A. Jacobs *et al.*, in preparation). Infrared spectra in the OH region frequently show weak bands which appear after dehydroxylation. These bands are generally believed to result from OH groups associated with either the NFA species[21] or vacancies created during the removal of Al[5,22,23]. There has been no unequivocal identification of these bands, but the species $Al(OH)^{2+}$, $Al(OH)_2^+$, AlO^+, $[Al_2O_2OH]^+$, $[Al_2O]^{4+}$ and $Al(OH)_3$ (refs 11, 14, 21, 24-29) have been proposed. Recently, ^{27}Al-NMR studies have indicated that the NFA species in zeolite Y contain octahedrally coordinated Al and are located in large cavities after deammoniation at 300 °C and are distributed in both the large cavities and the sodalite cages after treatment at 500 °C[20]. A distinction between low-condensed mobile NFA species formed at 300-500 °C under shallow-bed conditions and higher-condensed, immobile species formed at 500 °C under deep bed or moist conditions was made by Freude *et al.*[19] and Raatz *et al.*[13].

One sample of Na-Y for use in vacuum dehydroxylation was exchanged eight times with 1 M NH_4Cl to yield NH_4-Y-containing 0.99% Na (dry basis). Activation was carried out in a U-shaped quartz cell containing two 5-cm^2 frits. NH_4-Y (5 g) was heated in flowing O_2 (5 °C min^{-1}) from 25° to 300 °C to eliminate all hydrocarbons. The sample was then evacuated and held for 15 h at 300 °C to obtain H-Y. The quartz cell was opened in a glove box where 1 g of zeolite was transferred to a controlled atmosphere cell used to collect X-ray intensity data. The zeolite powder was pressed into a rectangular cavity in the inside of the back of the X-ray cell which was filled with Ar before removal from the glove box. Vacuum-dehydroxylated Y (HY-DHV) was prepared by transferring the H-Y used for data collection back to the U-tube, heating the sample to 650 °C for 6 h in a 10^{-5} torr vacuum. To minimize possible de-alumination by escaping NH_3 and H_2O, care was taken to heat the sample in a shallow-bed (1 mm thick) configuration and to increase the temperature slowly from 25 to 650 °C under vacuum. The sample was then retransferred to the X-ray sample holder in the glove box. A further sample of NH_4-Y containing 2.2% Na was prepared for steam dehydroxylation. NH_4-Y was heated under 100% H_2O at 600 °C, re-exchanged with NH_4Cl and reheated in 100% H_2O at 890 °C to give steam-dehydroxylated Y (HY-DHSt)[30].

Table 1 RDF spectra for non-framework Al species in HY-DH

d(Å)		
HY-DHV	HY-DHSt	Assignment
—	1.86	Al-O_t
—	2.10	Al-O_{br}
2.87	2.91	Al-Al, O-O
3.39	3.35	Al-O
4.12	4.17	Al-O
4 82	4.84	Al-Al, O-O, Al-O
5 79	—	Al-O, O-O

The X-ray diffraction patterns were recorded with a stabilized Philips diffractometer using a 2,400-W tube with molybdenum anode (λ(MoKα) = 0.710 Å) using a technique described earlier[31]. The radial distribution functions (RDFs) were calculated by Fourier transformation of the scaled intensities as described previously[32].

The calculations of the RDFs of the Al_3O_{14} clusters were made using the computer program RADMOR developed by D'Antonio and Konnert[33] after modification to run on a VAXS 11/780 computer. The program is described in detail in ref. 33 but we include above a brief description of the quantities calculated and parameters used in our calculations.

RADMOR calculates the following expression:

$$4\pi\rho(r) = \frac{1}{r}\sum_{i=1}^{n}\sum_{j=1}^{m}\frac{w_i w_j \bar{f}_i \bar{f}_j \exp[-(r-r_{ij})^2/2\sigma_{ij}^2(r)]\Delta r}{r_{ij}\sigma_{ij}(r)\sqrt{2\pi}}$$

where $4\pi\rho(r)$ is the probability, weighted by the scattering power of the atomic pair ij, $(f_i f_j)$, of finding atoms j in a shell of thickness Δr and radius r_{ij} from origin atoms i. w_i is the occupancy of atom i, $f_i = f_i(s=0)/[f^2(s=0)]^{1/2}$ where—$s = 4\pi \sin\theta/\lambda$—is the reduced, scattering angle independent, scattering factor for atom i, r_{ij} is the distance between atoms i and j and $\sigma_{ij}(r)$ includes both the r.m.s. displacement along r_{ij} and positional disorder. The first sum is carried out over the *n* atoms in the asymmetric unit for a crystalline material. The second sum is carried out over the *m* atoms in the structure that are separated from an atom in the asymmetric unit by less than the maximum distance selected for the calculation. For crystalline materials, space group transformations and unit cell translations are used to generate the atom positions in the second sum.

The pair correlation function, $G(r)$, for a spherical region of radius *t* is defined as:

$$G(r) = 4\pi r[\rho(r)/\varepsilon(r, R) - \rho_0]\varepsilon(r, t)$$

where for spherical particles $\varepsilon(r, R)$ is given by:

$$\varepsilon(r, R) = 1.0 - \frac{3}{4}\left(\frac{r}{R}\right) + \frac{1}{16}\left(\frac{r}{R}\right)^3 \quad \text{for } r \leq 2R$$

$$\varepsilon(r, R) = 0 \quad \text{for } r \geq 2R$$

When the input model is an infinite crystal, $\varepsilon(r, R) = 1$ and $\varepsilon(r, t)$ corrects for the fall-off in scattering near the diameter of a finite spherical particle. *R* is radius of model fragment.

The disorder parameters $\sigma_{ij} = 0.05$ at 1.5 Å and 0.19 at 10 Å were based on experimental values for SiO_2 glass obtained by Konnert and Karle[34] and Konnert *et al.*[35]. The RDFs of the Al_3O_{14} clusters were obtained using the published atomic coordinates of jadeite[36], chantalite[37] and $Al_2(OH)_2TeO_3SO_4$[38].

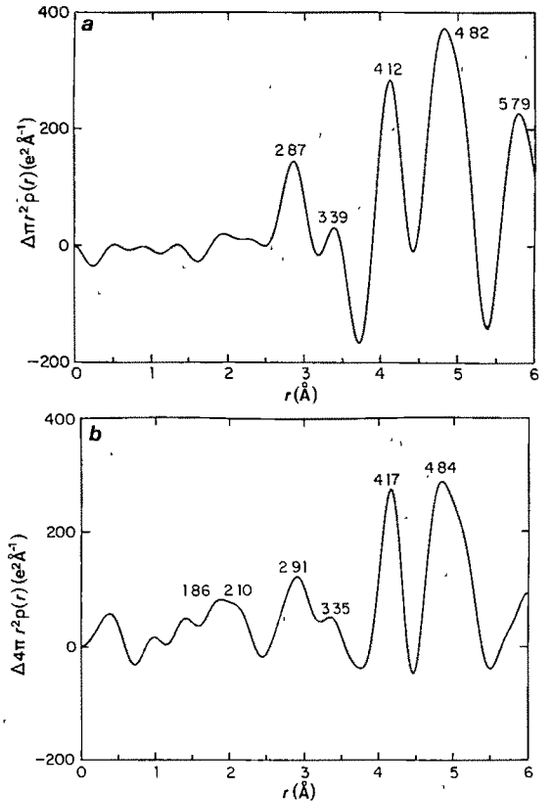


Fig. 2 RDFs for non-framework Al species in HY-DHV (*a*) and HY-DHSt (*b*)

Figure 1a shows a plot of $4\pi r^2\rho(r)$ against r for H-Y prepared at 300 °C in vacuum. The peaks were identified by comparison with calculated distances from the structure refinement of $H_{53\,8}Na_{2\,2}$-Y by Gallezot and Imelik[39]. The T-O value of 1.653 Å agrees well with the mean T-O distance of 1.648 Å calculated for $T = Al_{0\,29}Si_{0\,71}$ from ionic radii[40].

The RDF of HY-DHV (Fig. 1*b*) shows significant changes in peak position and poorer resolution of the T-T and O-O peaks at 3.13 and 2.69 Å and 4.39 and 3.86 Å, respectively.

More useful information is obtained, however, from subtraction of the HY RDF from the HY-DHV RDF Figure 2*a* shows that this difference spectrum is characterized by peaks at 2.87, 3.39, 4.12, 4.82 and 5.79 Å Because NMR studies of Freude *et al.*[19,20] show that extensive framework de-alumination occurs on dehydroxylation of H-Y at 650 °C in vacuum, we attribute the difference spectrum to the non-framework aluminium species.

To confirm that the difference spectrum of Fig. 2*a* is not an artefact, a further difference spectrum was obtained from the sample of Y dehydroxylated in steam at 890 °C (HY-DHSt) (Fig 2*b*) using the same H-Y as a reference[30]. The same prominent peaks as in Fig. 2*a* are present, but two additional peaks occur at 1.86 and 2.10 Å. It is apparent that a similar residual species is present in both HY-DHV and HY-DHSt.

The Al-O peak at 1.9 Å is only barely visible in HY-DHV. Although visible in HY-DHSt, its intensity is less than predicted from the calculated RDF. The increased intensity of the 1.9-Å peak and the generally narrower peaks for HY-DHSt probably reflect the increased degree of condensation and crystallinity of the NFA species in ultra-stable Y.

Table 1 summarizes the RDF peak positions from the NFA species in the two de-aluminated HY samples. The distances present in the NFA produced at 650 °C clearly correspond to a larger Al-OH cluster than the sample species listed earlier, that is, $Al(OH)^{2+}$, $Al(OH)_2^+$ or $[Al_2O]^{4+}$.

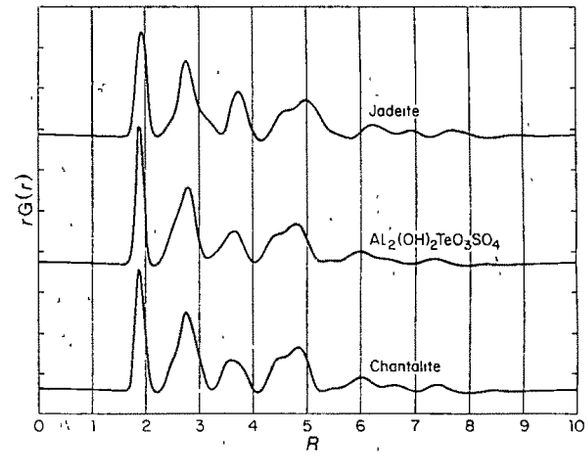


Fig. 3 Calculated RDFs for Al_3O_{14} clusters

To help identify the NFA species, we have calculated the RDFs of $Al_3(O,OH)_{14}$ groups extracted from the edge-shared octahedral chains in jadeite, chantalite and $Al_2(OH)_2TeO_3SO_4$. From the RDFs shown in Fig. 3, it can be seen that edge-shared octahedral clusters result in RDF peaks at positions similar to those shown in Table 1. The most prominent peaks occur at 1 9 Å (Al-O), 2.8 Å (O-O,Al-Al), 3.5 Å (Al-O), 3.8 Å (O-O), 4 4 Å (Al-O), 4.8 Å (O-O), 5.5 Å (O-O) and 5.9 Å (Al-O), where the values in parentheses indicate atom pair interactions calculated separately. This similarity suggests that the NFA species in HY-DHV and HY-DHSt contain edge-shared octahedral $Al_mO_n(OH)_p$ chains.

Two significant differences exist between the NFA peaks and those of the $Al_3(O,OH)_{14}$ clusters. First, the NFA peak at 1.9 Å is considerably broader and less intense than the 1.9-Å peak in the chains. The breadth could result from the considerable disorder caused by a large number of terminal OH groups expected in 10-Å clusters located in the supercages of Y zeolite. The peak at 1.86 Å probably corresponds to terminal OH groups whereas the peak at 2.1 Å would correspond to bridging OH or O atoms. Second, the NFA peak at 4.1 Å is shifted considerably from the typical value of 4 5 Å for the $Al_3(O,OH)_{14}$ clusters and its intensity is greatly increased. This suggests a different arrangement of the chains from the simple isolated species used for the calculations. Further calculations of RDF spectra for other $Al_mO_n(OH)_p(OH_2)_q$ clusters and the known crystalline forms of Al oxides and hydroxides are in progress with the hope that one of these will correspond to the difference RDFs of Fig. 2.

It should be emphasized that the NFA species formed in this study corresponds to an advanced stage of de-alumination under shallow-bed conditions. Dehydroxylation of H-Y occurs at 450 °C in shallow bed conditions and 300 °C in deep-bed conditions[19,20]. The de-alumination carried out in this study is in the region of substantial dehydroxylation and therefore de-alumination No attempt has yet been made to look at the de-alumination species obtained at lower temperatures The species formed at 300-550 °C may well be different and could correspond to some of the species proposed to form before condensation as emphasized by Freude *et al.*[19].

We thank P. D. Antonio and J. H. Konnert for making the program RADMOR available and K. H. Gardner for assistance in the RDF calculations.

Table 2 Peak positions in calculated partial RDFs for $Al_mO_n(OH)_p$

$Al_mO_n(OH)_p$	Compound	Al-O	O-O	Al-Al	Al-O	O-O	Al-O	O-O	O-O	Al-O	O-O
Al_3O_{14}	Jadeite	2.00	2 80	3 05	3 70	3 85	4 55	5 00			6.20
$Al_3O_{10}(OH)_4$	$Al_2(OH)_2TeO_3SO_4$	1.90	2.80	3 00	3 60	3.80	4 40	4.85		6.00	
$Al_3O_6(OH)_8$	Chantalite	1.90	2.80	3 00	3.55	3.80	4 45	4.85	5.50	6 00	6.10

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Kilometre-scale sheath fold at Mattmark and implications for transport direction in the Alps

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Folds were the first tectonic features of rocks to be studied and as such are taken as a reference for the interpretation of other structures. Intuitively, fold axes were assumed to be subperpendicular to the direction of transport. Nevertheless, many studies of metamorphic belts showing intense ductile deformation describe folds with axes close to the transport direction. In particular, sheath folds[1-9], are highly curvilinear folds elongated parallel to the direction of transport. We discuss here a spectacular large sheath fold situated in a crustal shear zone in the Monte Rosa nappe, Swiss Alps. Our analysis implies that both the geometry and significance of folds in the internal Alps must be re-examined. We further demonstrate the existence of crustal ductile shear zones in an area of the Alps where previously emphasis was generally placed on fold nappes; the movements on these shear zones is often tens of kilometres.

The Monte Rosa nappe (Fig. 1), Swiss Alps, representing the northernmost internal crystalline massif, is a spectacular example of deep ductile deformation. It is made up of Hercynian basement rocks, mainly gneiss and schists, intruded by late Hercynian granites[10] and unconformably covered by Permo-Carboniferous volcaniclastic rocks and by a series of Mesozoic sediments[10-12]. All these were intensively reworked during the Alpine tectonometamorphism with the formation of gneisses, micaschists and mylonites, and are tectonically overlain by the Zermatt Saas-Fee ophiolite thrust sheet[13].

The polyphase Alpine metamorphism may be divided into two major events: as a high-pressure eoalpine metamorphism[14,15], (the climax of this metamorphism occurred around 110 Myr ago in eclogitic conditions (15 kbar), followed by a decrease of pressure to 7-8 kbar until 60 Myr)[16,17], and the Alpine (s.s.) metamorphism at around 38 Myr ago[17,18], in greenschist to amphibolite conditions.

In the Monte Rosa area, as in all the pennine Alps, the intensity of the Alpine deformation is evidenced by large recumbent nappes[19,20] and the presence of a prominent Alpine foliation and intense microfolding[10,21-23]. However, such studies can only analyse fold geometry and usually describe several phases of folding[24]. The direction of transport is generally assumed to be perpendicular to the fold axes and the vergence of 'tectonic phases' is deduced from the apparent vergence of folds.

Our recent work[25,26] has attempted to study the deformation mechanisms using tectonic and microtectonic analysis. We obtained the following results: firstly, The most significant microstructure is the prominent stretching lineation. This is clearly recognized by the elongation of deformable markers (such as pebbles), the stretching of porphyroclasts or rigid inclusions (feldspars in gneissic rocks, dolomitic layer in marbles) and also by mineral elongation (biotite, phengite, recrystallized quartz, feldspar or calcite, elongation of pressure

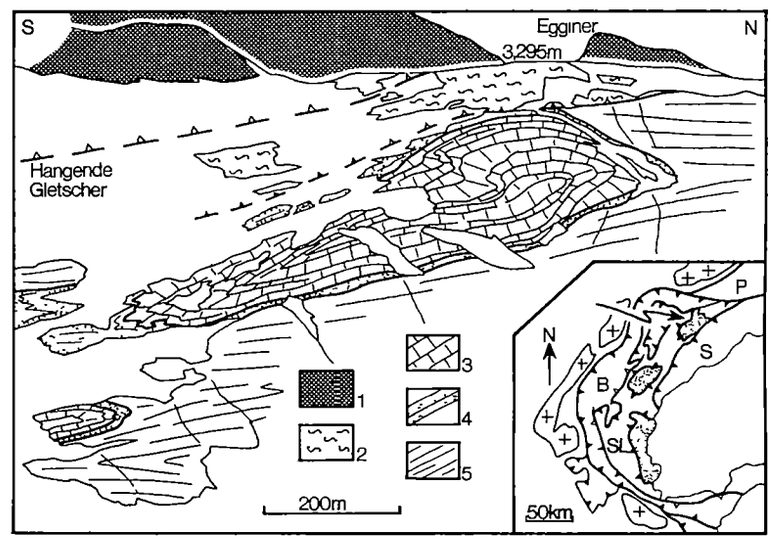


Fig. 1 Geological landscape of the 'eye fold' of Mattmark, viewed from the Gruenberghorn (3,015 m) towards the north-west. 1, Ophiolites; 2, Jurassic brown calcschists; 3, marbles, 4, quartzites; 5, paragneiss and schists Inset location of the studied area (Monte Rosa nappe) in the Western Alps. The internal crystalline massifs are shaded; crosses indicate the external crystalline massifs. B, Briançonnais zone; SL, *Schistes lustrés* and ophiolites, S, Sesia zone; P, pennine nappes of the Lepontine area.

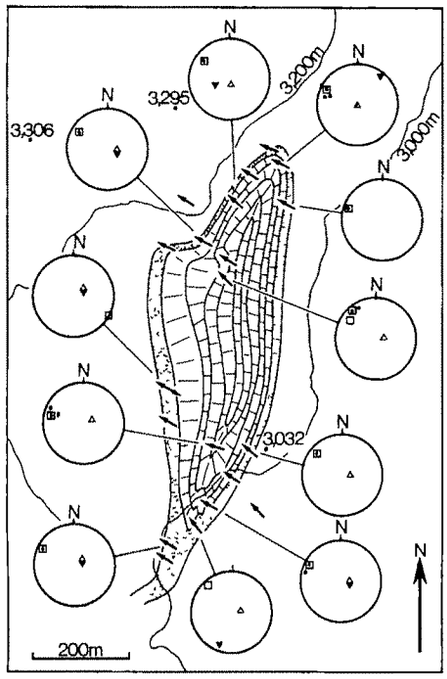


Fig. 2 Structural map of the 'eye fold'. The arrows represent the stretching lineations. In the Schmitt diagrams (lower hemisphere projection): △, foliation; ▲, stratification; □, stretching lineations; ●, intersection lineations (S_0/S_1 or S_0/S_{1-2}).

shadow crystallizations). These lineations represent finite extension. On average they trend N 110° E, plunging slightly to the west-north-west. Despite variations (from N 70° E to N 140° E) which may be related to heterogeneities of the deformation[27], they are regionally coherent. Secondly, the major deformation observed is generally intense and progressive, and shows evidence of non-coaxial deformation such as quartz petrofabrics[28], coherent displacement along shear zones[27], deformed porphyroclasts and pressure shadows[25], and shear band cleavages. Overall characteristics, the deformation resembles the result of simple shearing. As in shear zones the stretching lineation in the intensely deformed areas seems to lie close to the direction of shear.[29,30] On a regional scale, the lineations trace approximately straight paths. Two different units can be distinguished on the basis of the determined shear senses[25]. The first, the Monte Rosa nappe and its sedimentary cover, is characterized by shearing to the west-north-west associated with crustal ductile and major thrusting towards the west-north-west. The second unit, the Portjengrat unit, situated immediately south of the Mischabel backfold, shows east-south-eastward shears. We believe this deformation to be related to antithetic ductile thrusting[31]. Finally the observed progressive deformation mainly occurred during retrograde metamorphism at about 40 Myr. It may represent a late, although intense, phase of a complex deformation history and is probably related to major thrusting during and after continental collision.

In the vicinity of Mattmark in the Saas valley, a large-scale 'eye fold' has been recognized[32] and studied[25]. It is well exposed (Fig. 1), between 2,700 and 2,200 m, in a cliff oriented NNE–SSW and has been partly mapped[11,21]. It affects the sedimentary rocks of the Gornergrat zone with Upper Triassic marbles in the core of the fold, surrounded by Lower Triassic quartzites. This structure is in turn surrounded by schists and paragneisses (probably Permo-Carboniferous) and overlain by a slice of brown calcschists (*schistes lustrés* of supposed Jurassic age) and by the Zermatt Saas-Fee ophiolitic thrust sheet[13].

From the neighbouring summits (around 3,000 m) the 'eyed' structure is picked out by marble layers. On the outcrop both quartzite and marble layers surround the eye without discontinuity and define a closed structure (Fig. 1). The stretching lineation is generally prominent; in quartzites, very elongated

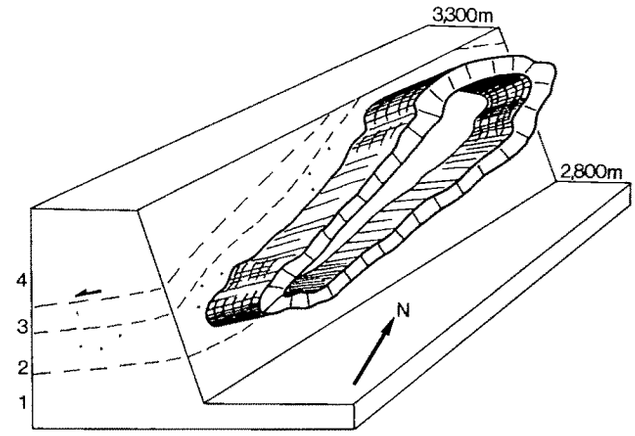


Fig. 3 Schematic structure of the 'eye fold' 1, Paragneiss and schists; 2, quartzites; 3, calcschists; 4, ophiolitic thrust sheet (mainly serpentinites)

pebbles, quartz rods and a mineral lineation (elongated phengite or quartz crystals) can be seen; in marbles or calcschists, the lineation is marked by boudinage of the dolomitic layers, the stretching of dolomitic inclusions or porphyroclasts (such as garnets) and elongation of minerals, and pressure shadows. These lineations show nearly constant direction in the studied area (Fig. 2) and on average trend N 120° E. Microfolds are isoclinal and more frequent in the north and south hinge zones of the eye fold (where we also observed small sheath folds and a mushroom-shaped fold) and also on the Jurassic calcschists. Both the fold axes (microfolds, north and south hinge zones of the eyed structure) and the intersection lineation, S_0/S_1 or S_0S_{1-2}, are parallel to the stretching lineation (Fig. 2).

The relative orientations of the bedding (S_0),and the fold axes suggest that the fold is cylindrical and that the eyed pattern is not a topographic effect (Fig. 3). Based on this structure and on the intensity, mechanism and kinematic of deformation, we interpret this eye fold to be a section lying subperpendicular to the axis of a large sheath fold[1,2]. This fold would be elongated parallel to the stretching lineation trend and of the order of several kilometres in size. It represents a synclinal structure with the younger rocks (marbles) in the core. There is no direct evidence for an east or west closure of this fold, that is, an eastward- or westward-facing nose. It is possible that a such large sheath fold is the result of a dramatic deformation associated with the westward crustal shearing rather than ductile backthrusting; if this is the case, the closure of the synclinal sheath fold should be to the east.

Other large folds exist in the Gornegrat zone. For instance, immediately north of the eye fold, we have described a refolded isoclinal fold of kilometre scale whose axis is always parallel to the stretching lineation[25]. On the metric scale, most folds show certain characteristics consistently over the whole area studied: they are highly isoclinal with very elongated limbs; the fold axes are generally straight and parallel to the stretching lineation; folds are clearly visible in sections perpendicular to the lineation where their apparent vergence is generally to the south-west. Nevertheless, folds showing opposite vergence (both to the south and north) on the same outcrop and mushrooom-shaped folds are relatively common. Two major folding phases (F_1 and F_2) with the same geometrical characteristics have previously been described[22].

Hence, the present deformation analysis indicates that folds in the Monte Rosa area (except obvious late folds) may represent either: (1) folds ("a folds")[33] with axes close to the long axis of the strain ellipsoid, as shown by the stretching lineation, which then approach the shear direction or (2) sheath folds; both result from or were strongly deformed during, the large progressive shearing.

The mechanism of formation of sheath folds during progressive deformation is well established[1,2]. They appear to be the

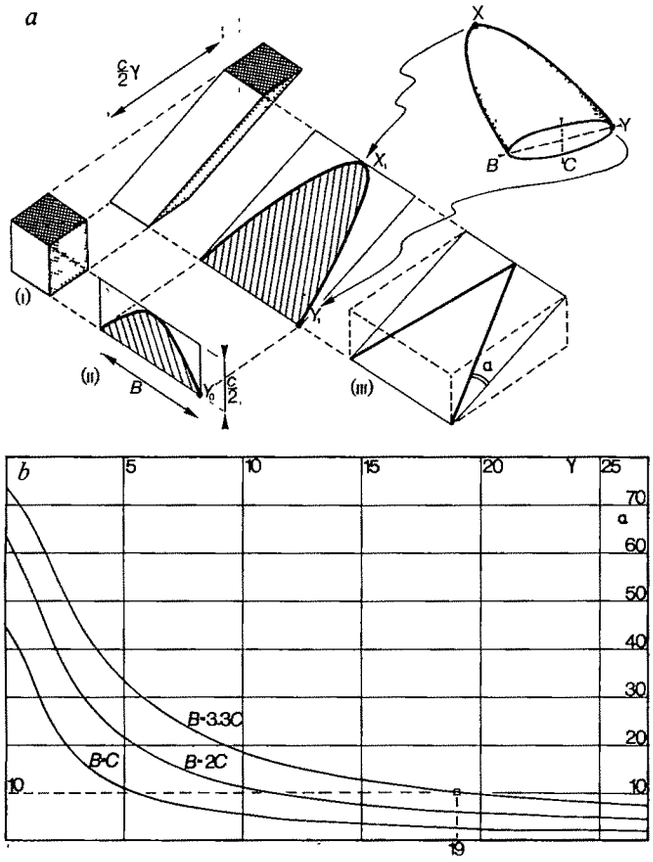


Fig. 4 Progressive reorientation of the limbs of a passive sheath fold. *a*, The model. (i) Idealized deformation of a cube of side. $C/2$ by simple shear (with shear strain γ); (ii) the same deformation applied to a cross-section of shape B, C representing the hinge zone of the fold; (iii) a simplification of this geometry to yield the angle α between the limbs and the long axis of the fold, as a function of γ and of the shape (B/C) of the fold. *b*, Curves of α versus γ. The curve $B=3.3C$ corresponds to the fold of Mattmark. γ is estimated to a minimum value of 19.

result of kinematic amplification of 'deflections' in the folded layers which produced curvilinear folds that became progressively more elongated during the deformation. The presence of a kilometre-scale sheath fold suggests that the shearing mechanism took place over a large area and caused very large deformation and transport. Commonly occurring folds lying parallel to the direction of shear were probably formed by reorientation, during the progressive deformation, of folds initially lying oblique to the shear direction[30,34]. Such oblique folds may have been induced during this deformation by the shearing of layers inclined relative to the shear plane[35,36].

In the absence of strain markers, an estimate of the shear strain can be obtained from a simple model of the geometry and evolution of the sheath fold. If exact, such an estimate would be more significant than a point-by-point strain analysis, because it would represent the average bulk shear strain for the whole volume of rock.

Assuming simple shear for the progressive deformation (Fig. 4*a*(i)), and a passive sheath fold, the shape of the hinge zone (Fig. 4*a*(ii)), represented in Fig. 4*a*(iii) by a triangle, is considered to be the result of kinematic amplification[2] of the deflection (Fig. 4*a*(ii)). We may calculate the angle α as a function of the shape of the sheath fold (B/C) and the shear strain (γ):

$$\alpha = \arctan\left(\frac{B}{C}\frac{1}{\sqrt{\gamma^2+1}}\right) \qquad (1)$$

Different curves, for different values of B/C, can now be plotted (Fig. 4*b*); the curve $B/C=10/3$ corresponds to the Mattmark fold We may then relate the characteristics of the observed sheath fold (the angle between the fold axes in the two hinge zones of the eye, which corresponds to 2α). Based on the approximately cylindrical shape of the Mattmark fold, we can consider the angle between the two axes to be $<20°$ (that is, $\alpha \leqslant 10°$). This leads to a minimum estimate for the bulk shear strain of $\gamma=19$, for a fold with the observed dimension $(B/C=10/3)$. Although this is an oversimplified model (which assumes simple shear, unknown shape of the deflection, and only passive reorientation), such an estimate may be a useful average value for the bulk shear strain. It agrees with the microstructural and textural characteristics of rocks such as: the elongation of quartz pebbles in conglomerates[25] (λ_1/λ_3 up to 50 and a K parameter close to 1 where λ_1 and λ_3 are finite strain axes and K is the shape parameter for the strain ellipsoid and is 1 when in plane strain); mylonitic microstructures[37]; and well-defined preferred lattice orientation of quartz.

In terms of transport, for a shear zone 1 km thick (minimum thickness for the intensely sheared area situated at the top of the Monte Rosa nappe), the value for γ obtained requires a minimum displacement of ~20 km. Note that much more movement may occur on discrete fault zones.

Such an analysis, associating folds with a deformation mechanism, has several implications.

First, for highly deformed areas, it seems logical to assume that folds perpendicular to transport ('b fold') cannot exist except in the case of late folds. This is true for mylonite zones[38] and also for deep parts of mountain belts such as the Himalayas[33], the Alps[39,40] or the North American Cordillera[41,42]. Hence, geometrical analyses of folds are not good kinematic indicators and must be used in association with analyses of ductile deformation mechanisms.

Second, this work demonstrates the importance of thrust displacements of several tens of kilometres along ductile shear zones, in an area where previously much emphasis was placed on fold nappes[19,22–24]. As has already been proposed for the French–Italian Alps[40], deformation analyses show that the evolution of the Swiss part of the belt is highly logical, and is dominated by thrusting towards the west-north-west, without major gaps between the external and internal Alps.

Finally, deformation analysis should be carried out on all the internal parts of the Alps. Some advances have been made on the French–Italian part[43,44], but in the famous Pennine nappes of the Swiss Alps[19,20] studies have concentrated on the superposed folding[23,24,45]. According to the highly deformed state of the rocks, fold geometry seems to be unrelated to the kinematic evolution, except in the case of late folds. It is probable that major deformations occurred in a shear context[46,47] with the formation of stretching lineations[48,49] tracing the shear directions. It would now be of great interest to study ductile deformation and its kinematics and to relate it to the fold geometries, with a view to analysing sheath folds[47] and folds subparallel to the shear directions.

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An early Cambrian rift to post-rift transition in the Cordillera of western North America

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The upper Proterozoic and lower Palaeozoic wedge of miogeoclinal strata in the North American Cordillera is widely regarded as evidence for a proto-Pacific passive margin[1–3]. The rifting history of this margin appears to have been protracted, possibly spanning >200 Myr in a tectonic setting that is not well understood[3]. Quantitative subsidence analyses of lower Palaeozoic strata between British Columbia and Utah, however, provide indirect evidence that the transition from rifting to post-rift cooling occurred within a relatively short interval of time, although probably not synchronously, between 600 and 555 Myr (refs 4–6). This age is significantly younger than that implied in previous studies[1,2]. We describe here new field data, which, together with published geological data, provide direct evidence of a latest Proterozoic or early Cambrian age for the rift to post-rift transition. The data support recent arguments for widespread initiation of passive margins around the edge of the North American craton close to the Cambrian–Precambrian boundary[7].

Indirect evidence for the ages of the rift to post-rift transition as well as for the final rifting phase were inferred from tectonic subsidence curves for early Palaeozoic strata in the Cordilleran miogeocline[4–6] (Figs 1, 2). The strata (ranging from early Cambrian to late Ordovician in age) are laterally continuous, stable-shelf deposits and are regarded as part of an extensive post-rift continental terrace[1–3]. The tectonic subsidence curves have the same form as the age–depth curve for ocean floor (Fig. 2). This indicates that, as in modern passive margins, the post-rift tectonic subsidence was controlled mainly by contraction of the lithosphere resulting from the decay of thermal anomalies generated during rifting[4–6]. We emphasize, however, that the curves do not distinguish between margins adjacent to an open ocean or a back arc basin. The steep slopes of the curves, which are a function of the rates of heat loss, place the maximum age for the beginning of post-rift cooling at ~600 Myr (refs 4–6). This is because older ages for initiation of post-rift cooling, at 650 Myr, for example, would require anomalously high rates of heat loss from the Cambrian ocean floor, reaching at least 160%

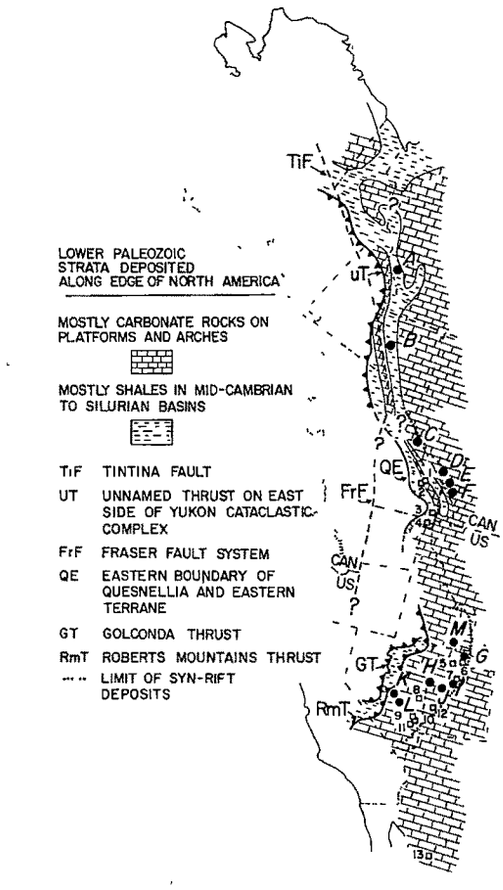


Fig. 1 Distribution of the lower Palaeozoic miogeoclinal facies in western North America (modified from ref. 5). UT, QE, GT and RMT are major structural boundaries of various terranes accreted to western North Amerca in post-early Palaeozoic time[3]. These boundaries mark the westernmost preserved edge of the lower Palaeozoic North American continent. Ti–FrF is a major right-lateral transcurrent fault system[3]. The dotted outline of western North America gives the position of this region after restoration of the right-lateral transcurrent movement on the Tintina–Fraser fault system[5]. Heavy dotted lines indicate the approximate eastern limits of the latest Proterozoic or earliest Cambrian post-Windermere rift deposits in the southern Canadian Rockies and in Utah–Nevada. ●, Locations of sections in which tectonic subsidence has been calculated[4,5]. □, Locations where mafic extrusive rocks occur in post-Windermere rift deposits that record the final (latest Proterozoic or early Cambrian) rifting in the margin. Sources: 1,2,3, Hamill Group[15,16,35]; 4, Three Sisters Formation (although the volcanics may be in the Windermere Supergroup, F. Miller, personal communication); 5, Mutual Formation (Crittenden, unpublished mapping); 6, Browns Hole Formation[23]; 7, Tintic Quartzite[30]; 8, 12, Prospect Mt Quartzite[26,29]; 9–11 Stirling Quartzite[26]; 13, Sonora[33,34].

of that of the modern ocean floor[5]. The high rates of post-rift cooling further indicate that relatively little heat was lost during rifting, and in terms of finite rifting models[9], the duration of the final rifting phase probably did not exceed 10–20 Myr. The age of final rifting, therefore, could not be much older than our inferred maximum estimate for the rift to post-rift transition.

The subsidence data do not preclude earlier extensional and heating events, which are indicated by geological evidence and have profoundly influenced the structural evolution of the margin, probably since the Middle Proterozoic[2,3,10]. However, igneous rocks thought to be associated with late Proterozoic rifting in the margin have radiometric ages of ~900–750 Myr (refs 8, 10, 11), and clearly are too old for the final rifting of the margin. Our mapping in the southern Canadian Rockies and in Utah, together with a synthesis of published data, have led to the identification of a widespread younger rifting event

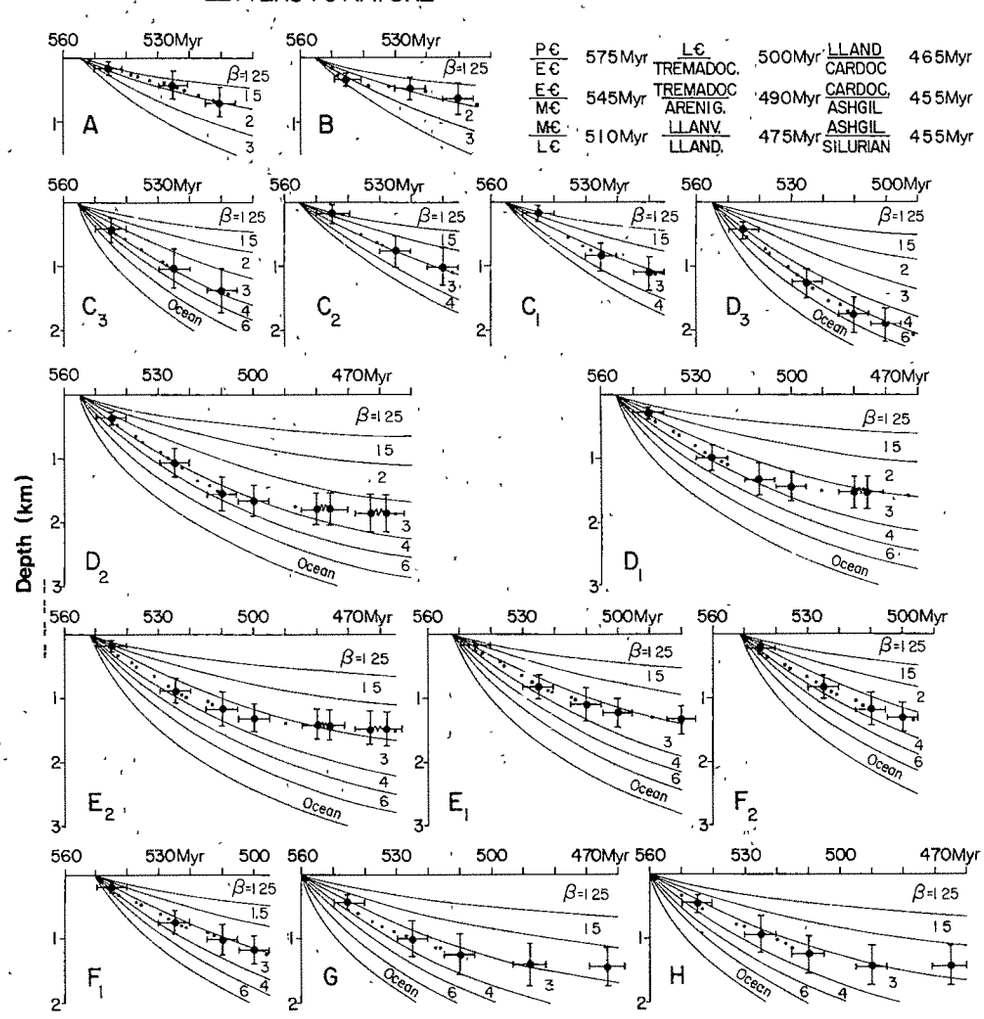


Fig. 2 Tectonic subsidence (●) for sections located in Fig. 1 compared with thermal subsidence curves from the McKenzie stretching model[36] (solid curves). Large dots are stratigraphical boundaries to which numerical ages have been assigned. Small dots are formation boundaries positioned by assuming constant sedimentation rates between dated boundaries. Vertical bars give the range between maximum and minimum values of tectonic subsidence[4]. Horizontal bars give an assumed ±5-Myr error for stratigraphical correlations with numerical ages β, Crustal stretching factors from the McKenzie stretching model; β values corresponding to the subsidence curves are not necessarily the stretching factors for the passive margin. Letters with subscripts designate different sections lying along east–west lines at locations that are too close to indicate separately in Fig. 1. The higher subscript numbers designate the more westerly sections. Most of the sections with subscripts are >15 km apart on a non-restored base Numerical ages assigned to the stratigraphical boundaries[20] are shown in the upper right-hand corner. Stratigraphical boundaries are from ref. 37.

whose age appears to be compatible with the high rates of post-rift cooling in the margin.

In the southern Canadian Rockies, limited evidence for younger rifting has been reported from the Hamill Group west of Golden[4,12] and from the McNaughton Formation near Jasper[13] (Fig. 3). Both deposits underlie strata with early Cambrian trilobites and overlie the Windermere Supergroup (Fig. 4*a*), a widespread stratigraphical unit that was deposited during rifting in late Proterozoic time (refs 2, 3, 10, 11). Both the Hamill Group and McNaughton Formation contain distinctive thick sequences of feldspathic sandstones and conglomerates[13–16] (Fig. 4*a*). In the Hamill Group west of Golden (Fig. 3), shallow-marine sediments pass abruptly into deeper marine deposits that contain resedimented sandstones, pillowed lavas and mafic volcaniclastic debris[12,15,16]. These facies changes are regarded as evidence of syn-depositional faulting accompanied by extrusion of mafic rocks[12]. Similarly, near Jasper, abrupt changes in thickness in the McNaughton Formation have been interpreted as evidence of syn-depositional faulting[13]. Strata of the Hamill Group and the McNaughton Formation pass gradationally upwards into the lower Cambrian mature quartz sandstones with *Skolithos* that are part of the early post-rift strata of the margin (Fig. 4*a*). Neither, however, is overlain by post-rift strata that are suitable for quantitative analysis, and their precise stratigraphical postion relative to a rift to post-rift transition cannot be inferred from subsidence data.

Recent field work in areas east and south of the exposures of the McNaughton Formation and Hamill Group (between 51°45′ and 52°30′, Fig. 1) has indicated that at least two of the sections analysed, SF and MtWi, and probably HL7b and GL as well (Fig. 3), are underlain conformably by a rift-like deposit that is at least 500 m thick. This deposit forms a distinctive basal unit of the Gog Group and is broadly correlative with the McNaughton Formation and at least the lower part of the Hamill Group[17] (Fig. 4*a*). The exact stratigraphical relation of this deposit to formally recognized strata in the region is not yet established, however, and we refer to it informally as a basal conglomeratic unit of the Gog Group.

A rift origin of the basal conglomeratic unit is suggested by a striking succession of cross-bedded, poorly sorted arkosic to subarkosic sandstones and conglomerates interbedded with thin layers of red shales and siltstones in the lower two-thirds of the unit (Fig. 4*a*). The sandstones and conglomerates contain ~30–80% quartz, 15–50% feldspar, more than half of which is a distinctive pink alkali feldspar as much as 2 cm across, and 5–20% rock fragments consisting mainly of quartzite, chert and granitic clasts. A total of 219 measured orientations of trough axes and large-amplitude (50 cm to 2 m) cross-beds, corrected for tilting, indicate mainly unimodal westerly current flow. The coarse grain size, arkosic composition, abundance of red shales together with westerly palaeocurrent directions suggest a fluvial or alluvial fan environment and a strongly uplifted source in crystalline basement to the east. An easterly provenance in crystalline basement has also been suggested for the McNaughton Formation based on palaeocurrent measurements and sandstone compositions[13,18]. The abrupt terminaton of the conglomeratic unit between Mt Chephren and Bow Pass (Figs 3, 4*a*) could reflect deposition against a fault scarp or hinge zone flanking the easterly basement source. The conglomeratic unit of the Gog Group grades upward into mature quartz sandstones with *Skolithos*, which are correlative with the post-rift deposits in the upper part of the McNaughton Formation and in or above the upper part of the Hamill Group (Fig. 4*a*) These *Skolithos*-bearing strata form the base of the sections used in subsidence analyses.

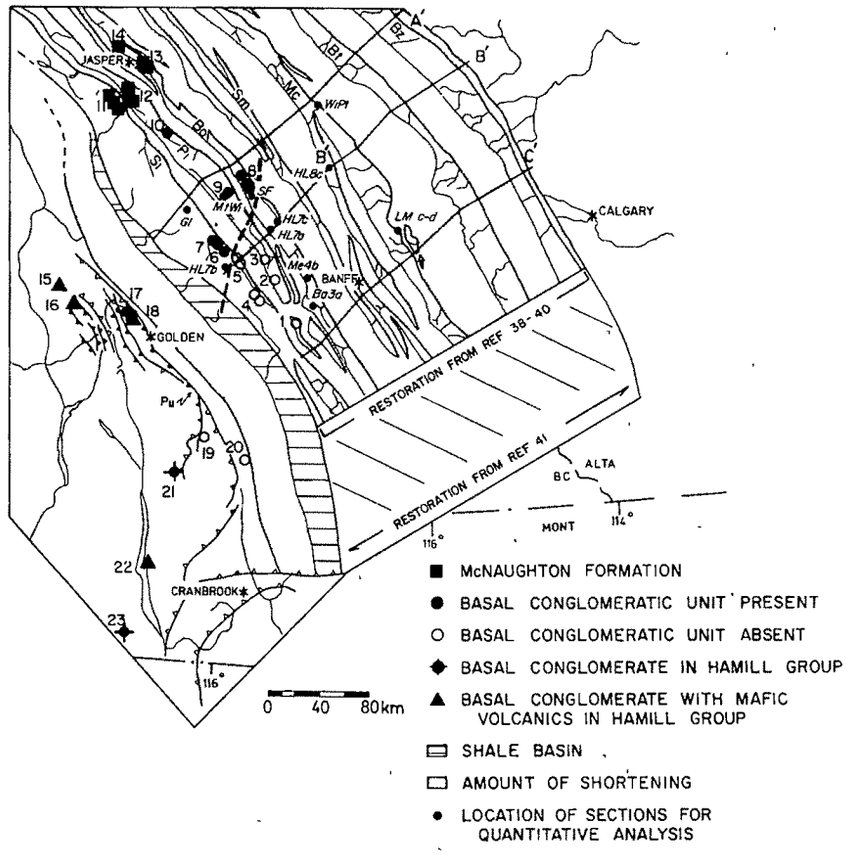


Fig. 3 Distribution, on a palinspastic restoration, of the uppermost Proterozoic to lowest Cambrian strata that are interpreted to record the final rifting and the transition to post-rift subsidence in the southern Canadian Rockies. Large filled circles, solid circles, solid circles with cross, solid squares, open circles and solid triangles mark localities examined in the field Open circles west of Banff are the Gog Group and south of Golden are the Cranbrook Formation. Stippled area is the amount of shortening between thrust panels that are unpatterned The shortening in the shale basin has not been determined from balanced sections and is assumed here to be equal to the amount of shortening in the carbonate bank (a factor of 2). The displacement on thrusts west of the shale basin has not been estimated. Italic letters correspond to analysed sections shown in Fig. 1. *WiPt*, *SF*, *MtWi*, *GL* are at locality D, Fig. 1; *HL8c*, *HL7c*, *HL7a*, *HL7b* are at locality E, Fig 1, and *LMC-D*, *Me4b* and *Ba3a* are at locality F, Fig. 1. Lines A′, B′ and C′ are lines of palinspastic restoration which are control for restored positions of thust plates[38-42]. The dashed line is the approximate position of the southeastern edge of the basal conglomeratic unit.

In the McNaughton Formation, the change from coarse feldspathic sediments to mature, marine quartz sandstones, which we assume is approximately correlative with the end of final rifting and the transition to post-rift subsidence, occurs in the lower part or below the early Cambrian *Fallotaspis* trilobite zone[19] (Fig. 4*a*). The transition to post-rift subsidence, therefore, lies near the base of the Atabanian Stage or in the Tommotian Stage. These stages are not well dated but are thought to range from slightly younger than 570 Myr to ~590 Myr (ref. 20). If the correlation of the McNaughton Formation with the basal conglomeratic unit and the Hamill Group suggested in Fig. 4*a* is even approximately correct, there is excellent agreement between the stratigraphical position of the rift to post-rift transition and the age of that transition inferred from the subsidence data.

There is also evidence, from work in progress, for syn-depositional rifting in the western United States, in uppermost Proterozoic and lower Cambrian rocks that are broadly correlative with the succession in the southern Canadian Rocky Mountains (Figs 1, 4*b*). The principal evidence for rifting is the occurrence of feldspathic sandstones and conglomerates, relatively abrupt lateral facies and thickness changes, regionally variable palaeocurrent directions and the presence of mafic volcanic flows and volcaniclastic conglomerates. Few chemical data are available for the volcanic rocks, but the rocks appear to be of tholeiitic to alkalic affinities, consistent with continental rifting[1,21]. The feldspathic sandstones and conglomerates occur sporadically, but in places constitute units several hundred metres thick[22,23]. Sequential changes in mineralogical maturity and grain size are locally abrupt, consistent with episodic uplift and exposure of crystalline basement. In Utah, for example, medium- to fine-grained, well-sorted, shallow-marine (?) quartzite of the upper Browns Hole Formation is overlain with sharp contact by arkosic, poorly sorted fluvial (?) conglomerate of the Geertsen Canyon Quartzite[23] (Fig. 4*b*). In general, the occurrence of lateral facies and thickness changes tends to be obscured by formational nomenclature which emphasizes the regional persistence of gross stratigraphical units. However, in one well-documented example, the middle member of the Wood Canyon Formation in southeastern California[22], strata thicken and coarsen to the north-east and contain south-west-directed palaeocurrents, whereas associated stratigraphical units thicken to the west or north-west, and yield west- to north-directed and polymodal palaeocurrents[24,25].

The inferred rift deposits in Utah and Nevada overlie strata that are thought to be correlative with the Windermere Supergroup in the Canadian Cordillera[26,27], and they pass upward into lower Cambrian mature quartz sandstones with *Skolithos* that lie at the base of the post-rift strata from which the subsidence data were collected (Figs 1, 2, 4*b*). In the Delamar area of eastern Nevada, lithostratigraphical correlation with a section in the White-Inyo Mountains, California, suggests that the transition upward into post-rift mature quartz sandstones occurs near the base of or below the *Fallotaspis* trilobite zone[26,28]. In the Wah Wah[29] and East Tintic[30] sections, the transition to post-rift strata is not well dated but seems to occur at about the same stratigraphical level relative to the base of the middle Cambrian as in the Delamar area[26]. In the Huntsville section[23,27], the transition may be slightly lower stratigraphically but is probably broadly correlative with that to the south. In the western United States, therefore, as in the southern Canadian Rockies, the stratigraphical position of the final rifting to post-rift transition is in good agreement with the age of that transition inferred from the subsidence data.

The age of the final rifting episode can also be estimated from stratigraphical relations at several localities in the miogeocline. Near Jasper, in the southern Canadian Rockies, the uppermost 500 m of the underlying Windermere Supergroup (Miette Group, Fig. 4*a*) contains the trace fossils *Platysolenites* and *Didmaualichnus* and a microbiota with *Campitius* and *Volborthella* spp.[13,31]. These are reported to occur in the Tommotion Stage (~570–590 Myr) and possibly younger stages of the early Cambrian[13,32], but their range is not well established. The rift deposit clearly lies above the Windermere Supergroup and only a short distance beneath probable earliest Cambrian strata, suggesting a latest Proterozoic age for the deposit. Simlarly, in Utah and Nevada, the mafic volcanic rocks and feldspathic deposits occur a short distance below the *Fallotaspis* zone in

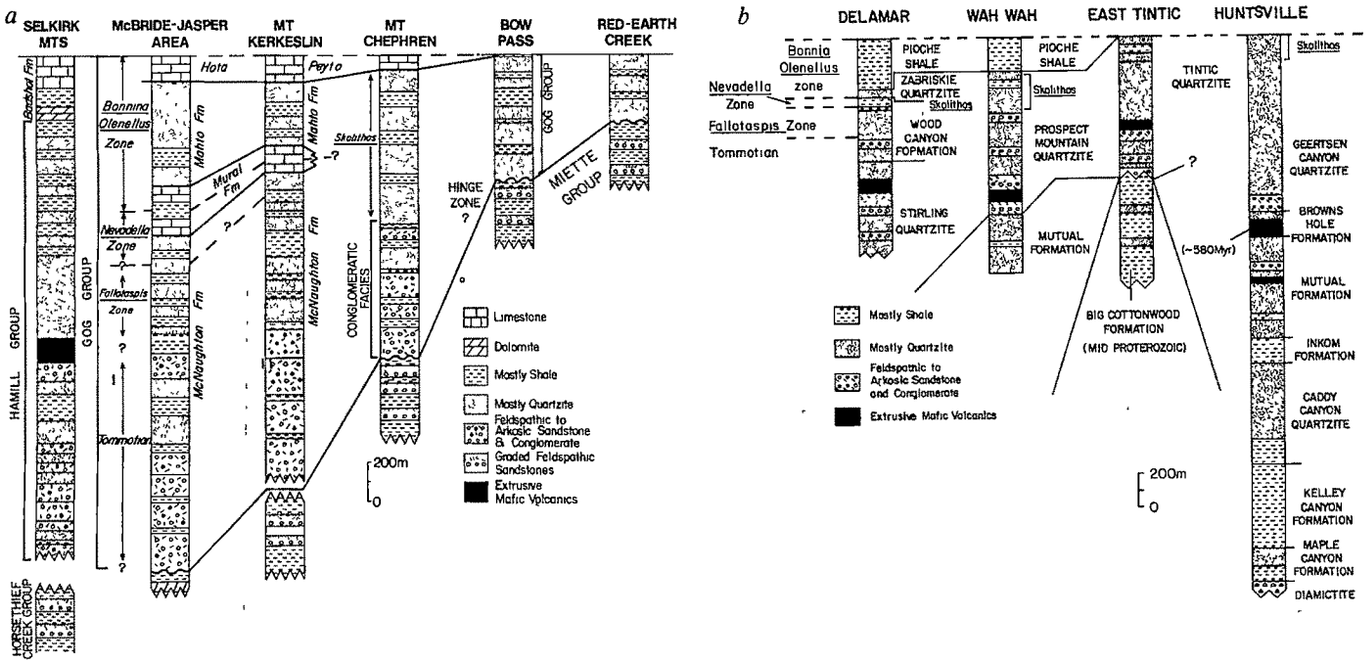


Fig. 4 *a*, Respresentative sections of the basal conglomeratic unit of the Gog Group and its probable equivalents in the McNaughton Formation and the Hamill Group. Columns show the transition of these strata to post-rift deposits of the miogeocline. These strata are interpreted as evidence of post-Windermere rifting and latest Proterozoic to earliest Cambrian transition to the post-rift phase in the margin. The Horsethief Creek Group and the Miette Group compose the Windermere Supergroup west and east of the Rocky Mountain Trench, respectively. Dash-dot lines are approximate boundaries between trilobite zones. The base of the middle Cambrian (uppermost dash-dot line) is the reference datum of the sections. The sections are located in Fig. 3 as follows: 1, Red Earth Creek; 5, Bow Pass; 7, Mt Chephren; 12, Mt Kerkeslin; 14, McBride-Jasper area; 17, 18, Selkirk Mts. Sources: Red Earth Creek, Bow Pass and Mt Chephren from G.C.B., Ma and M.A.K. (field work 1982 and 1983); Mt Kerkeslin from refs 13, 43; Selkirk Mts from ref. 16. Faunal zones are from ref. 19. *b*, Representative sections from Utah and Nevada indicating inferred final rift deposits and the transition to post-rift strata for comparison with similar successions in the southern Canadian Rockies. Dash-dot lines are approximate boundaries between trilobite zones. The ages in the Delamar section are based on lithostratigraphical correlation with biostratigraphically dated strata in the White–Inyo Mountains of California, about 300 km to the west. The upper part of the Stirling Quartzite is correlated with strata assigned a Tommotian age in California; the base of the Tommotian stage has not been established in the western United States[34]. Sections located in Fig. 1 and sources as follows: 11, Delamar[26]; 12, Wah Wah[29]; 7, East Tintic[30]; 6, Huntsville[23,27].

strata that are probably Tommotian in age (Fig. 4*b*), which, together with the 580-Myr Ar/Ar age from the volcanic rocks in northern Utah[21], suggests that rifting occurred in the latest Proterozoic and/or earliest Cambrian. Finally, in Mexico, post-rift carbonate rocks and quartz sandstones of early Cambrian age pass gradationally downward into nearly 300 m of strata containing mafic lava and mafic volcaniclastic rocks. These volcanic deposits seem to be correlative with, or slightly older than, the lower part of the *Fallotaspis* zone[33,34]

Large parts of the Cordilleran miogeocline from Canada to Mexico, therefore, contain evidence of late Proterozoic rifting events that led to the formation of an extensive proto-Pacific passive margin. These events began perhaps as early as 800–900 Myr but appear to have terminated in a final, distinct and short-lived phase near the Cambrian–Precambrian boundary. The age is compatible with ages for the final rifting and rift to post-rift transition inferred from quantitative subsidence data. The results of the subsidence analyses were fundamental to this research, not only focusing on the problem of the ages for rifting, but also in predicting the stratigraphical position where the final rift deposit and the transition to post-rift strata should occur. This greatly facilitated the search for these deposits in the field. We have now identified these deposits in a large region in the southern Canadian Rockies and in an even larger area between southeastern Idaho and southeastern California (indicated by the dotted lines in Fig. 1). It seems that final rifting along a large portion of the proto-Pacific margin of western North America occurred at about the same time as rifting in the early Palaeozoic miogeocline in Arctic Canada and along an extensive portion of the Iapetus margin in the US Appalachians and Newfoundland[7]. The contemporaneity of these events support recent arguments for rifting around nearly all of the perimeter of North America close to the Cambrian–Precambrian boundary[7].

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Direct measurements of secondary currents in a meandering sand-bed river

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Natural channels often adopt a meandering course. Water flow in meander bends is three-dimensional, consisting of primary velocities which are tangential to the bend, and secondary velocities, which are in the radial plane. The pattern of secondary flow strongly affects the distribution of primary velocities. This in turn affects the distribution of erosion and deposition in the bend and the way in which the channel shifts and changes shape. Measurements of primary and secondary flows in a meandering gravel-bed river[1,2] show that, in addition to the widely recognized main secondary circulation driving surface water outwards and bed water inwards, there can be a small cell of reverse rotation at the outer bank. Further data have been collected in a sand-bedded river at low, intermediate and high discharges. The results confirm the existence of the main and outer bank cells but also indicate that in some bends the main cell does not extend to the inner bank. In fact, secondary flow at the inner bank of wide, shallow bends is directed radially outwards over the whole flow depth at all in-channel flows. This indicates that some models of bend flow and channel development may be significantly in error.

Secondary currents are defined as currents that occur in a plane normal to the axis of primary flow[3]. In meander bends, they develop by skewing of cross-stream vorticity into a long-stream direction[3,4]. The resulting skew-induced secondary circulation carries fast surface water towards the outer bank and slower bed water towards the inner bank.

At the outer bank, the primary flow, secondary circulation, and bank interact to produce a small cell of reverse rotation to the skew-induced cell. This cell occupies the channel to a distance one or two times the bank height away from the bank. Although this is a small proportion of the cross-section in most rivers, the outer bank cell is still important because it strongly affects the distribution of boundary shear stress, thereby influencing bank erosion processes[5,6].

In the central part of the channel, helical skew-induced flow produces inward velocities near the bed, which sweep bedload towards the inner bank. Sediment accumulation as a point bar at the inner bank gives the channel an asymmetrical cross-section. The balance between the transverse, upslope component of fluid drag on a bedload particle, and the transverse, downslope component of particle weight has been used as the basis for models of bed topography in bends[7,8]. This view of secondary flow-point bar interaction has been challenged recently[9]. Dietrich and Smith suggest that secondary flow above the point bar is directed radially outwards over the whole flow depth and that the helical skew-induced cell is confined to the deepest, or thalweg, portion of the cross-section Sediment accumulation is

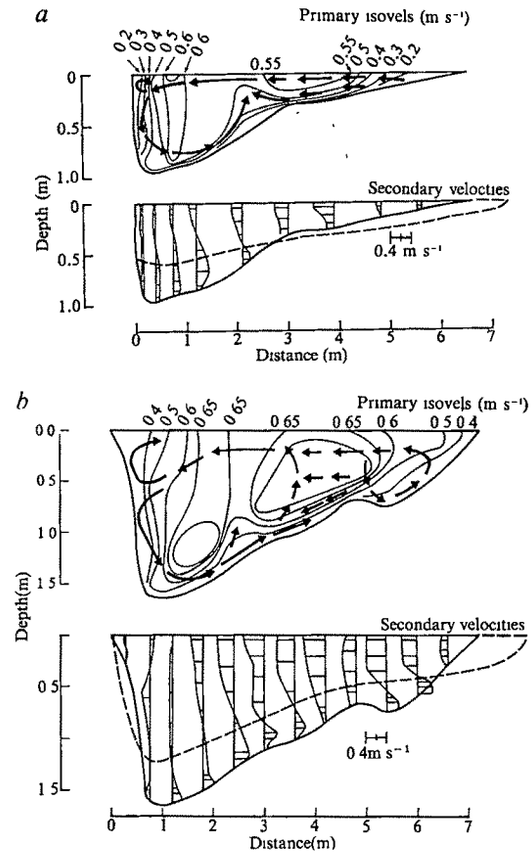


Fig. 1 Upstream bend apex, discharge: *a*, 1.7 $m^3 s^{-1}$; *b*, 4.0 $m^3 s^{-1}$. Dashed channel section represents that at the previous measurement section, ~6 m upstream.

then concentrated on a steep transverse slope called the point bar face, between the upper point bar (point bar platform) and the thalweg, where near bed flow converges and there is strong upwelling. Bridge[10] suggests that this pattern of flow and point bar building is a characteristic of point bar emergence at flows below about two-thirds bankfull. He suggests that at such low stages the point bar topography causes flow convergence at the bend entrance, but that such patterns are replaced by helical flow at formative (that is, bankfull) discharge.

To help to resolve this discussion, data were collected in this study over a range of discharges in meander bends of the Fall River in Rocky Mountain National Park, Colorado. The reach studied has a bankfull capacity of about 4 $m^3 s^{-1}$ and well developed meanders with a sinuosity of 2.2. The annual hydrograph is dominated by snowmelt runoff, resulting in long periods of almost steady bankfull flow in May, June and July. The gradient is ~0.1%. The bed material is sand, $D_{50} = 1.0$ mm, moving in dunes, ripples and as suspended load. Sediment availability has been greatly increased by erosion associated with the failure of Lawn Lake Dam on the Roaring River (an upstream tributary) on 15 July 1982. As a result, the bed is mobile at all discharges and point bars in the bends respond quickly to changes in flow stage.

Measurements were made at low, intermediate and bankfull stages, corresponding to discharges of 1.7, 2.8, and 4.0 $m^3 s^{-1}$. Long and cross-stream velocities were measured using an electromagnetic current meter capable of measuring two mutually perpendicular velocity components with an accuracy of ± 3 mm s^{-1}. All measurements were made from temporary bridges aligned at right angles to the outer bank, and care was taken to work only over dune crests, avoiding separation zones on the lee side of dunes. Data were collected at 18 sections evenly spaced along the channel through two consecutive bends. The complete data set is available[11,12] but because of limitations of space, only data for the intermediate and high flow at the

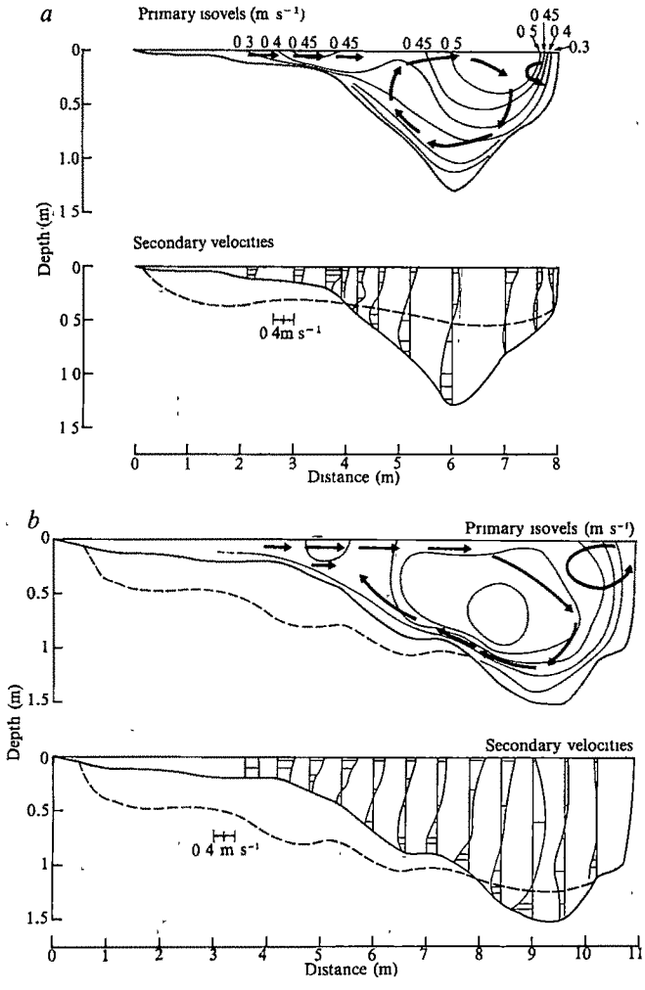


Fig. 2 Downstream bend apex, discharge: *a*, 1.7 $m^3 s^{-1}$; *b*, 4.0 $m^3 s^{-1}$. Dashed channel section represents that at the previous measurement section, ~4 m upstream.

bend apices are reported here. However, these sections do typify the different patterns found in the two bends. The long and cross-stream velocities were resolved into primary and secondary components using the method based on three-dimensional continuity recommended by Dietrich and Smith[9]. The results are shown in Figs 1, 2.

At both sections and flows the salient feature of the secondary circulation is the helical, skew-induced cell. However, close to the outer bank, a small cell of reverse rotation is clearly present, especially at bankfull flow. These cells are associated with distortion of the primary isovels and depression of the maximum primary velocity below the free surface in the outer bank region. The outer bank cell occupies the channel to a distance 1–2 times the bank height away from the outer bank, as observed elsewhere[5,9,10] and predicted theoretically[13].

The pattern of primary and secondary flows in the outer half of the channel changes little with stage at a bend, and is similar at both apices. This robustness is not present in the flow pattern in the inner half of the channel, where the two bends react differently to changing stage.

At intermediate flow, secondary flow over the point bar is directed radially outwards over the whole depth, as predicted and observed by Dietrich and Smith and by Bridge[9,10]. The reason for this is the dominance of the centrifugal force acting outwards over the hydrostatic pressure gradient, acting inwards. Also, the flow is shelving and narrowing in the longstream direction at the inner bank, as indicated in Figs 1*a* and 2*a* by the dashed lines showing the cross-section just upstream from the bend apex. Consequently, water is driven radially outwards to maintain continuity, leading to outward secondary flow. These arguments agree with the explanation put forward by the previous researchers[9,10].

At bankfull stage, the two bends show different flow patterns in the inner half of the channel. At the upstream bend, outward flow has been replaced by helical flow over most of the width, as predicted by Bridge[10]. The point bar has been scoured and reprofiled by the high flow and this, combined with the increased stage at almost constant width, has significantly increased the importance of the cross stream hydrostatic pressure gradient, which is now able to drive bed flow inwards. This is not the case at the downstream bend. Here, outward flow over the point bar persists up to bankfull discharge. The reason for this is that the width increases markedly with stage at the downstream bend, so that both the depth and the hydrostatic pressure gradient over the point bar remain small (Fig. 2*b*). Also, the point bar is more prominent in the downstream bend, so that shelving in the downstream direction is maintained at bankfull flow (Fig. 2*b*). This is not the case at the upstream bend, where high flow scouring produces deepening downstream at the inner bank (Fig. 1*b*).

We conclude that both patterns of flow in the inner half of the channel are possible and do occur in nature, depending on the morphology of the channel cross-section. Of particular importance is the stage–width relationship. Where there is marked widening with stage, outward flow persists to bankfull flow, but where width is almost constant with stage, helical flow expands almost to the inner bank. A quick survey of the Fall River bends revealed about equal numbers of bends falling into each category. As all the bends have the same bankfull discharge, sediment load, and bed and bank materials, there seems to be no obvious reason for the different morphologies, raising the possibility of there being two stable bend sections for a given set of independent controls. Clearly, point bar-flow interaction is a topic deserving further consideration and study.

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Enhancement of marine primary production by nitrogen-enriched acid rain

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The effect of acid rain on the *p*H of freshwater habitats is well known[1]. The acidity of rainwater is largely due to hydrated oxides of sulphur, nitrogen and carbon[2], which also constitute nutrients essential for plant growth. Most freshwater and marine environments contain sufficient quantities of inorganic carbon and sulphur to satisfy plant growth needs, but nitrogen inputs can at times fall short of growth demands[3,4]. Nitrogen limitation of phytoplankton growth is widespread and chronic in some marine waters[5–7]. I

report here that nitrogen-limited conditions in North Carolina near-surface waters are ameliorated following rainfall inputs; the most acidic rainfall events led to the greatest stimulation of phytoplankton growth. Continentally-derived acid rain consistently contained more inorganic nitrogen (NO_3^-, NO_2^- and NH_4^+) than near neutral rainfall generated from oceanic fronts. Marine primary production can thus be influenced by the source of rainfall in marginal environments.

Nutrient limitation and impacts of rainfall on phytoplankton growth were examined at three Atlantic Ocean locations on the east coast of North Carolina. Bogue Sound, a euhaline (30–34 parts per 10^3 (p.p.t)) sound separating the mainland from a barrier island (Bogue Island) served as an inshore location. Continental slope waters ~1 km offshore from Bogue Banks provided a near-shore habitat, while Gulf Stream waters 25 km offshore represented a more pelagic habitat All three locations commonly receive both acidic (continental origin) and near-neutral (oceanic origin) rainfall. Continental rain *p*H characteristically ranged from *p*H 3.5 to 5.5, while oceanic rain ranged from *p*H 5.5 to 6.8. Surface waters from these locations were collected in 20-l pre-cleaned polyethylene carboys throughout 1983–84, immediately brought to the Institute of Marine Sciences (IMS) and analysed for the soluble nutrients $NO_3^- + NO_2^-$, NH_3/NH_4^+, PO_4^{3-} and chlorophyll *a* content. Samples were collected during periods of minimal riverine runoff (salinities of at least 33 p.p.t.); however, Bogue Sound was occasionally sampled after freshwater runoff episodes, as experienced after the landfall of hurricane Dianna during mid-September 1984 (salinity = 27 p.p.t.).

Rainwater was routinely collected on the rooftop of IMS using pre-cleaned polyethylene buckets. Rainfall amounts and *p*H were recorded and nutrient chemistry was performed on rainwater used in bioassays.

Bioassays were performed in triplicate in 1-l pre-cleaned polyethylene Cubitainers, which were 85% transparent to photosynthetically active radiation (PAR = 400–700 nm). They were incubated in natural surface light and temperature conditions in a saltwater pond at IMS. Incubation periods lasted between 1 and 7 days. Freshly collected seawater was dispensed, followed by additions of either 10 or 20% v/v rainwater (RW) or distilled deionized water (DDW). Total bioassay volume was 900 cm^3. Parallel $N\text{-}NO_3^-$ (100 and 200 parts per 10^9 (p.p.b.), $P\text{-}PO_4^{3-}$ (50 and 100 p.p.b.), and combined NO_3^-/PO_4^{3-} additions were also made A 10-μCi aliquot of $^{14}C\text{-}NaHCO_3$ (68 μCi μmol ICN Corporation) was also added to each Cubitainer to monitor photosynthetic $^{14}CO_2$ assimilation as a growth-response parameter. All ^{14}C assimilation results were corrected for abiotic ^{14}C precipitation and liquid scintillation quenching. The fluorometric determination of chlorophyll *a* in 90% v/v $MgCO_3$-buffered acetone extracts served as an additional phytoplankton growth-response parameter.

Both oceanic and continental rain significantly ($P < 0.001$) stimulated phytoplankton primary production as observed by both $^{14}CO_2$ fixation and chlorophyll *a* production (Fig. 1). Stimulation was evident with both 10 and 20% v/v additions. The more acidic continental rainfall led to much higher magnitudes of stimulation (Fig. 1). Typically, 30–70% higher phytoplankton growth yields were observed in response to acidic as opposed to near-neutral rainfall additions. Parallel NO_3^- and PO_4^{3-} additions consistently revealed exclusive NO_3^- stimulation of phytoplankton production during 1983–84. Acidic rain also led to more sustained periods of stimulation. Generally, enhanced (over control) phytoplankton growth, as monitored by chlorophyll *a* production, continued for 6–7 days in response to acid rain additions (Fig. 2). Near-neutral rainfall led to initial growth stimulation, which decreased after 2–3 days (Fig. 2) Differential growth stimulation was not directly attributable to *p*H; neutralized (with NaOH) acid rain yielded results identical to untreated acid rain. Similarly, near-neutral rain made more acidic did not reveal greater stimulation. Furthermore, the addition of either acid or neutral rain failed to alter ($P < 0.001$) the pre-existing *p*H of seawater significantly (*p*H = 8.36). Accordingly, I attribute differential stimulation among, as well as between, treatments and controls to contrasting levels of dissolved inorganic nitrogen enrichment.

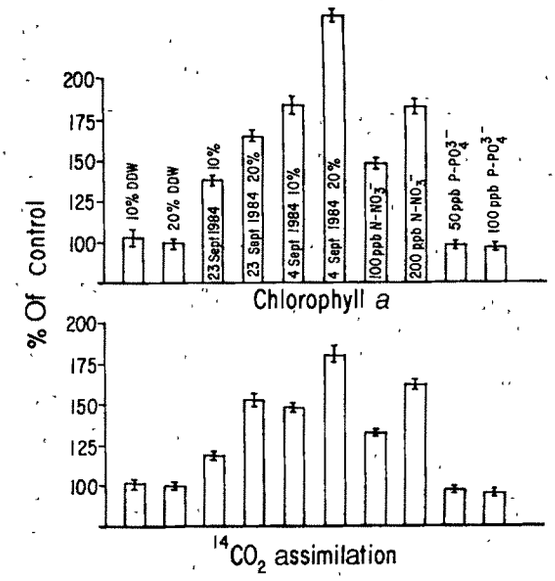


Fig. 1 Impacts of rainwater on Bogue Sound chlorophyll *a* production, and $^{14}CO_2$ assimilation following a 5-day *in situ* Cubitainer bioassay. All treatments were compared with controls (no additions). Both 10% and 20% v/v additions are shown. Rainwater collected on 4 September 1984 had a *p*H of 4.05, while 23 September 1984 rainwater had a *p*H of 5.85. Parallel 10% and 20% v/v distilled deionized water (DDW) additions revealed the impacts of equi-volume nutrient-free freshwater dilutions on Bogue Sound Water. Parallel $N\text{-}NO_3^-$ (100 and 200 p.p.b.) and $P\text{-}PO_4^{3-}$ (50 and 100 p p.b.) additions consistently showed exclusive stimulation of phytoplankton growth by nitrogen. Error bars indicate variability among triplicate samples for each treatment. Biostimulation by all rainwater and $N\text{-}NO_3^-$ additions was significant ($P < 0.001$) among the two growth parameters.

Chemical analyses of sea water from all sampling locations and bioassays demonstrated: (1) consistent NO_3^- depletion but PO_4^{3-} sufficiency during the main phytoplankton growth period (April–October); (2) rapid depletion of NO_3^- and NH_3/NH_4^+ enrichments supplied in rainwater, but a lack of concurrent PO_4^{3-} depletion either supplied in rainwater or as an individual nutrient, and (3) good agreement between the initial amount of NO_3^-, NH_3/NH_4^+ present in rainwater and the duration of phytoplankton growth stimulation. Lastly, a positive correlation existed between $NO_3^- + NO_2^-$ content and acidity of rainfall samples collected ($r^2 = 0.87$; $n = 15$).

Five additional bioassays, complementing those shown in Figs 1, 2, revealed that phytoplankton growth was nitrogen limited throughout the spring and summer of 1984. Atomic ratios of dissolved inorganic N : P ranged from 1 to 5 (weighted mean of 2.8), further evidence for consistent nitrogen-depleted conditions of these waters.

The choice of 10–20% (v/v) rainwater additions was based on the impacts of rainwater dilutions on near-surface (0–5 m depth) salinities following large (1–5 cm) rainfall events in coastal waters, before the dilution observed by enhanced terrigenous freshwater runoff. Near-surface salinities were commonly depressed by 2–3 p.p.t. immediately after such rainfall events. A 10% v/v bioassay rainwater addition depressed the salinity of coastal water by ~2.2 p.p.t. Hence, such additions provide reasonable approximations of natural rainwater dilution effects on near-surface seawater. Large storm systems are likely to mix rainfall inputs to depths >5 m; therefore, the estimates presented here apply to more localized short-lived storm activities, including the numerous summer thunderstorms and rapidly dissipating winter cold fronts often observed in this area.

Periodic offshore chlorophyll *a* samplings before and after storm events in 1984, substantiate bioassay-derived conclusions,

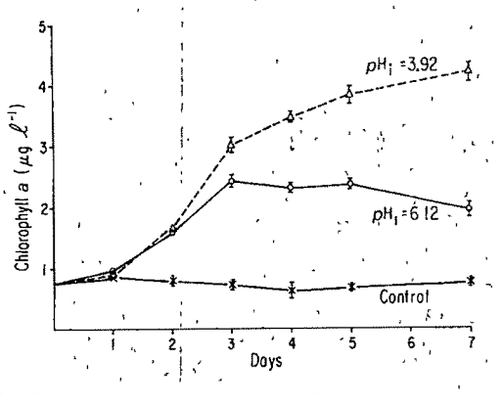


Fig. 2 Chlorophyll *a* production in Cubitainer bioassays subsampled daily following acidic versus near-neutral rainwater (10% v/v) additions. Acidic rainfall ($pH = 3.92$) characteristically showed higher magnitudes and longer-lasting stimulatory effects than near-neutral (pH 6.12) rainfall. Chemical analyses of acidic rainfall yielded the following results $N\text{-}NH_4^+ = 233$ p.p.b., $N\text{-}NO_3^- + NO_2^- = 459$ p.p.b., $P\text{-}PO_4^{3-} = 9.1$ p.p.b. Near-neutral rainfall: $N\text{-}NH_4^+ = 251$ p.p.b., $N\text{-}NO_3^- + NO_2^- = 219$ p.p.b., $P\text{-}PO_4^{3-} = 3.7$ p.p.b. Control was untreated Bogue Sound water incubated in parallel. Error bars show variability among triplicate samples. Beyond day 2, all treatments were significantly ($P < 0.001$) different from each other as well as from the control.

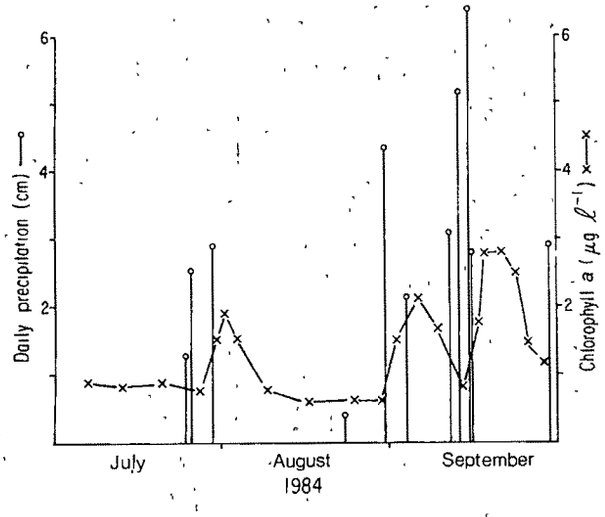


Fig. 3 Major (>0.1 cm per day) precipitation events recorded at Morehead City, North Carolina versus surface chlorophyll *a* concentrations sampled at an offshore loction 5 km south-east of Bogue Banks during July, August and September 1984. All chlorophyll *a* samples were from non-turbid waters free of runoff plumes and sediment resuspension. Extensive rainfall during mid-September was due to the passage of hurricane Dianna near the North Carolina coast.

namely, that precipitation nitrogen inputs lead to significant localized enhancement of phytoplankton production in these nitrogen-depleted waters. Characteristically, a 2-day lag occurs between extensive rainfall and enhancement of near-surface chlorophyll *a* levels (Fig. 3). Chlorophyll *a* enhancement can last from a few days to several weeks. The initial (within 3 days) enhancement closely follows direct precipitation inputs, leading to small but detectable increases in NO_3^- and NH_3/NH_4^+ concentrations. Freshwater runoff sources, as delineated by suspended fine-sediment plumes and the appearance of brackish water phytoplankton species and detritus, typically do not appear in these waters until 2–3 days after the onset of chlorophyll *a* enhancement. While direct precipitation inputs seem to be responsible for initial chlorophyll *a* enhancement, terrigenous runoff probably sustains such enhancement beyond a period of several days.

Experimental and observational findings indicate that localized inorganic nitrogen enrichment attributable to direct precipitation inputs can lead to enrichment of marine primary production in near-surface waters. Annually, chlorophyll *a* concentration ranges from >1 to 5 μg l^{-1} in these waters. The observed buildup of chlorophyll *a* concentrations following direct precipitation inputs (Fig. 3) therefore constitutes a substantial fraction of maximum levels. Acidic rainfall, being enriched in NO_3^- and NO_2^-, preceded the highest magnitudes of primary production stimulation. I therefore conclude that although pH impacts of acid rain are largely insignificant in well-buffered marine habitats, nutritive impacts are both detectable and may be of long-term consequence in shaping both patterns and magnitudes of phytoplankton production. Such potential impacts are expected to be of greatest importance in waters bordering the eastern margins of land masses supporting extensive industrial and urban development. These would include the eastern seaboards of the United States and the United Kingdom, the Baltic region and the Western Pacific waters bordering Japan, Korea and China.

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Weak neutral current and β radiolysis effects on the origin of biomolecular chirality

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The possibility of a connection between the parity non-conserving weak interactions and the handedness of biomolecules has received recent attention[1]. Although the asymmetric effects of the weak interactions in molecules have been estimated to be very small[2–9], Kondepudi and Nelson (KN)[10–13] have shown that autocatalytic chemical systems that spontaneously break chiral symmetry[14–19] can effectively amplify systematic chiral perturbations as small as those produced by weak neutral currents[2–4,20], despite the simultaneous presence of larger randomly fluctuating chiral perturbations, and hence provide a mechanism for producing the observed homochirality of biomolecules. I show here, in the light of new estimates of the asymmetric decomposition of racemic mixtures by β radiation[9], that the chiral selection parameter due to β radiolysis can be up to six orders of magnitude larger than that resulting from weak neutral currents. This suggests that β radiolysis is more likely to be the selector of biomolecular chirality than weak neutral currents.

The same chemical kinetic model is used as in KN[10–13] but the reactants S and T in the model are taken to be chiral, rather than achiral as assumed by KN, and only homochiral combinations of reactants with each other and with the autocatalytically formed product X are assumed to occur, for example, $S_L + T_L + X_L \rightarrow 2X_L$; $S_D + T_D + X_D \rightarrow 2X_D$. Here S and T could be, for example, chiral monomers which react to form a dimer X. Alternatively, this simple model could be interpreted as an

abbreviated approximation of a more complicated mechanism for the formation of a polymer X. Because all known biomolecules capable of catalysing their own formation (the protein/nucleic acid systems) are polymers formed from homochiral monomers, the assumption of chiral reactants rather than achiral ones seems a reasonable modification of the original KN model considered as a description of the autocatalytic formation of prebiomolecules.

The kinetic model gives the expression $dX_L/dt = k_1S_LT_L - k_{-1}X_L + k_2S_LT_LX_L - k_{-2}X_L^2 - k_3X_LX_D$ for the rate of formation of X_L and a similar expression (with L and D interchanged) for formation of X_D, where S_L, T_L, and X_L denote molar concentrations of the corresponding substances. According to these equations, the enantiomers X_L and X_D are formed at different rates if the concentrations of reactants, assumed by KN[10-13] and in the present work to be constant or slowly varying in time, are such that S_LT_L is not equal to S_DT_D. The production of this inequality by asymmetric β radiolysis is considered below.

An inequality in constant reactant concentrations is equivalent to an inequality in rate constants. With the definitions $\eta = (S_L - S_D)/(S_L + S_D)$ and $S = (S_L - S_D)/2$ for reactant S, which imply $S_L = (1+\eta)S$ and $S_D = (1-\eta)S$, and with similar definitions for reactant T, the term $k_1S_LT_L$ appearing in the rate equation can be written correct to first order in η as $k_1(1+2\eta)ST = k_{1L}ST$, where it has been assumed for simplicity that η has the same value for both S and T, and where the last equation defines k_{1L}. Similarly, $k_1S_DT_D = k_{1D}ST$ with $k_{1D} = (1-2\eta)k_1$. In the same way as for k_1, the terms containing k_2 are replaced by ones containing $k_{2L} = (1+2\eta)k_2$ and $k_{2D} = (1-2\eta)k_2$. Finally, the selection parameter, defined by KN as $g = 2(k_{1L} - k_{1D})/(k_{1L} - k_{1D})$, is found to be related to η simply as $g = 4\eta$.

With the above definitions, the resulting rate equations are identical to those of the original KN model[10-13], but the physical origin of the asymmetries in the effective rate constants k_1 and k_2 is quite different. In the original KN model, weak neutral currents produce inequalities $k_{1L} \neq k_{1D}$ and $k_{2L} \neq k_{2D}$ which in typical organic molecules give a resulting selection parameter $g = \Delta E/RT \sim 10^{-17}$, where ΔE is the parity nonconserving energy difference between enantiomers[2-4]. In spite of its smallness, KN have demonstrated that this value of g is sufficiently large to determine the eventual dominance of one of the enantiomers of X (refs 10-13). For comparison, we now consider the production of concentration inequalities $S_L \neq S_D$ and $T_L \neq T_D$ by β radiolysis[5-9] of a racemic mixture of reactants (β radiolysis of X was also calculated and found to be less important). Here the value of η created by the asymmetric decomposition of the enantiomers depends on the strength of the β-ray source, the irradiation time, and the temperature. For organic compounds exposed to likely prebiotic sources of β radiation and temperatures below 80 °C, η has been estimated[9] to attain values as high as 10^{-12}. It follows from the relation $g = 4\eta$ that the value of g resulting from β radiolysis can be up to five or six orders of magnitude larger than the value of g resulting from weak neutral currents. This dominance by β radiolysis is also expected for most chiral molecules containing heavy atoms, where the values of g are predicted to be larger for both types of interaction[2-4,8].

Hence, it is predicted that β radiolysis can produce a much larger systematic chiral perturbation than produced by weak neutral currents. If a racemic mixture of reactants is exposed for a sufficiently long period of time to sufficiently strong β radiation sources such as those which may have been present in prebiotic times[9], and if a symmetry-breaking autocatalytic chemical reaction of the type analysed by KN[10-13] then occurs, the chirality of the resulting product is more likely to be determined by β radiolysis than by weak neutral currents. Provided that an effective autocatalytic chemical reaction can be realized, an experimental test of this prediction seems possible using existing intense sources of β radiation[9].

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Osmotic stress mimics effects of vasopressin on learned behaviour

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It has been suggested that arginine vasopressin (AVP) is involved in the retention of learned responses, in addition to its classical physiological functions of water retention and modulation of blood pressure. AVP administered subcutaneously (s.c.), intraventricularly or intracerebrally can prolong extinction of active avoidance behaviour[1,2] and can enhance retention in inhibitory (passive) avoidance[3-5]. These effects have been interpreted as a direct action of AVP on the central nervous system to facilitate memory consolidation[3,4]. AVP also has facilitatory effects on cognitive function in humans[6], and marked deficits in AVP function have been associated with certain types of psychopathology[7]. Alternative hypotheses for the behavioural actions of AVP have involved motivational constructs such as arousal[8], and our recent work has focused on the role of arousal resulting from the activation of peripheral visceral signals in the behavioural effects of peripherally administered AVP[9-12]. The development of a specific antagonist for AVP[13], 1-deaminopenicillamine-2-*O*-methyl tyrosine arginine vasopressin (dPTyr(Me)AVP), which can reverse the behavioural effects of exogenously administered AVP[9-12], has provided a powerful tool for examining the role of AVP in the behavioural responses produced by physiological challenges known to release vasopressin. However, the relationship between the behavioural effects of exogenously administered AVP and the behavioural function of endogenously released AVP has not been evaluated. We report here that a potent peripheral osmotic stimulus, the intraperitoneal (i.p.) injection of hypertonic saline, at doses known to release AVP both centrally[14] and peripherally[15], will produce behavioural effects similar to those of exogenously administered AVP. Furthermore, the prolongation of active avoidance induced by this osmotic stimulus is reversed by pretreatment with dPTyr(Me)AVP, suggesting that endogenously released AVP may also produce behavioural effects.

Male Wistar rats (150-220 g), each handled and weighed once previously, were trained to avoid shock in a pole jump apparatus as described previously[2]. Briefly, during the initial training of the active avoidance response, the rats were subjected to 10 trials per day where a 5-s conditioned stimulus preceded a 0.3-0.5-mA footshock to the floor. A response during the 5-s conditioned stimulus (light illumination) was recorded as an

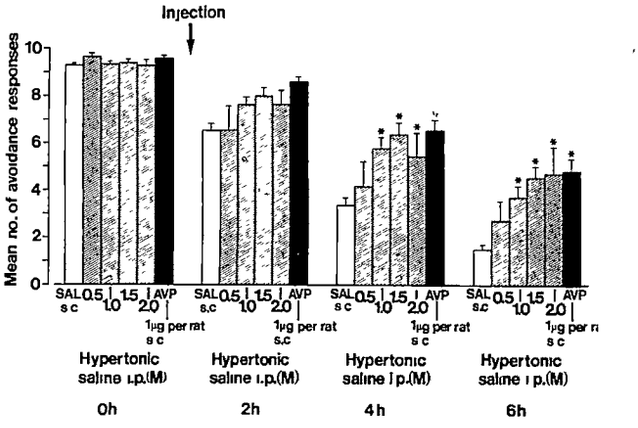


Fig. 1 Effects of different doses of hypertonic saline on extinction of active avoidance Rats were injected with saline i p immediately after the first 10 extinction trials on day 4 of testing. * Significantly different from the saline group, $P < 0.05$, Student's t-test following a significant overall analysis of variance for each separate extinction session. Numbers of subjects were 83, 11, 52, 34, 11 and 42 in the saline, 0.5 M, 1 0 M, 1 5 M, 2 0 M NaCl and AVP (1 μg per rat, ~5 4 μg per kg) groups, respectively

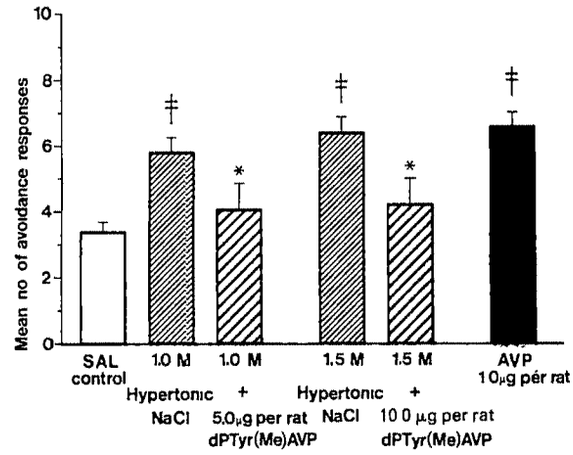


Fig. 2 Effects of the AVP antagonist on the prolongation of extinction produced by hypertonic saline. Data shown represent only those from extinction session III, but results from extinction session IV were identical. * Significantly different from hypertonic saline alone, $P < 0$ 05, Student's t-test following a significant overall analysis of variance for each logical grouping; ‡ significantly different from saline, $P < 0.05$. Numbers of subjects were 83, 52, 21, 34, 10 and 42 for the saline, 1.0 M NaCl only, 1.0 M NaCl + 5.0 μg per rat (~27 μg per kg) dPTyr(Me)AVP, 1 5 M NaCl only, 1.5 M NaCl + 10 μg per rat (~54 μg per kg) dPTyr(Me)AVP, and AVP, respectively. Saline, 1.0 M NaCl, 1.5 M NaCl and AVP groups are the same as in Fig. 1 for extinction session III.

avoidance and the inter-trial interval was 40, 60 or 80 s, randomly assigned across trials. Following 3 days of acquisition, rats were subjected to a fourth day of extinction testing with all conditions identical except that the shock was turned off. Only those rats meeting the previously established acquisition/extinction criteria[1,2] were treated; these rats all showed ≥14 successful avoidance responses during acquisition and ≥8 avoidances during the first 10 extinction trials. Only these animals were used in the experiment. This criterion was similar to that used by De Wied and associates[1] and eliminated 10% of the rats. Four blocks of 10 trials each were run; after the first 10 trials, each rat was treated with either 0.9% saline s.c., 1 μg of vasopressin dissolved in 0.9% saline, or different concentrations of hypertonic saline (0.25–2.0 M) injected i.p. in a volume of 2 ml.

Extinction testing was continued for three more blocks of 10 trials, one block every 2 h. Drinking water was available during the testing period. In a second experiment, the procedure was identical except that rats received two injections immediately after the first 10 trials of extinction: 0.9% saline or the AVP pressor antagonist was injected s.c.[13], followed immediately by 1.0 or 1.5 M NaCl as an osmotic stimulus. All testing was done under 'blind' conditions, that is, the experimenter did not know which rats received which treatments. Each 4-day testing series consisted of 20 rats randomly assigned to the treatment groups. Each series always contained rats receiving 0.9% saline and AVP s.c., and groups receiving hypertonic saline injections combined with the antagonist were always tested with a group receiving hypertonic saline alone. As a result, many more rats were included in the 0.9% saline (control) group and the AVP group.

The hypertonic saline was injected i.p. to replicate previous studies which measured AVP levels and plasma osmolality. These doses are similar to those used by others[15] to show dose-dependent increases in plasma osmolarity and plasma AVP levels. In that study, a dose identical to our 1.0 M NaCl dose in rats of the same weight produced plasma osmolarity of 305 mosm per kg and a plasma AVP level of 10 pg ml^{-1}. Measurements of AVP in the blood in our laboratory (S. Deyo, W. J. Shoemaker, A. Ettenberg, F.E.B. and G.F.K., unpublished results) revealed plasma levels of 13.1 ± 2.2 to 28.4 ± 3.5 pg ml^{-1} 20 min after s.c. injection of behaviourally effective doses of AVP (0.1–1.0 μg per rat). Thus, the osmotic stimuli used here produce plasma AVP levels within the range of those produced with exogenous injection. A similar osmotic challenge (2 and 4 ml of 2 M NaCl) in anaesthetized rats provoked a dramatic increase in AVP release into a push-pull cannula localized to the lateral septum[14].

Hypertonic saline injection produced a dose-dependent prolongation of active avoidance, reaching significance at doses of 1.0 and 1.5 M NaCl (Fig. 1); data from 0.25 M NaCl were not different from controls and are not shown. These results were virtually identical to those produced by 1 μg AVP injected s.c. Following the injection of hypertonic saline, the rats showed some irritability and occasionally some vocalization, but these effects disappeared within 2–3 min. Note that the rats receiving this hypertonic saline injection showed no greater ill effects than those observed following AVP. For example, exogenously administered AVP in behaviourally effective doses produces taste aversions in rats[12] and hypertonic saline at the higher doses used in these avoidance experiments (1.5 and 2.0 M NaCl) produced similar taste aversions (R.D. and G.F.K., unpublished results).

At 5 μg per rat, the vasopressin antagonist produced an attenuation of this effect of hypertonic saline (Fig. 2). A similar reversal was seen with 10 μg antagonist after treatment with 1.5 M NaCl (Fig. 2). Preliminary results indicate that this effect may be dose-dependent, in that 2.5 μg antagonist did not attenuate the prolongation of extinction produced by 1 M NaCl (mean ± s.e.m., number of avoidances for extinction session III were 5.90 ± 1.17 for 2.5 μg per rat compared with 5.79 ± 0.49 for 0 μg per rat).

These results demonstrate that an interoceptive osmotic challenge (hypertonic saline), known to release AVP, can mimic the effects of exogenous AVP on learned behaviour. Further, this behavioural effect is reversed by an AVP receptor antagonist, suggesting a causal relationship between the endogenously released AVP and the behavioural change. Similar doses of the AVP antagonists have been used to reverse the behavioural effects of exogenously administered AVP in various learning tasks[9–10].

It is unlikely that the antagonist effect in the present experiment is due to an action of the antagonist itself, independent of endogenously released AVP, for example by a general sedative or tranquillizing action. Except for the 100 μg dose[2], we have observed no effect with this antagonist in reversing prolongation of active avoidance produced by high shock levels during training. Also, the AVP antagonist did not significantly alter caffeine-stimulated locomotion at doses from 2.5 to 10 μg per rat (~17–67 μg per kg, see Table 1*a*). Analysis of variance of these data

Table 1 Effects of AVP antagonist dPTyr(Me)AVP on caffeine-stimulated locomotor activity (*a*) and performance in a multiple-schedule operant conflict test (*b*)

		dPTyr(Me)AVP dose		
a	Saline	2.5 μg (17 μg per kg)	5.0 μg (33 μg per kg)	10.0 μg (67 μg per kg)
	1,238 ± 103 $n = 6$	1,032 ± 145 $n = 6$	1,512 ± 283 $n = 5$	1,487 ± 294 $n = 7$
			dPTyr(Me)AVP dose	
b	Component	Saline	10 μg (27 μg per kg)	20 μg (54 μg per kg)
	Random interval 30-s schedule	108 ± 18	117 ± 18	111 ± 29
	Conflict	122 ± 30 $n = 5$	113 ± 19 $n = 5$	113 ± 22 $n = 5$

a, Activity was measured automatically in standard photocell activity cages in continuous 10-min epochs for 90 min following injection of dPtyr(Me)AVP s.c. and 10 mg per kg caffeine (base). For details see ref. 30 *b*, Food-deprived rats previously trained on the conflict multiple schedule were given dPTyr(Me)AVP s.c. 5 min before an 18-min test session. Schedule and testing procedure are described in ref 16

showed no dose effect and no dose × time interaction (both $F < 1$). The AVP antagonist also failed to produce any sedative-like actions in an operant Geller-Seifter conflict test[16] that is very sensitive to minor tranquillizers. It did not alter high-rate responding maintained by the random interval 30-s schedule, and also failed to produce a release of punished responding in the conflict component (Table 1*b*). Finally, the antagonist did not reverse apomorphine-induced taste aversion (R.D., R. M. Bluthe, G.F.K. and M. Le M., unpublished observations). Although it is possible that the hypertonic saline produced elevations in blood pressure that contributed to the behavioural effects, NaCl-induced changes in blood pressure are also reversed by these vasopressor AVP antagonists[17], indicating that such a role for pressor responses as an intervening variable with NaCl would be similar to that seen with exogenously administered AVP[12].

Finally, these and other recent data[9-12] reopen the question of the nature of brain-pituitary interactions. One unresolved problem concerns the mechanism mediating this AVP behavioural response. For example, there is little evidence that AVP itself crosses the blood-brain barrier, particularly at doses that would be liberated from the pituitary in the conditions of the present experiment[18-22]. Furthermore, central AVP pathways exist independently of the classical hypothalamo-posterior pituitary projection[23], and both central and peripheral AVP are released by the osmotic challenge used here[14,15]. De Wied and co-workers have argued for a central site of action based on the observation that a new analogue of vasopressin (pGlu^4, Cyt^{6}AVP(4-8)), when injected peripherally, is much more active than AVP itself in facilitating passive avoidance[24]. This analogue has no pressor activity and its effects can be reversed by an even more potent AVP pressor antagonist[25] injected in nanogram amounts intracerebroventricularly. Our earlier work showed that dPTyr(Me)AVP can reverse the effects of centrally administered AVP[26], but will only reverse the behavioural effects of peripherally injected AVP when injected centrally in doses sufficient to reverse the peripheral pressor effects of the same doses[27,28]. From this we hypothesize an effect of AVP from both central and peripheral sources acting independently but in a homologous fashion[29]. Thus, in the present experiment, the behavioural change following osmotic challenge could result from a release of AVP from the pituitary and/or the central nervous system.

These results also have important implications for our conceptualization of the role of AVP in behaviour and of brain-body interactions in learning. Clearly, an AVP reserve sufficient to change or even mobilize behaviour, is available in situations of extreme challenge. It will be critical to determine whether other cues can activate this sytem, and how this mechanism works, if at all, in normal behaviour.

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Bidirectional control of cytosolic free calcium by thyrotropin-releasing hormone in pituitary cells

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It is now established that a key step in the action of calcium-mobilizing agonists is stimulation of the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) to 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (InsP_3)[1,2]. The latter substance acts as a second messenger, controlling the release of calcium from intracellular stores (see ref. 3 for review). The bifurcating nature of the signalling system is exemplified by the fact that the other product of PtdIns(4,5)P_2 hydrolysis, 1,2-diacylglycerol, can alter cellular function by activating protein kinase C, the cellular target for several tumour-promoting agents such as the phorbol esters[4,5]. In various tissues, including GH_3 pituitary tumour cells, a synergistic interaction between calcium ions and protein kinase C underlies agonist-induced changes in cell activity[4,6-10]. The data presented here suggest that when GH_3 cells are stimulated by thyrotropin-releasing hormone (TRH), an agonist inducing PtdIns(4,5)P_2 hydrolysis[11-14], the two limbs of the inositol lipid signalling system interact to control free cytosolic calcium levels ($[Ca^{2+}]_i$). At low levels of TRH receptor occupancy, $[Ca^{2+}]_i$

increases rapidly, then declines relatively slowly. As receptor occupancy increases, the calcium signal becomes more short-lived due to the appearance of a second, inhibitory, component. This latter component, which is enhanced when $[Ca^{2+}]_i$ is elevated by high potassium depolarization, is mimicked by active phorbol esters and by bacterial phospholipase C. It seems likely that protein kinase C subserves a negative feedback role in agonist-induced calcium mobilization.

When TRH was added to GH_3 cells which had been preloaded with the fluorescent calcium indicator, Quin-2 (ref. 15), there was a rapid increase in fluorescence, indicative of an elevation in $[Ca^{2+}]_i$ (refs 16-20). Figure 1*a* shows, in confirmation of previous results[20], that the kinetics of the TRH-stimulated calcium signal is concentration-dependent; low concentrations of TRH (10^{-9} M) stimulate a relatively long-lived response, while higher concentrations of the peptide give a larger, more transient response. The reversal of the calcium signal with elevated TRH concentrations was more readily seen when the peptide was added following stimulation with high KCl, which has been shown to increase $[Ca^{2+}]_i$ by opening voltage-sensitive calcium channels[16-19,21,22]. Low concentrations of TRH had no effect in these conditions, while with higher concentrations, an initial increase in fluorescence was rapidly superceded by an inhibitory component (Fig. 1*b*). The calcium dependence of the inhibitory mechanism is examined in the experiments summarized in Fig. 1*c*. Pre-stimulation with different concentrations of KCl allows $[Ca^{2+}]_i$ to be titrated to various levels. In the experiment shown in Fig. 1*c*, the $[Ca^{2+}]_i$ values immediately before TRH addition were 110, 205 and 440 nM when the concentration of the depolarizing stimulus was 5, 20 and 40 mM KCl, respectively. Subsequent addition of TRH (10^{-7} M) led to a marked inhibitory effect only with 40 mM KCl (Fig. 1*c*).

There is considerable evidence that phorbol esters activate cellular processes by mimicking the effects of 1,2-diacylglycerol on protein kinase C[5,23-25]. Addition of active phorbol esters such as 4β-phorbol 12-myristate 13-acetate (PMA, 10^{-9}-10^{-6} M) to GH_3 cells had no significant effect on the resting $[Ca^{2+}]_i$ (Fig. 2) or on the cellular content of $InsP_3$ (Fig. 2 legend). Addition of PMA (10^{-6} M) to GH_3 cells 5 min before the addition of TRH resulted in a partial inhibition of the TRH-induced rise in $[Ca^{2+}]_i$ (Fig. 2) and a reduction in the ability of the peptide to increase $InsP_3$ levels (Fig. 2 legend). The inhibition of both responses was especially marked when low concentrations of TRH (10^{-10}-10^{-9} M) were used.

When PMA (5×10^{-7} M) was added to GH_3 cells previously stimulated with 40 mM KCl, $[Ca^{2+}]_i$ decreased after a lag of 30 s (Fig. 3*a*). This inhibitory effect, unlike that following TRH addition (Fig. 3*a*), was not preceded by an increase in $[Ca^{2+}]_i$. Other phorbol esters such as 4β-phorbol 12,13-didecanoate also produced a reduction in $[Ca^{2+}]_i$ in the above conditions, whereas agents which are inactive or only weakly active in stimulating protein kinase C[5,26-28], such as 4β-phorbol or 4α-phorbol 12,13-didecanoate, were inactive at concentrations of 2×10^{-6} M (data not shown).

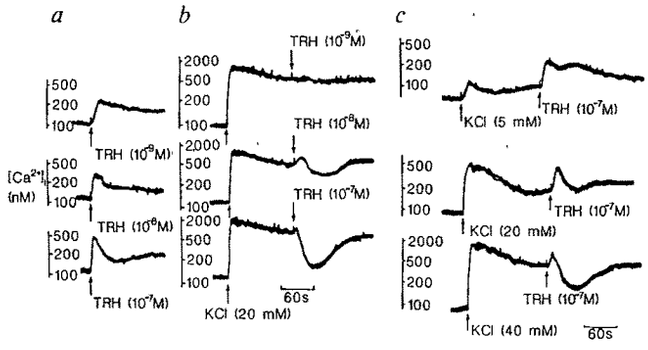


Fig. 1 Effect of TRH on cytosolic free calcium levels in untreated GH_3 cells and in GH_3 cells previously exposed to depolarizing concentrations of KCl *a*, Response of Quin-2-loaded GH_3 cells to different TRH concentrations Note that the decay of the calcium signal is considerably enhanced with high concentrations of the peptide. *b*, Response when TRH was added 2 min after addition of a depolarizing concentration of KCl Addition of a similar concentration of NaCl had no effect on $[Ca^{2+}]_i$. *c*, GH_3 cells were exposed to different concentrations of KCl before the addition of TRH. Note that TRH had a marked inhibitory effect only when the highest concentration of the depolarizing stimulus was used **Methods.** GH_3 cells were grown as described previously[13] in Ham's F10 medium supplemented with antibiotics, horse serum and fetal calf serum. For all experiments, the cells to be used were suspended in a balanced salt solution (BSS)[13]. Cytosolic free calcium was estimated using Quin-2[15] Cells ($3-5\times10^6$ ml^{-1}), loaded with 2×10^{-5} M Quin2/AM, the tetraacetoxymethyl ester of Quin-2, for 15 min at 37 °C, were washed twice in BSS and stored until use at room temperature in a darkened container. Immediately before use, the cell samples were centrifuged (14,000 *g*, 5 s) and resuspended in fresh BSS (pre-equilibrated at 37 °C) at a cell concentration of $3-5\times10^6$ ml^{-1}. Fluorescence measurements were made at 37 °C on stirred samples (2 ml) of these cells using a Perkin-Elmer LS-3 spectrophotofluorimeter; excitation wavelength, 339 nm, emission wavelength, 492 nm. At the end of the analysis, 5×10^{-5} M digitonin was added to measure the maximum fluorescence (F_{max}) and then excess alkaline EGTA was added to measure minimum fluorescence (F_{min}) The values of $[Ca^{2+}]_i$ were calculated from the equation: $[Ca^{2+}]_i = K_D\times(F-F_{min})/(F_{max}-F)$, taking a value of 115 nM as the K_D of Quin-2 for Ca^{2+} (ref 15) Neither TRH nor any of the other drugs used in this study had any effect on the fluorescence of GH_3 cells which had not been loaded with Quin-2 (data not shown).

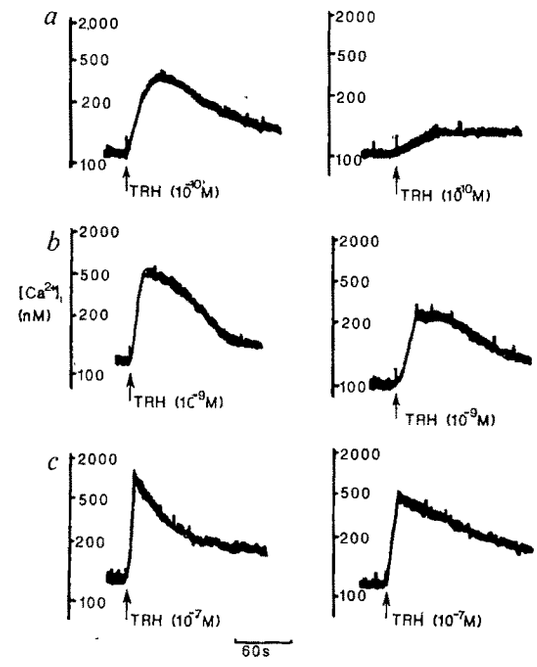


Fig. 2 Effect of 4β-phorbol 12-myristate 13-acetate (PMA) on basal and TRH-stimulated cytosolic free calcium levels in GH_3 cells The cytosolic free calcium concentration of Quin-2-labelled GH_3 cells was measured as described in Fig. 1 legend. The traces in *a*, *b* and *c* show the effects of preincubation of GH_3 cells for 5 min with dimethyl sulphoxide (vehicle of PMA, 0.1% (v/v) left) or PMA (10^{-6} M) (right) Dimethyl sulphoxide, at this concentration, did not alter the basal level of $[Ca^{2+}]_i$ or affect the response of the cells to TRH (data not shown). Note that although PMA did not significantly reduce basal levels of $[Ca^{2+}]_i$ in GH_3 cells, it did reduce the response (and, in particular, the rate of rise) to TRH (10^{-10}-10^{-7} M). The GH_3 cell content of $InsP_3$, measured as described in Table 1 legend, was unaffected by 5 min preincubation with PMA (10^{-6} M) (4,622 ± 533 d p.m. per sample, control; 4,517 ± 286 d p.m. per sample, PMA-treated). This value rose within 5 s of TRH addition to 5,866 ± 177 (10^{-9} M TRH) and 9,777 ± 711 (10^{-7} M TRH) d.p.m. per sample. Corresponding values in PMA-treated cells were 5,016 ± 187 (5 s, 10^{-9} M TRH) and 6,933 ± 177 (5 s, 10^{-7} M TRH) d p m. per sample.

Table 1 Effect of bacterial phospholipase C on the GH_3 cell content of 1,2-diacylglycerol and inositol trisphosphate

Addition	Incubation time (s)	1,2-Diacylglycerol (d.p.m. per sample)	$InsP_3$
Control	—	1,756±80	1,079±182
Phospholipase C (0.1 U ml^{-1})	180	7,756±869*	1,278±100
Phospholipase C (0.1 U ml^{-1})	360	12,624±367*	1,427±98
Phospholipase C (0.5 U ml^{-1})	30	3,188±203†	1,079±132
Phospholipase C (0.5 U ml^{-1})	180	17,800±221*	1,560±50
Phospholipase C (2.5 U ml^{-1})	30	8,824±363*	1,328±66
Phospholipase C (2.5 U ml^{-1})	180	36,450±1610*	2,872±282†

The GH_3 cell content of 1,2-diacylglycerol and $InsP_3$ was measured in cells which had been pre-labelled to isotopic equilibrium with ^{3}H-glycerol (25 μCi per dish) or ^{3}H-inositol (5 μCi per dish) respectively. The methods have been described previously[13,14]. Briefly, GH_3 cell suspensions (0.5 ml), pre-labelled for 3–4 days with ^{3}H-glycerol or ^{3}H-inositol (Amersham International), were incubated with phospholipase C (from *Clostridium perfringens*, Sigma lot 53F-9007) for the times indicated The reaction was stopped by the addition of 1.5 ml chloroform/methanol (1:2 v/v; for 1,2-diacylglycerol extraction) or 0.5 ml 20%(w/v) trichloroacetic acid (for $InsP_3$ estimation). The cell content of the two products of $PtdIns(4,5)P_2$ hydrolysis were then measured as outlined in refs 13 and 14 In different experiments, the samples of cell suspension contained 0.4–0.6 mg of cellular protein. The values shown are the means±s.e m. for three or four samples.

* $P<0.001$; † $P<0.01$, significantly different from the untreated control

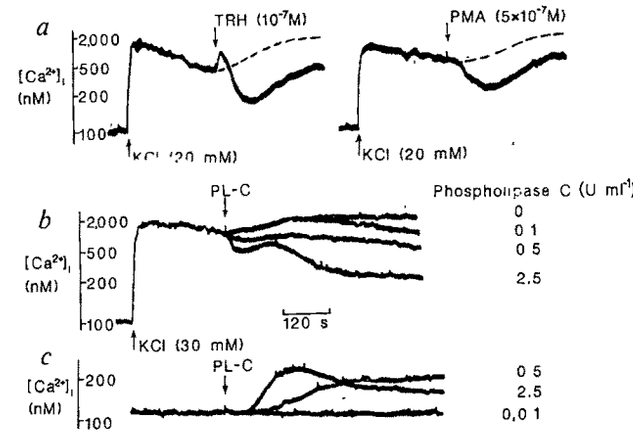


Fig. 3 Effect of agents that stimulate protein kinase C activity on cytosolic free calcium levels in GH_3 cells. The cytosolic free calcium concentration of Quin-2-labelled GH_3 cells was monitored as described in Fig. 1 legend. The traces in *a* show representative responses of GH_3 cells to the addition of TRH and PMA. The dotted line indicates the control response Note that the phorbol ester elicited a reduction in $[Ca^{2+}]_i$ without a preceding rise. *b*, *c*, Effect of addition of bacterial phospholipase C (PL-C) The enzyme (from *C perfringens*, Sigma lot 53F-9007) elevated $[Ca^{2+}]_i$ itself after a lag of ~60 s (panel *c*) when high activities were used. In cells whose $[Ca^{2+}]_i$ had previously been elevated with KCl, phospholipase C induced a reduction in the level of the cation (panel *b*). The onset of the response was dependent on the activity of the enzyme used. Corresponding changes in the cell content of 1,2-diacylglycerol and $InsP_3$ are shown in Table 1

Addition of bacterial phospholipase C to GH_3 and various other cells stimulates 1,2-diacylglycerol formation and activates protein kinase C-dependent protein phosphorylation and cellular responses[9,29–32]. We found that low activities of the enzyme (≤100 mU ml^{-1}) led to a reduction in $[Ca^{2+}]_i$ after a lag of 3 min (Fig. 3*b*). At this time, cellular 1,2-diacylglycerol levels increased significantly, with no change in $InsP_3$ content (Table 1) and, as reported elsewhere[32], protein kinase C-dependent protein phosphorylation also increased. Higher enzyme activities led to more rapid increases in 1,2-diacylglycerol content in the absence of changes in cell $InsP_3$ levels (Table 1), and this was also associated with a decrease in $[Ca^{2+}]_i$ (Fig. 3*b*). When the incubation time was lengthened, however, phospholipase C (>500 mU ml^{-1}) did elicit an elevation in $InsP_3$ (Table 1). As might be expected from the effects of $InsP_3$ on various cells[3], this was associated, in the absence of depolarization, with a rise in $[Ca^{2+}]_i$ (Fig. 3*c*) or, in the presence of depolarization, with a temporary stabilization of $[Ca^{2+}]_i$ (Fig. 3*b*).

There is now strong evidence that TRH, acting on GH_3 cells, produces the initial rise in $[Ca^{2+}]_i$ by a mechanism dependent on $InsP_3$-stimulated release of intracellular calcium[20,33]. Other studies show that this mechanism is fully activated at relatively low levels of receptor occupancy[20]. The second, inhibitory, mechanism, which predominates at high levels of receptor occupancy, seems to be under the control of protein kinase C, as it can be mimicked by treatments that activate the enzyme in the conditions used. Moreover, at the time the inhibitory phase is becoming evident, 1,2-diacylglycerol levels have already been elevated by TRH[12,13]. It is possible that the inhibitory mechanism is effected by: (1) stimulating the intracellular sequestration of calcium, (2) stimulating calcium extrusion from the cell, (3) inhibiting calcium mobilization or (4) blocking calcium entry across the plasma membrane. The latter two explanations seem unlikely, as the inhibitory effect occurs with either TRH or depolarization as the stimulus for calcium elevation; there is good evidence[16,21] that the source of calcium differs in the two cases, with intracellular calcium the source for the TRH effect and influx of extracellular calcium the mechanism by which high K^+ elicits its effect. Indeed, the TRH-induced calcium signal still fades rapidly with high concentrations of the peptide in the presence of 2 mM EGTA (data not shown). It is evident, nevertheless, that activators of protein kinase C can inhibit the TRH-induced rise in $InsP_3$ and $[Ca^{2+}]_i$ in GH_3 cells and diminish calcium mobilization and phosphatidate accumulation in other cells[34,35]. It is unlikely, however, that this effect underlies the inhibitory phase of TRH action because: (1) this phase is mimicked by phorbol esters in conditions in which no $InsP_3$ formation occurs (with high K^+ as the stimulus), (2) levels of $InsP_3$ remain elevated in response to TRH for at least 30 min after peptide addition, although the calcium signal fades rapidly with high concentrations of TRH[36] and (3) such a block of $InsP_3$ production cannot reasonably explain why TRH is able to decrease $[Ca^{2+}]_i$ below the elevated steady-state value achieved after high K^+ depolarization; only the increment due to TRH would be expected to be reversed by this mechanism. It seems likely that the inhibition of $InsP_3$ formation represents an additional mechanism which may have a general role in limiting information flow through the inositol lipid signalling system in chronically stimulated cells, rather than over the initial seconds of the agonist-induced response. The most plausible mechanism for the inhibitory phase of TRH action, as studied here, is that sequestration or extrusion of the cation is facilitated by protein kinase C. ATP-dependent calcium transport across both intra- and extracellular membranes has been reported to be sensitive to protein kinase C[37,38]. Further work is under way to clarify this point.

Whatever the mechanism, it is evident that both elevated $[Ca^{2+}]_i$ and 1,2-diacylglycerol levels are required for the negative feedback system to operate effectively. This suggests that the mechanism by which calcium is removed must be primed with an appropriate amount of calcium before it can be modulated by protein kinase C—an elegant and economical way of buffering excessive changes in $[Ca^{2+}]_i$ after agonist stimulation. These data demonstrate the flexibility of control which is a major advantage of the bifurcating inositol lipid signalling system.

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Intracellular Ca indicator Quin-2 inhibits Ca^{2+} inflow via Na_i/Ca_o exchange in squid axon

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Until recently, intracellular free calcium has been amenable to measurement and investigation only in cells large enough to permit either microinjection of a suitable Ca sensor such as aequorin or arsenazo III or insertion of a Ca-sensitive microelectrode[1,2]. This constraint on cell size was removed by the development of the fluorescent Ca^{2+}-sensitive dye Quin-2 and its acetoxymethyl ester, which can be introduced into a wide range of cell types[3,4]. A major requirement of any intracellular Ca^{2+} indicator is that it should not disturb intracellular Ca^{2+} homeostasis and Quin-2 is generally considered to be satisfactory in this respect[5]. We now report that injection of Quin-2 into squid (*Loligo forbesi*) axons can almost completely abolish one component of Ca^{2+} entry—intracellular Na^+ (Na_i)-dependent Ca^{2+} inflow, which occurs via Na/Ca exchange[6]. Mixtures of Ca and Quin-2 that buffer an ionized Ca^{2+} at close to physiological concentrations also block Na_i-dependent Ca^{2+} influx but these same mixtures fail to block the extracellular Na^+ (Na_o)-dependent extrusion of Ca^{2+}, showing that Quin-2 acts specifically on Ca^{2+} inflow.

Depending on the size and direction of the electrochemical gradient for Na^+, the Na/Ca exchange system can effect net movement of Ca^{2+} either into or out of the cell. For squid axons the exchange fluxes are most easily monitored either as the Ca_o-dependent ^{22}Na efflux (Ca_o/Na_i exchange mode) or the Na_o-dependent ^{45}Ca efflux (Na_o/Ca_i exchange mode)[2]. Because external Na^+ and Ca^{2+} compete for the exchanger, Ca_o-dependent ^{22}Na efflux is most conspicuous in Na^+-loaded axons immersed in Na^+-free media[6], although a small component is detectable in physiological conditions. Figure 1 shows an experiment in which both the Ca_o-dependent ^{22}Na efflux (Ca_o/Na_i exchange) and the K_o-dependent ^{22}Na efflux (Na/K exchange pump) were monitored before and after injection of Quin-2 to give a final axoplasmic Quin-2 concentration of ~300 μM. Within 2 min of injecting the Quin-2, the Ca_o-dependent ^{22}Na efflux started to fall and after 10 min <10% of the initial Ca_o-dependent ^{22}Na efflux was detectable. Subsequent examination of the K_o-dependent ^{22}Na efflux revealed that the Na/K pump was essentially unaltered, showing that Quin-2 selectively inhibits the Ca_o/Na_i exchange flux. Essentially similar results were obtained with final axoplasmic Quin-2 concentrations as low as 150 μM. Inhibition by Quin-2 is not confined to the Ca_o-dependent ^{22}Na efflux: the associated Ca^{2+} influx is also

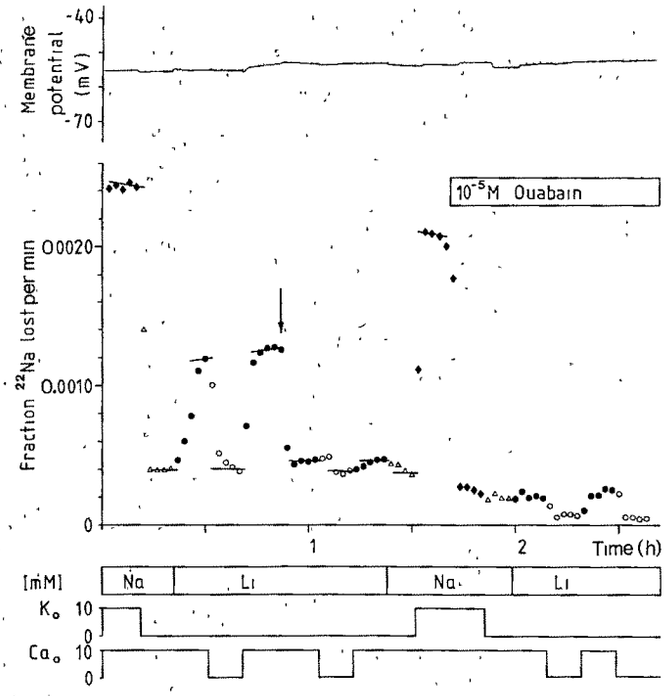


Fig. 1 Effect of Quin-2 injection on the various components of the ^{22}Na efflux from an axon of *Loligo forbesi*. The magnitude of the sodium pump flux was first determined as the reduction of the ^{22}Na efflux brought about by removing external K^+. In the absence of K^+, subsequent iso-osmotic replacement of NaCl by LiCl activates the ^{22}Na efflux and the whole of this activated flux is dependent on external Ca^{2+}. Injection of Quin-2 selectively inhibits the Ca_o-dependent ^{22}Na efflux, because subsequent re-introduction of potassium still activates a large ouabain-sensitive ^{22}Na efflux. The different symbols reflect solution changes, as indicated at the bottom

Methods. After dissecting away the surrounding small nerve fibres, the giant axon was cannulated and injected with a small patch of ^{22}Na. The injector was removed, loaded with 10 mM Quin-2 (tetrapotassium salt; Calbiochem) in 100 mM MOPS buffer, *p*H 7.4 and reinserted. Sampling of ^{22}Na in the superfusate began 65 min after injection of isotope. The membrane potential was monitored continuously by means of an axial intracellular microelectrode. At the time indicated by the arrow, Quin-2 was injected so that the column of Quin-2 overlapped the region of axon containing ^{22}Na. The artificial sea water contained 400 mM NaCl, 100 mM $MgCl_2$, 2.5 mM $NaHCO_3$, *p*H 7.8 with potassium and calcium as indicated. Periods for which NaCl was replaced iso-osmotically by LiCl are indicated at the the bottom, and the membrane potential (uncorrected for any junction potential) at the top. The axon was from a squid killed at sea and brought to the laboratory in refrigerated sea water; this results in appreciable Na^+-loading of the axon, which increases the size of the Ca_o/Na_i exchange flux[6]. Axon diameter 880 μm. Temperature 17 °C.

inhibited Thus the Ca^{2+} influx into control axons preinjected with morpholinopropanesulphonic acid (MOPS; 3.5 mM final concentration, *p*H 7.4) and subsequently immersed in Li-seawater was reduced to 25% of its initial value after injection of the same buffer containing Quin-2 to give an axoplasmic Quin-2 concentration of ~350 μM. As Na_i/Ca_o exchange is only one component of the measured Ca^{2+} influx, this may reflect a larger inhibition of the Na_i/Ca_o exchange moiety.

It has been known for some years that injection of EGTA into axons can inhibit Ca_o-dependent ^{22}Na efflux and the associated Ca^{2+} influx[7,8], and the data of Fig. 1 shows that the chemically related Quin-2 closely resembles EGTA in this respect. Both Quin-2 and EGTA are still effective inhibitors when injected with sufficient calcium to give a buffered free Ca^{2+} concentration close to the physiological value of 100 nM. Tests with other chelators revealed that injection of BAPTA (bis(*O*-aminophenoxy)ethane-*N*, *N*, *N'*, *N'*-tetraacetic acid), EDTA or NTA (nitrilotriacetic acid) to give a final axoplasmic concentration in the range 0.5–1.0 mM also inhibits selectively the Ca_o/Na_i exchange. In the same concentration range, arsenazo III exhibits a transient inhibition only. Of particular interest is the finding that injection of the chelator 1,2-dihydroxybenzene-3,5-disulphonate (Tiron), 1 mM final concentration), which has a very low affinity for Ca^{2+} but fairly high affinity for many heavy metal divalent cations[9], fails to block Ca_o/Na_i exchange. Although this result suggests that those chelators which block do so by stabilizing free Ca^{2+} and not by removing another essential divalent cation[10], other possibilities are not completely excluded.

While the present experiments demonstrate that calcium-chelators can block Na^+ and Ca^{2+} movements via Ca_o/Na_i exchange, the same chelators have little or no effect on Na_o/Ca_i exchange. Thus, injection of a mixture of 50 mM Quin-2 and 25 mM $CaCl_2$, which buffers the free Ca^{2+} close to the physiological concentration, does not alter the properties of the Na_o-dependent ^{45}Ca efflux (Na_o/Ca_i exchange). Essentially similar results are seen after injection of Ca^{2+}-EGTA mixtures. It would seem that Ca_o/Na_i exchange is particularly vulnerable to inhibition by intracellular chelators.

Experiments on dialysed axons provide a possible explanation for these findings[11]. Ca_o/Na_i exchange requires micromolar amounts of intracellular for Ca^{2+} for activation. It is unknown why Ca_i is needed to promote Ca^{2+} inflow but it seems to constitute a form of positive feedback which can be disrupted by the presence within the cell of a strong chelator[2].

These findings have two important implications: (1) experiments on Quin-2-loaded cells may fail to reveal Ca^{2+} inflow via Ca_o/Na_i exchange, especially when the Quin-2 level in the cell is ≥150 μM, which is quite common in Quin-2 studies. (2) Loading cells with millimolar amounts of Quin-2 (as the acetoxymethyl ester) might provide a relatively simple means of effecting selective inhibition of the Ca_o/Na_i exchange mode of the Na/Ca exchanger, it might, for example, be a useful way of examining the contribution of the Ca_o/Na_i exchange system, which is known to be voltage-sensitive[12], to ionic currents in tissues such as the heart.

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Expression of a mammalian Na–H exchanger in muscle fibres of the giant barnacle

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It is now well established that the internal *p*H (*p*H$_i$) of mammalian cells is regulated by means of a plasma membrane transport system that exchanges extracellular Na^+ for intracellular H^+ (ref. 1). Furthermore, modulation of the activity of the Na–H exchanger seems to have a crucial role in the action of various mitogens and growth factors[2–4]. The possibility that such a mammalian Na–H exchanger might be efficiently expressed in a giant invertebrate cell was suggested to us by recent results of Barnard[5] and Miledi and colleagues[6,7], who demonstrated in frog oocytes the expression of various plasma membrane channels that presumably were encoded by the mammalian messenger RNA with which the oocytes had been injected. We used muscle fibres of the giant barnacle, which normally have no demonstrable Na–H exchanger activity[8], and report here that, when injected with poly(A)$^+$ RNA isolated from rabbit liver, the muscle fibres express a Na–H exchanger. No such expression is observed, however, when the injected material is pretreated with ribonuclease A. As hepatocytes are known to possess a Na–H exchanger[9], the most straightforward interpretation of our data is that a mammalian Na–H exchanger has been expressed in the muscle fibre of an invertebrate.

Muscle fibres of the giant barnacle (*Balanus nubilis*) were isolated as described previously[10], with one end of the fibre attached to the shell and the opposite (tendon) end free. The tendon of an individual fibre was cannulated, and through this cannula a glass injection pipette (~125 μm diameter) was advanced about 3 cm into the muscle, along the fibre's longitudinal axis. As the injection pipette was withdrawn from the fibre, fluid was deposited in the cell at the rate of ~1 μl per cm of fibre length. Fibre diameter was ~1 mm. In the first series of experiments, the injected material contained ~1 μg μl^{-1} of poly(A)$^+$ RNA (for a total dose of 3 μg RNA per fibre), isolated from rabbit liver as described in Fig. 1 legend. After the injection, the muscle fibre was incubated in artificial sea water (ASW) at 16 °C for ~18 h. Immediately before the assay for Na–H exchange, the fibre was soaked for ~15 min in Ca-free ASW, and then severed at the tendon and shell ends. The central portion of the fibre, ~2.5 cm in length, was transferred to a standard internal-dialysis chamber, cannulated at both ends. A length of dialysis tubing was inserted through one cannula, down the length of the fibre, and out of the opposite cannula. By perfusing this tubing with dialysis fluid of known composition, we were able to achieve good control over the intracellular environment[11]. A *p*H-sensitive microelectrode of the design of Hinke[12] was inserted through one cannula, and its tip advanced to the centre of the fibre. An open-tipped reference electrode filled with 3 M KCl was advanced similarly through the opposite cannula, permitting the continuous monitoring of pH_o, which was plotted on a strip-chart recorder. The actual assays for Na–H exchanger activity were performed in Ca-containing ASW.

Our strategy for detecting a mammalian Na–H exchanger in barnacle muscle was to block selectively the fibre's normal pH_i-regulating system. Regulation of pH_i in this cell[10,13,14], as well as in giant cells from molluscs[15,16], is mediated by a plasma membrane transport system that seems to exchange external

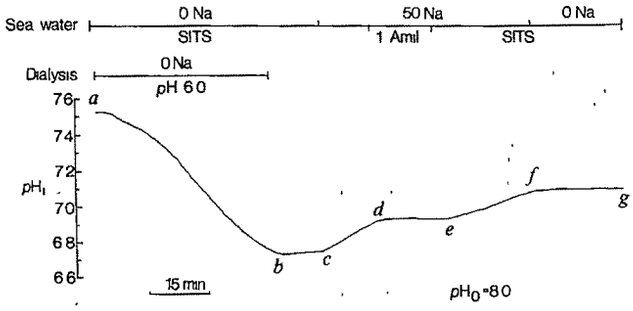


Fig. 1 pH_i recovery in a barnacle muscle fibre injected with poly(A)$^+$ RNA. See text for a detailed explanation of the experiment. The *p*H 7.4 dialysis fluid (DF) had the following composition (in mM): 243 K^+, 7 Mg^{2+}, 8 $Tris^+$, 34 Cl^-, 175 glutamate, 2 EGTA, 4 ATP, 44 of the anionic form of HEPES, 56 of the neutral form of HEPES, 348 mannitol and 0.5 phenol red. The osmolality was adjusted to ~975 mosm per kg with mannitol or water, and the *p*H to 7.4 with KOH or HEPES. The *p*H 6.0 or 6.5 DF had a similar composition, except that the buffer was 100 mM 2-(*N*-morpholino)ethanesulphonic acid (MES). The 0-Na ASW had the following composition (in mM): 440 *N*-methyl-D-glucammonium ($NMDG^+$), 10 K^+, 11 Ca^{2+}, 44 Mg^{2+}, 55 Cl^-, 5 of the anionic form of *N*-2-hydroxyethylpiperazine-*N*-3-propanesulphonic acid (EPPS), and 5 of the neutral form of EPPS. The *p*H was 8.0 and the osmolality ~975 mosm per kg. In the 50-Na or 440-Na ASW, $NMDG^+$ was replaced with Na^+ on a mole-for-mole basis. 0.5 mM SITS or 1 mM amiloride were added as powders immediately before use. The poly(A)$^+$ RNA was prepared as follows. Fresh rabbit liver was minced in cold phosphate-buffered saline and then the tissue was homogenized in a Waring blender. SDS was added to a final concentration of 0.2%, and the tissue was disrupted in a Dounce homogenizer with the loose-fitting pestle. The volume was increased to about 10 times that of the original tissue by the addition of a solution containing 100 mM NaCl, 10 mM Tris-HCl and 5 mM EDTA (*p*H 7.5). The suspension was extracted three times with water-saturated phenol. The combined interfaces from the phenol extractions were extracted once with 100 mM Tris-HCl (*p*H 9.0) to recover polyadenylated RNA which might have partitioned into the interface. This high-*p*H extract was combined with the original aqueous phase, and the nucleic acids were precipitated overnight after the addition of 0.3 M sodium acetate (*p*H 6.0) and 2.5 vols of ethanol. The precipitate was dissolved in 10 mM Tris-HCl (*p*H 7.5) and the RNA was selectively precipitated at −20 °C overnight with 3 M NaCl. The precipitate was washed three times with 3 M sodium acetate (*p*H 6.0) and once with 90% ethanol, and then dried. The crude RNA was dissolved in distilled water and fractionated by chromatography on oligo(dT)-cellulose, as described previously[18], to isolate the poly(A)$^+$ RNA. The peak fractions from the column were precipitated with ethanol, dried and resuspended either in *p*H 7.4 DF or a solution containing 2 mM EGTA, 100 mM HEPES and 0.5 mM phenol red (*p*H ~7.5, osmolality ~975 mosm per kg). The final concentration of poly(A)$^+$ RNA was 1 μg μl^{-1} in the six experiments similar to that shown here and 0, 0.1 or 0.32 μg μl^{-1} in the 11 additional experiments of the dose-response study.

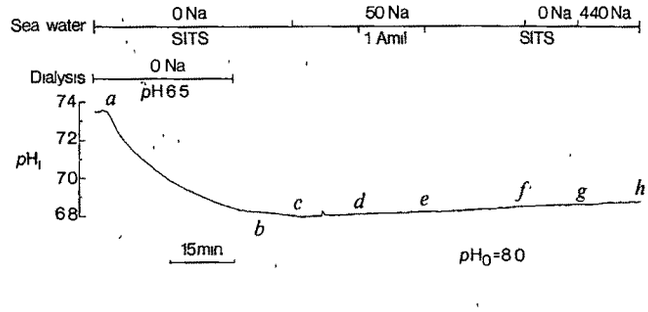


Fig. 2 pH_i recovery in a barnacle muscle fibre injected with fluid pretreated with ribonuclease. See text for details of the experiment. Poly(A)$^+$ RNA was isolated from rabbit liver, as outlined in Fig. 1 legend. We verified that this sample of RNA, when injected into a muscle fibre, caused the expression of a Na–H exchanger. The remainder of the poly(A)$^+$ RNA was incubated with ribonuclease A (200 μg ml^{-1}) at 21 °C for 2 h in a solution buffered to *p*H 8.5 with Tris. The solution was extracted first with phenol and then with chloroform/butanol. Sodium acetate was added to the aqueous phase to a concentration of 0.3 M (*p*H 6). After the addition of 2.5 vols of ethanol, the mixture was incubated for 2 h at −20 °C, and then centrifuged. The precipitate was resuspended at a concentration equivalent to 1 μg of original poly(A)$^+$ RNA per μl.

Na^+ and HCO_3^- for internal Cl^- and H^+. This Na/HCO_3–Cl/H exchanger is functionally quite distinct from mammalian Na–H exchangers[8]. The Na/HCO_3–Cl/H exchanger has an absolute requirement for Na^+, HCO_3^- and Cl^-, is blocked by disulphonic stilbenes such as 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate (SITS), and is unaffected by the diuretic amiloride. Na–H exchangers, on the other hand, require Na^+ (but not Cl^- or HCO_3^-), are inhibited by amiloride and are insensitive to SITS.

Figure 1 shows the result of an experiment on a muscle fibre that had been injected with 3 μg poly(A)$^+$ RNA ~18 h previously. An *in vitro* translation assay had previously shown that this RNA sample stimulated protein synthesis. The fibre was pretreated for 23 min by dialysing it with a Na-free fluid at *p*H 7.4. During this time, the fibre was also exposed to a nominally Na^+- and HCO_3^--free ASW containing 0.5 mM SITS. As the barnacle muscle's own pH_i-regulating mechanism requires HCO_3^- and is blocked by SITS[10], our treatment should have prevented any contribution of the barnacle's Na/HCO_3–Cl/H exchanger to pH_i. At point *a* in Fig. 1, when the *p*H of the dialysis fluid was lowered to 6.0, pH_i slowly fell. Dialysis was halted as pH_i reached ~6.75, returning control of pH_i to the fibre (point *b*). In the absence of external Na^+, pH_i drifted slowly upwards (points *b*, *c*). The addition of 50 mM Na^+ to the ASW caused pH_i to increase much more rapidly (points *c*, *d*); this pH_i recovery, which is typical of an active acid-extruding mechanism, was completely blocked by 1 mM amiloride (points *d*, *e*), a known inhibitor of Na–H exchange[17]. Furthermore, removal of amiloride allowed the pH_i recovery to continue (points *e*, *f*). Finally, the removal of external Na^+ blocked further recovery (points *f*, *g*).

The Na-dependent, amiloride-sensitive pH_i recovery observed in this experiment is indicative of Na–H exchange. In 17 similar experiments, we studied expressed Na–H exchanger activity at injected poly(A)$^+$ RNA doses of 0–3 μg per fibre, and fitted the data to the Michaelis–Menten equation using a nonlinear least-squares method. The apparent maximal pH_i recovery rate was 0.66 *p*H units per h, with the half-maximal rate occurring at a dose of 0.6 μg per fibre. In two experiments with the potent amiloride analogue 5′-(*N*-methyl-*N*-isopropyl)-amiloride (not shown), 10 μM of the drug inhibited the Na-dependent pH_i recovery by an average of 71%. Na–H exchanger activity was also found in barnacle muscle fibres injected with 0.95 μg per fibre of poly(A)$^+$ RNA isolated from rat liver. The average pH_i recovery rate in two experiments was 0.40 *p*H units per h, comparable to that observed for fibres injected with the same dose of rabbit RNA. In one such experiment, the recovery was blocked by 1 mM amiloride, and inhibited 74% by 10 μM 5′-(*N*-methyl-*N*-isopropyl)-amiloride.

To determine whether it was the injected mRNA, rather than some other substance, that was responsible for the expression of the Na–H exchanger, we performed six experiments in which the injected material was treated with ribonuclease A in order to degrade the mRNA (see Fig. 2 legend for details). After the muscle fibre had been acid-loaded, the addition of 50 mM Na^+ to the ASW produced little if any increase in pH_i (Fig. 2, points *c*–*f*). Amiloride produced no detectable inhibition (points *d*, *e*). Even the addition of 440 mM Na^+ to the ASW (points *g*, *h*) failed to elicit a sizeable pH_i recovery. In six such experiments, the mean Na-dependent pH_i recovery rate was 0.05 ± 0.08 *p*H units per h, which is not significantly different from zero ($P > 0.35$). Thus, the expression of a Na–H exchanger requires the injection of a ribonuclease-sensitive substance, presumably mRNA.

The RNA we injected is a mixture of mRNA for many different proteins. Until the mRNA responsible for the expression of the Na-H exchanger is isolated, it will be impossible to determine whether the injected RNA caused the expression of a native but previously unexpressed Na-H exchanger, or whether the injected RNA directly encoded a Na-H exchanger. If the latter explanation is correct, this would be the first example of the expression of a mammalian ion exchanger or co-transporter in a foreign system. Our approach for expressing a Na-H exchanger could have important implications for studying the properties and regulation of Na-H exchange, and suggests an approach for cloning the gene(s) for the Na-H exchanger.

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Structural organization of the *bcr* gene and its role in the Ph′ translocation

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The Philadelphia (Ph′) chromosome, an abnormal chromosome 22 (ref. 1), is one of the best-known examples of a specific human chromosomal abnormality strongly associated with one form of human leukaemia, chronic myelocytic leukaemia (CML). The finding[2] that a small region of chromosome 9 which includes the c-*abl* oncogene is translocated to chromosome 22 prompted studies to elucidate the molecular mechanisms involved in this disease. We have demonstrated previously that the chromosome 9 of one patient with CML contains a breakpoint 14 kilobases (kb) 5′ of the most 5′ v-*abl*-homologous exon[3]. These data suggest a role for c-*abl* in CML, a theory supported by the presence of an abnormally sized *abl* messenger RNA[4,5] and protein[6] in the CML cell line K562. The region involved in the translocation on chromosome 22 has also been identified: all Ph′-positive patients examined to date have a breakpoint within a 5.8-kb region, for which we have proposed the name 'breakpoint cluster region' (*bcr*)[7]. To determine whether *bcr* contains protein-encoding regions, probes from *bcr* were tested for their ability to hybridize to complementary DNA sequences. A 0.6-kb *Hin*dIII/*Bam*HI *bcr* restriction enzyme fragment proved suitable for isolating several cDNA clones from a human fibroblast cDNA library[8]. Using *bcr* cDNA sequences, we obtained data strongly suggesting the presence of a chimaeric *bcr/abl* mRNA in the leukaemic cells of Ph′-positive CML patients. The recent isolation of cDNA clones containing *bcr* and *abl* sequences confirms this finding[12]. Because the *bcr* part of the chimaeric mRNA could be required to induce the transforming activity of the human c-*abl* oncogene, we have now initiated studies to characterize the normal '*bcr* gene' and to determine the effect of a translocation within its coding domain. We demonstrate that as a result of the Ph′ translocation, a variable number of *bcr* exons are included in the chimaeric *bcr/abl* mRNA. The *bcr* gene sequences in this mRNA could be responsible for the transition of the *abl* cellular proto-oncogene into an oncogene.

The largest cDNA, V1-3, containing an insert of 2.2 kb, was characterized in detail by restriction enzyme mapping (Fig. 1*a*) and sequence analysis (Fig. 1*b*). The cDNA contains one long open reading frame, starting at the poly(G) tail at the 5′ end and continuing to nucleotide 1,770, where a stop codon is encountered. All other reading frames have many stop codons within the entire region. The long open reading frame has the coding capacity for 589 amino-acid residues, corresponding to a protein of relative molecular mass (M_r) ~65,000; at the 3′ end, a polyadenylation signal occurs at nucleotide 2,182 followed by a poly(A) tail beginning at base 2,208 indicating that the cDNA contains the complete 3′ end of the gene. Although translational start sequences are encountered at the 5′ end, it is unlikely that this cDNA contains a complete copy of the mRNA, as Northern blot hybridizations indicate the presence of *bcr* mRNAs of ~4.0 and ~6.5 kb. Computer searches of newly isolated protein sequences derived directly from proteins or deduced from cDNA nucleotide sequences frequently result in the identification of proteins with partial homology. Such information is valuable, frequently allowing the assignment of a preliminary function to an unknown protein. Therefore, the PIR FASTP program[10] was used to search for *bcr*-homologous proteins; no proteins with significant homology were found, indicating that the *bcr* protein exhibits an as yet unidentified cellular function.

To determine the orientation of the *bcr* gene on chromosome 22, 5′ and 3′ probes were prepared from the V1-3 cDNA and hybridized to cosmids[7] containing human chromosome 22 sequences. This established that the 5′ end of the *bcr* gene is towards the centromere of chromosome 22 and is retained after the Ph′ translocation; the 3′ end of the *bcr* gene lies in the direction of the telomere and is translocated to chromosome 9 in the t(9; 22) translocation. The cDNA hybridizes to restriction enzyme fragments distributed over a region of up to 45 kb of chromosome 22 DNA (Fig. 2*a*). Within this region, a minimum of 13 exons are present. To determine the exact position and number of exons within the breakpoint cluster region, all hybridizing regions in *bcr* were sequenced and compared with the V1-3 cDNA. Four relatively small exons, designated 1-4, were present within *bcr*, varying in size from 76 to 105 base pairs (bp) (Fig. 2*b*); in the cDNA, these exons correspond to nucleotides 483-836 (Fig. 1*b*). As *bcr* was defined as the area on chromosome 22 in which the Ph′ breakpoints are found, we conclude that the breakpoints occur within a gene.

Having determined the position of the exons within *bcr*, we investigated whether the breakpoints occur in exon or intron regions. For CML DNA such as that of CML patient C481, this was readily determined. We had previously demonstrated[7] a breakpoint within a 1.2-kb H/Bg *bcr* fragment in several CML DNA samples, including that of patient C481. As no coding sequences are located within this region (see Fig. 2), patients such as C481 must have a chromosomal breakpoint in the intron between the exons designated 3 and 4.

A less simple situation was encountered in the DNA of patients 0311068 and 7701C. Nonetheless, cloning of $9q^+$ breakpoint fragments from these DNAs (data not shown) and restriction enzyme analysis followed by Southern hybridization enabled us to locate the breakpoints between exons 2 and 3 (see Fig. 2) The breakpoints in the previously cloned[3,7] $9q^+$ breakpoint fragments of patients 0319129 and 02120185 were analysed by DNA sequencing. In addition, we cloned the $22q^-$ breakpoint

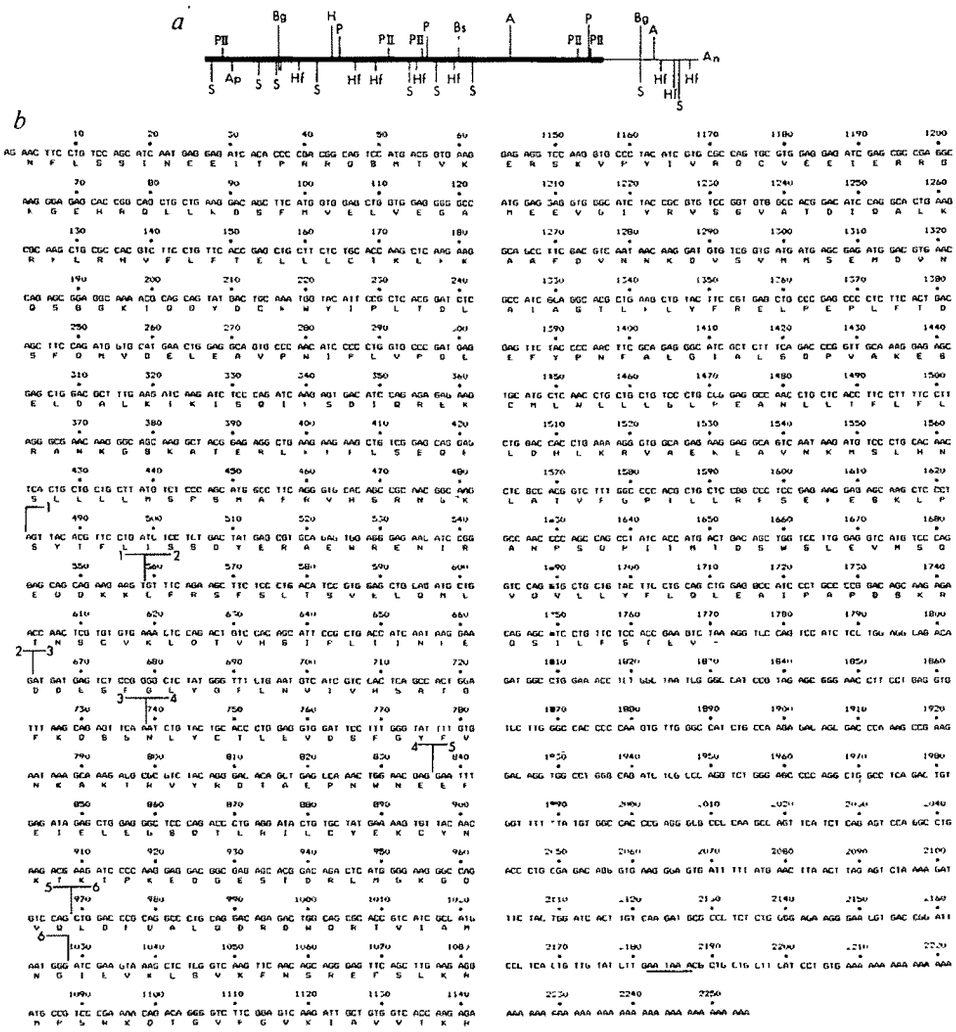


Fig. 1 *bcr* cDNA V1-3 *a*, Restriction enzyme map of V1-3 The black bar indicates the open reading frame sequences, the thin line non-translated sequences. The 3′ end of the cDNA containing the poly(A) tail is depicted as A_n. Restriction enzymes include A, *Ava*I; Ap, *Apa*I, B, *Bgl*II; Bs, *Bst*EII; H, *Hin*dIII, Hf, *Hin*fI; P, *Pst*I, PII, *Pvu*II, S, *Sau*3A. *b*, DNA sequence and translation of V1-3 sequence. Amino-acid residues are indicated with a one-letter symbol below the sequence The exact position of exons designated 1–6 (see Fig 2*a*) within V1-3 *bcr* cDNA is shown by the numbers above the sequence. DNA sequencing was performed using the dideoxy-chain termination method[14] on restriction enzyme fragments of the cDNA subcloned into M13 phage[15]. All regions were sequenced on both strands.

fragments of patient 0319129 (unpublished results) and of the cell line K562. DNA sequence analysis of the breakpoint regions of these fragments and that of the sequence of the corresponding chromosome 22 regions enabled us to define the point of translocation for these DNAs (see Fig. 2). None of the breakpoints analysed here could be located within an exon, indicating that in the Ph′ translocation, breakpoints occur within intron regions of *bcr*. For four of the six DNA samples analysed, the translocation would result in the transcription of a mRNA that includes *bcr* exons 1 and 2 (see Fig. 2), in two CML DNAs the third exon would be additionally incorporated in the chimaeric *bcr*/c-*abl* mRNA. Exon 3 encodes only 25 amino acids, not substantially increasing the size of the chimaeric protein.

A knowledge of the DNA sequence of the translocation junction point may provide additional information about the mechanism of chromosomal translocation. Figure 3*a* shows the sequence of the region containing the crossover point for translocation in the DNA of patient 0319129 and compares this with normal chromosome 22 and 9 DNA sequences. In 0319129 DNA, the chromosomal break has occurred in a rather 'precise' manner, leading to the generation of a $22q^-$ and $9q^+$ sequence exactly reflecting the sequence of the normal chromosome 22 and 9 DNA sequences. However, at the breakpoint, a C nucleotide is found in both the $9q^+$ and $22q^-$ sequences, whereas the chromosome 22 sequence contains a G at that position. Note that both chromosomes 9 and 22 contain limited stretches of homology near the break (Fig. 3*a* underlined); a DNA search revealed homology of this region to human *Alu* repetitive sequences.

No such similar stretches of homologous sequences between chromosomes 9 and 22 occur at the breakpoint in the DNA of patient 0212015 (Fig. 3*b*) or in that of K562 DNA (data not shown). In the $9q^+$ DNA of patient 0212015, sequences are found between the breakpoints on chromosomes 9 and 22 (Fig. 3*b*) which are not present in the sequenced region of the normal chromosomes 9 and 22. This suggests that in the $9q^+$ chromosome a secondary event has taken place; it is possible that after translocation of chromosome 22 sequences to chromosome 9, a deletion affecting chromosome 9 sequences occurred. The arrow indicating the breakpoint on chromosome 9 (see Fig. 3*b*) would then represent the point of deletion. In addition, compared with the normal chromosome 9 sequences, the chromosome 9 sequences of the $9q^+$ fragment contain 13 nucleotide changes within an 81-bp stretch. These changes may reflect differences between individuals (the control chromosome 9 sequences were isolated from a human lung carcinoma cosmid library); however, the number of nucleotide substitutions would be very high in such an event. Moreover, the chromosome 22 part of the $9q^+$ fragment contains no nucleotide changes in either intron or exon sequences, favouring the explanation of inefficient DNA repair on deletion of chromosome 9 sequences.

These results indicate that *bcr* is part of a gene oriented with its 5′ end towards the centromere of chromosome 22. In the Philadelphia translocation, a break occurs within *bcr*, and sequences from chromosome 9 which contain the human c-*abl* oncogene in all Ph′-positive cases examined[7,9] are translocated to the 3′ of the truncated *bcr* gene. The joining of *bcr* and c-*abl* sequences is highly specific for CML, as this configuration has been found in complex translocations[9] and even in the leukaemic cells of one CML patient cytogenetically lacking the Ph′ chromosome[11]. Because the orientation of c-*abl* on chromosome 9 is also centromere–5′–3′–telomere, *bcr* and c-*abl* are joined in a

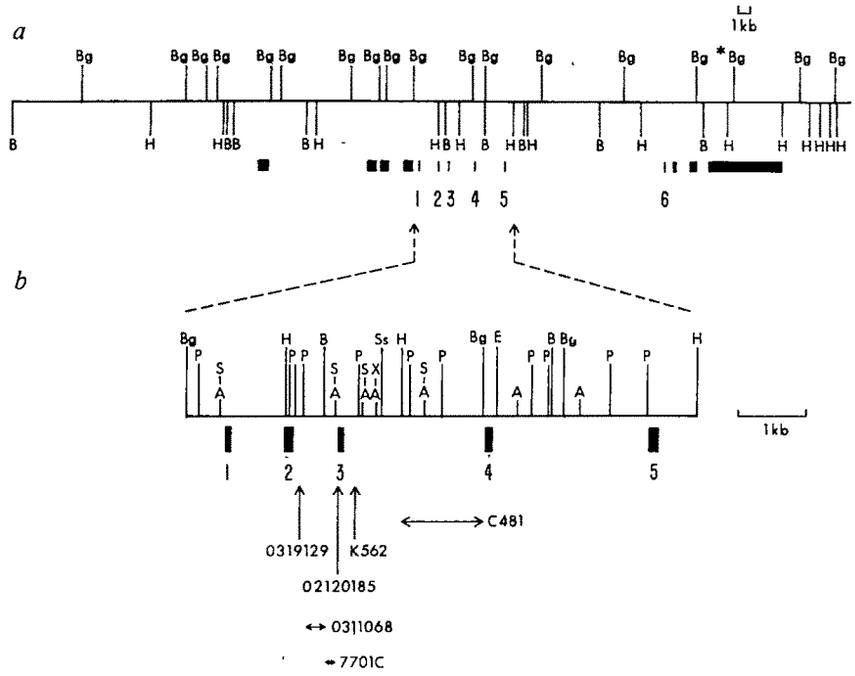


Fig. 2 Genomic organization of the *bcr* gene. *a*, Restriction enzyme map of chromosome 22 sequences encompassing *bcr*. Exons are indicated by black boxes below the restriction enzyme map. The position of the numbered exons has been determined by sequencing; all other exons were located by hybridization to the V1-3 cDNA. The asterisk indicates a polymorphic *Bgl*II restriction enzyme site. *b*, Restriction enzyme map of the breakpoint cluster region, with the exons as indicated in *a*. Below the map, the approximate positions of the breakpoints in different CML DNAs are indicated by horizontal or vertical arrows. Restriction enzymes include: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI, H, *Hin*dIII; P, *Pst*I; S, *Sma*I, Ss, *Sst*I, X, *Xho*I. The breakpoint of the 22q$^-$ chromosome from DNA 0319129 was cloned as a 9.5-kb *Bgl*II fragment in Charon 30 according to previously described methods[16]. 9q$^+$ fragments were isolated by cloning a 7.2-kb *Bam*HI fragment and a 7.7-kb *Eco*RI fragment into Charon 30 and λgtwes, respectively.

head-to-tail fashion on the Ph′ chromosome. Although the distance between the most 5′ v-*abl* homologous to v-*abl* and the physical breakpoint may vary from 14 kb (patient 0319129) to >100 kb (K562), the effect of the translocation on the expression of the *bcr* gene and c-*abl* seems to be very similar in different patients: in K562 and in Ph′-positive CML patients, abnormal RNA transcripts of ~8.5 kb are detected, which hybridize to both c-*abl* and 5′ *bcr* exon probes. The molecular cloning of a chimaeric cDNA from K562 cells has provided definitive proof for the existence of chimaeric mRNA[12]. The chimaeric mRNAs must be the result of transcription initiating at the promoter of the *bcr* gene; depending on the exact location of the breakpoint, the transcript will include all 5′ exons in addition to either exons 1 and 2 or exons 1, 2 and 3 of *bcr*. Recent sequence analysis of K562 *bcr*/*abl* cDNA confirms the variable presence of the third exon. In K562 we found that, as predicted, the third exon is present in the cDNA, immediately preceding c-*abl* sequences. In contrast, Southern blot analysis of the molecularly cloned 22q$^-$ genomic DNA fragment of, for example, patient 0319129 unambiguously demonstrates that in this patient exon 3 has been removed from the Ph′ chromosome and will not be included in a chimaeric *bcr*/*abl* transcript. Transcription continues into the c-*abl* oncogene, including, as a minimum, the most 5′ v-*abl*-homologous exon and all exons 3′ of it, including the phosphotyrosine acceptor site[13]. We do not know whether the inclusion of exon 3 in the chimaeric mRNA has an effect on the progression of the disease.

Chromosomal aberrations may be generated by specific events involving recombination-prone DNA sequences. Alternatively, such recombination events could occur almost at random. In either case, a very limited number of translocations will result in gene alterations leading to the disruption of normal growth and differentiation. In the Ph′ translocation, we have found that breakpoints on chromosome 9 are spread over a region of up to 100 kb. The breakpoints on chomosome 22 occur within a smaller region of around 50 kb. Nonetheless, no sequence homology can be found between breakpoint regions of different CML patients or coding regions of c-*abl* and *bcr* genes. Therefore, we may conclude that the processes underlying the Ph′ translocation are random recombination events. Once such recombinations result in a genomic configuration that allows the transcription of chimaeric *bcr*/*abl* mRNA, malignant proliferation of specific cell types may occur. It seems highly likely that this chimaeric mRNA is translated into protein because an



Fig. 3 Breakpoint sequences of the DNAs of two CML patients. *a*, Sequence of 0319129 DNA; the sequences are in a 5′–3′ orientation. Normal chromosome 9 sequences (first line) are from non-CML DNA, the 9q$^+$ and 22q$^-$ sequences (second and third lines) are from DNA of patient 0319129. Normal chromosome 22 sequences (fourth line) are from non-CML DNA An arrow indicates the breakpoint on chromosomes 9 and 22; the nucleotide C found in both the 9q$^+$ and 22q$^-$ sequences at the breakpoint is boxed Limited regions of homology between the normal chromosome 9 and 22 sequences are underlined *b*, Breakpoint sequence of 02120185 DNA; normal chromosome 9 and 22 sequences (first and third lines in each set) were from non-CML DNA The 9q$^+$ sequence on the second line contains an area boxed to indicate that it does not originate from the normal chromosomes 9 or 22 sequenced in the present experiments. Dots above the chromosome 9 sequences indicate nucleotide differences at those positions from the 9q$^+$ chromosome. The beginning of exon 3 (see Fig 2*b*) in the 9q$^+$ and 22 sequence is indicated in the figure Small restriction enzyme fragments containing the breakpoints were chosen for sequence analysis, based on restriction enzyme mapping data and comparison with normal chromosome 9 and 22 maps.

abnormally sized 210,000-M_r c-*abl* protein was detected in K562 cells. In contrast to the normal c-*abl* protein, the P210 has tyrosine kinase activity[6].

It is tempting to speculate that the *bcr* moiety of the fusion protein is responsible for this effect. However, although the consequences of the Ph' translocation on a molecular level are becoming evident, it remains to be established whether this phenomenon is actually the cause or merely one of the steps that eventually result in CML.

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Cloning, sequencing and expression of cDNA for a novel subunit of acetylcholine receptor from calf muscle

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The nicotinic acetylcholine receptor (AChR) from fish electric organ has a subunit structure of $\alpha_2\beta\gamma\delta$, and this is thought to be also the case for the mammalian skeletal muscle AChR[1–3]. By cloning and sequencing the complementary or genomic DNAs, we have previously elucidated the primary structures of all four subunits of the *Torpedo californica* electroplax[4–6] and calf muscle AChR[7–10] and of the α- and γ-subunits of the human muscle AChR[7,11]; the primary structures of the γ-subunit of the *T. californica* AChR[12] and the α-subunit of the *Torpedo marmorata* AChR[13,14] have also been deduced elsewhere. We have now cloned DNA complementary to the calf muscle messenger RNA encoding a novel polypeptide (the ε-subunit) whose deduced amino-acid sequence has features characteristic of the AChR subunits and which shows higher sequence homology with the γ-subunit than with the other subunits. cDNA expression studies indicate that the calf ε-subunit, as well as the calf γ-subunit, can replace the *Torpedo* γ-subunit to form the functional receptor in combination with the *Torpedo* α-, β- and δ-subunits.

The initial cDNA clone for the calf ε-subunit was isolated by cross-hybridization with a calf γ-subunit cDNA probe in an experiment aimed at cloning an upstream cDNA sequence for the calf γ-subunit by primer extension (see Fig. 1 legend). Figure 1 shows the nucleotide sequence of the mRNA encoding the calf ε-subunit precursor, deduced from the cDNA sequence, together with the predicted primary structure of the polypeptide. We deduce that the ε-subunit precursor consists of 491 amino acids including a hydrophobic prepeptide[15] of 20 amino acids, and calculate the relative molecular masses of the ε-subunit and its precursor to be 52,568 and 54,562, respectively.

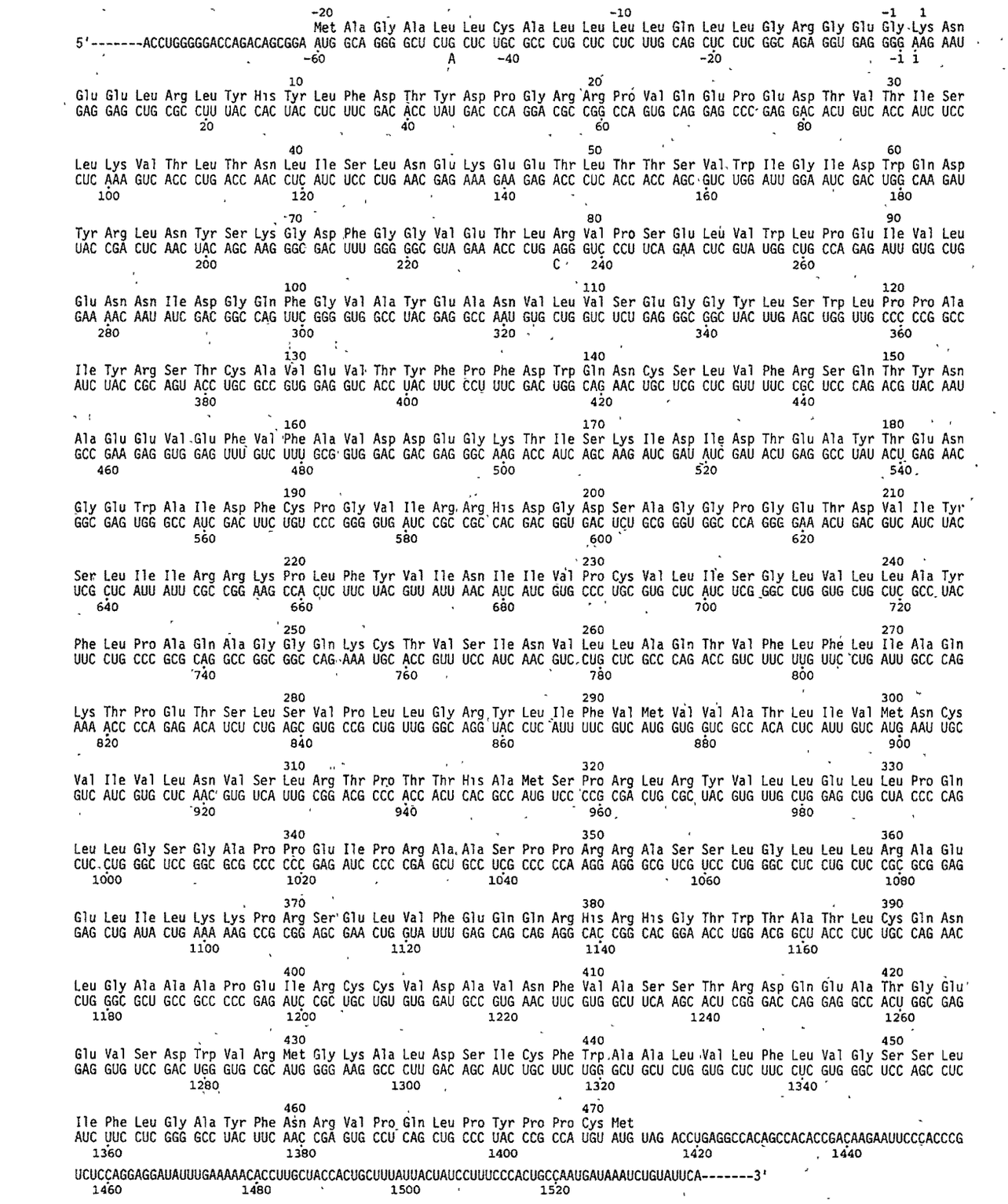
Figure 2 shows the alignment of the amino-acid sequences of the calf ε-subunit precursor and the calf, human and *T. californica* γ-subunit precursors. The mature calf ε-subunit shows 53, 53 and 57% sequence homology with the mature calf, human and *Torpedo* γ-subunits, respectively, whereas the degrees of its homology with other mature AChR subunits are as follows: calf, human and *Torpedo* α-subunit, 30% (refs 4, 7), calf β-subunit, 37% (ref. 8), *Torpedo* β-subunit, 39% (ref. 5), calf δ-subunit, 45% (ref. 10) and *Torpedo* δ-subunit, 44% (ref. 5). Homology among the mature γ-subunits is 92%, 56% and 55%, respectively, for the calf/man, calf/*Torpedo* and man/*Torpedo* pairs. Because the calf ε-subunit shows similar degrees of homology with the calf, human and *Torpedo* γ-subunits, the divergence of the ε- and γ-subunits may have occurred around the time of separation of mammals and fish. Thus, it is possible that fish have a second, undetected gene encoding a γ/ε-family subunit, which may or may not now be active.

The calf ε-subunit shares structural features common to all four subunits of the fish and mammalian AChR[4–12,14]. It contains four strongly hydrophobic segments[4–12,14] (M1–M4) and an amphipathic segment[16–18] (MA). Our expression studies using site-directed mutagenesis of the α-subunit cDNA indicate that these five putative α-helical transmembrane segments are all required for the operation of the ionic channel[18]. The hydrophilicity profile[19] and the predicted secondary structures[20] of the calf ε-subunit are also similar to those of the other AChR subunits[4–12,21]. These findings suggest that the ε-subunit is oriented across the membrane in the same manner as the four other subunits. Three cysteine residues (aligned at positions 128, 142 and 231) and one potential *N*-glycosylation site[22] (aligned at position 141) are conserved in all AChR subunits whose sequences are known[4–14]. The calf ε-subunit contains two additional potential *N*-glycosylation sites (aligned at positions 66 and 307), one of which (position 66) is assigned to the extracellular side of the membrane[4–12,14]. Because our expression studies are consistent with the presence of a disulphide bridge between the conserved cysteine residues 128 and 142 of the α-subunit[18], the corresponding cysteine residues of the ε-subunit may also form a disulphide bond.

To examine whether the ε-subunit can function as an AChR subunit, we synthesized the mRNA specific for this subunit (or the γ-subunit) *in vitro*[23,24] using the cDNA template and injected the mRNA, combined with the *Torpedo* α-, β- and δ-subunit-specific mRNAs synthesized similarly[18], into *Xenopus* oocytes. The oocytes were then tested for response to acetylcholine (ACh) applied ionophoretically by recording the intracellular potentials. All of the 61 oocytes injected with the calf ε-subunit and the three *Torpedo* mRNAs and 38 of the 56 oocytes injected with the calf γ-subunit and the three *Torpedo* mRNAs responded to ACh (Fig. 3) with mean ACh sensitivities (for the responsive oocytes) of 510 and 8 mV μC^{-1}, respectively. None of 60 control oocytes injected only with the *Torpedo* α-, β- and δ-subunit-specific mRNAs was responsive ($<\sim 0.2$ mV μC^{-1}). The mean ACh sensitivity of 70 responsive oocytes out of 72 oocytes injected with the *Torpedo* α-, β-, γ- and δ-subunit-specific mRNAs[18] was 1,300 mV μC^{-1}. The atropine-resistant and (+)tubocurarine-sensitive nature of the observed ACh responses (Fig. 3) indicates the formation of the nicotinic AChR. These results show that the calf ε-subunit, as well as the calf γ-subunit, can be functionally substituted for the *Torpedo* γ-subunit.

Blot hybridization analysis using ε-subunit cDNA probe of poly(A)$^+$ RNA from the skeletal muscle of a 4-month-old fetal calf revealed a hybridization-positive species with an estimated size of ~4,200 nucleotides (Fig. 4*A*, lane *a*). No hybridizable species was detected, however, in poly(A)$^+$ RNA from the skeletal muscle of a 7-month-old fetal calf (Fig. 4*A*, lane *b*), a 9-month-old fetal calf or a newborn calf or from adult bovine diaphragm (data not shown).

Figure 1



The AChR is present predominantly at the neuromuscular junction in adult muscle, whereas in embryonic muscle and denervated adult muscle the AChR also occurs in the extrajunctional membrane[1,2]. Some reports suggest that the two types of AChR are structurally different, the junctional AChR being slightly more acidic than the extrajunctional AChR[25]. Our amino-acid sequence data reveal that the calf ε-subunit contains more acidic residues and less basic residues than does the calf γ-subunit[9]; the numbers of charged residues contained in the mature ε- and γ-subunits are 20/18 Asp, 33/28 Glu, 26/31 Arg, 12/14 Lys and 5/11 His, respectively. However, the content of both the γ- and ε-subunit mRNAs in fetal calf muscle, like that of the β-subunit mRNA[8], decreases with increasing age of the calf (Fig. 4), although the relative contents of the ε- and γ-subunit mRNAs in other age groups were not studied systematically. Muscle contains much smaller amounts of ε-subunit mRNA than of γ-subunit mRNA (Fig. 4), and the latter is present in similar amounts to the α-, β- and δ-subunit mRNAs[7–10]. It thus remains uncertain whether the differential presence of the γ- and ε-subunits is responsible for the difference in the junctional and extrajunctional forms of the AChR. Alternatively, the ε-subunit (or the γ-subunit) may be a

Fig. 1 (opposite) Primary structure of the mRNA encoding the ε-subunit precursor of the calf muscle AChR. The nucleotide sequence of the mRNA was deduced by sequence analysis[28] of the cDNA inserts in clones pcACRε64, pcACRε1 and pcACRε2. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the codon specifying the amino-terminal residue of the mature ε-subunit, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. Because the 3′-untranslated sequence presented contains no polyadenylation signal[29] and is not followed by a poly(A) tract, this sequence is incomplete, as is the 5′-untranslated sequence shown. The amino-acid sequence, deduced by using the reading frame of the mRNA corresponding to the known amino-acid sequences of the AChR subunit precursors[4–14], is shown above the nucleotide sequence. Amino-acid residues are numbered beginning with the amino-terminal residue of the mature ε-subunit, and the preceding residues are indicated by negative numbers. The possibility that the initiating methionine is located upstream of the 5′ end of the cDNA sequence shown cannot be excluded. The nucleotide differences observed between the individual clones are as follows: G (pcACRε64) or A (pcACRε2) at position −46; A (pcACRε64) or C (pcACRε1) at position 235; the G at position 279 and the C at position 285 of pcACRε1 (instead of A and T, respectively, in pcACRε64), derived apparently from the synthetic primer, are not shown. **Methods.** Total RNA was extracted from newborn or fetal calf skeletal muscle, and poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography. The oligodeoxyribonucleotide 5′-GTTGTTCTCCAGCAC-3′, complementary to residues 271–285 of the calf γ-subunit mRNA[9], was synthesized by the triester method and used for specific priming of reverse transcription. Single-stranded cDNA was synthesized using 5 μg of this primer and 100 μg of newborn calf muscle poly(A)$^+$ RNA as template and was converted to double-stranded cDNA, which was cloned in pBR322 plasmid by inserting it into the *Pst*I site using poly(dG)·poly(dC) homopolymeric extensions. *Escherichia coli* χ1776 or HB101 was used for transformation, and $\sim 3 \times 10^5$ tetracycline-resistant transformants were screened by hybridization at 60 °C with the *Hin*cII(88)/*Ava*II(202) fragment excised from clone pcACRγ5 (ref. 9) to yield one positive clone (pcACRε1 carrying nucleotides 84–285); the restriction endonuclease sites are identified by numbers indicating the 5′-terminal nucleotide generated by cleavage; all hybridization probes were labelled by nick-translation with [α-^{32}P]dCTP. Another batch of cDNA clones prepared similarly ($\sim 1.5 \times 10^5$ transformants) was then screened by hybridization at 60 °C with the *Mbo*II(152)/*Fnu*4HI(260) fragment from clone pcACRε1 to give one positive clone (pcACRε2 carrying nucleotides −46 to 203). The procedures used for the above experiments have been described previously[30]. A cDNA library was next constructed by the procedure of Gubler and Hoffman[31], using 10 μg of oligo(dT)$_{12-18}$ primer and 13 μg of a fraction of fetal (4-month-old) calf muscle poly(A)$^+$ RNA (>~4,000 nucleotides; see Fig. 4) obtained by centrifugation in a gradient of 5–25% (w/v) sucrose[32]. Screening of $\sim 8 \times 10^5$ tetracycline-resistant transformants by hybridization at 60 °C with the *Hin*fI(−46)/*Hin*fI(171) fragment from clone pcACRε2 yielded one positive clone (pcACRε64 carrying nucleotides −82 to 1,539); the *Hin*fI(−46) site was generated in pcACRε2 at the junction of the 5′ end of the cDNA insert and the poly(dG)·poly(dC) tail.

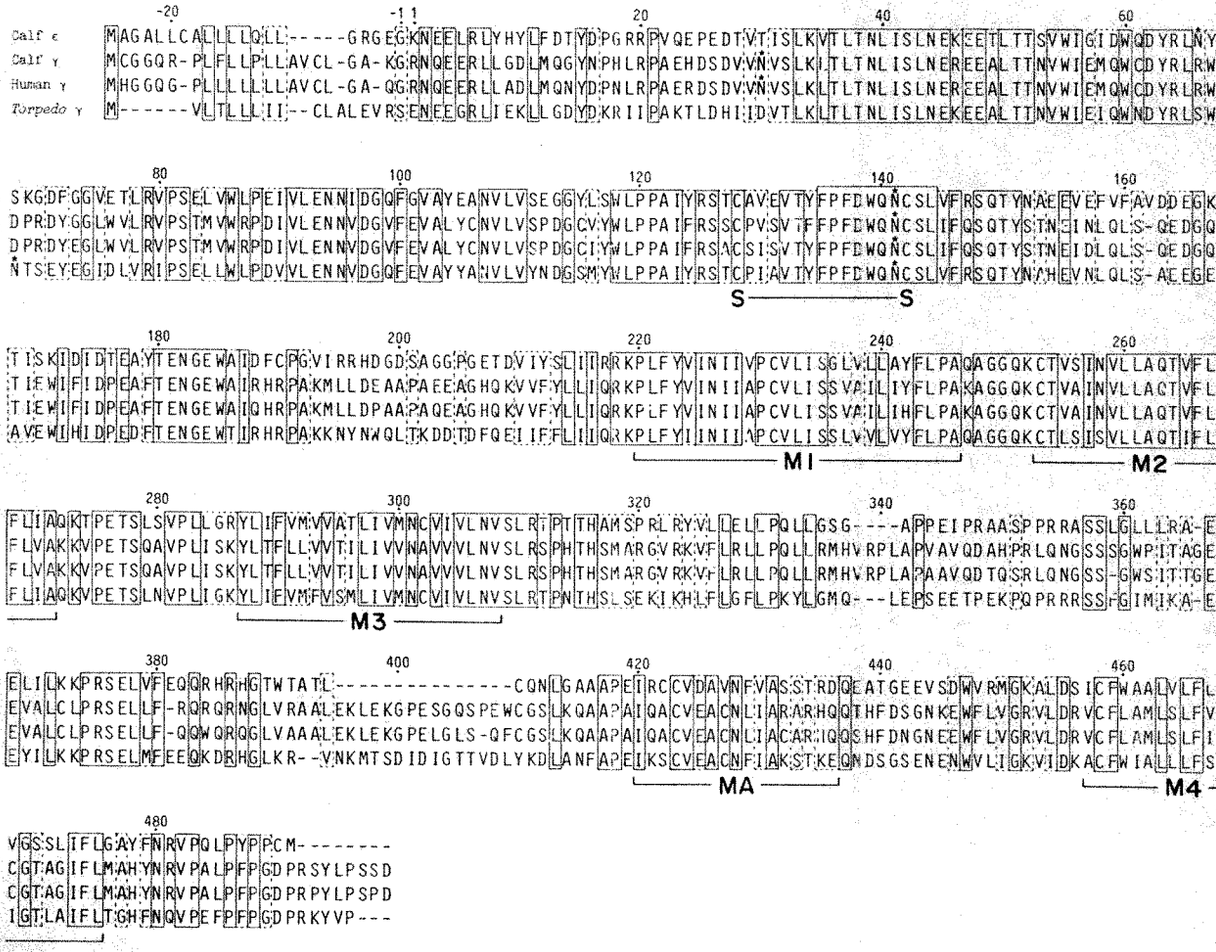


Fig. 2 Alignment of the amino-acid sequences of the calf AChR ε-subunit precursor (top) and the calf (second row), human (third row) and *T. californica* AChRγ-subunit precursors (bottom). The one-letter amino-acid notation is used. The sequence data for the calf, human and *T. californica* γ-subunit precursors have been taken from refs 9, 11 and 6, respectively. Sets of four identical residues at one aligned position are enclosed by solid lines, and sets of four identical or conservative residues at one aligned position by dashed lines. Conservative amino-acid substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W. Gaps (-) have been inserted to achieve maximum homology. The degrees of homology given in the text were calculated on the basis of the alignment of the amino-acid sequences of the 11 AChR subunits (calf, human and *Torpedo* α, calf and *Torpedo* β, calf, human and *Torpedo* γ, calf and *Torpedo* δ and calf ε), gaps being counted as one substitution regardless of their length. The positions in the aligned sequences including gaps are numbered beginning with the amino-terminal residue of the mature subunits, and the preceding positions are indicated by negative numbers. The asparagine residues as potential glycosylation sites are marked with asterisks; only those preceding segment M1 are shown. The putative disulphide bridge (S—S) and the putative transmembrane segments (M1, M2, M3, M4 and MA) are indicated; segment MA has been assigned by comparison with that of the *Torpedo* α-subunit defined by deletion mapping[18].

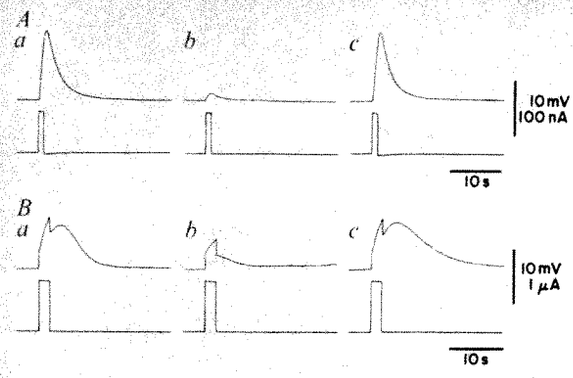


Fig. 3 ACh potentials recorded from *Xenopus* oocytes injected with mRNA specific for the calf AChR ε-subunit (*A*) or γ-subunit (*B*) together with the *T. californica* AChR α-, β- and δ-subunit-specific mRNAs. The recordings were made at room temperature in Ringer's solution containing 1 μM atropine and 5 mM $MgCl_2$ instead of $CaCl_2$: upper traces, ACh potentials; lower traces, currents used for ionophoresis of ACh (backing current, 12 nA). The membrane potential was held at −70 mV. Responses to ACh were recorded before (*a*) and 6–8 min after perfusion with 2 μM (+)tubocurarine (*b*) and 8–9 min after washing out the antagonist (*c*).

Methods. The 320-base-pair (bp) fragment extending from the *Ava*II site generated at the 5′ end of the cDNA insert to the *Ava*II(238) site and the *Ava*II(238)/*Eco*RI (1,444) fragment, derived from clone pcACRε64, and the ~520-bp fragment of clone pACRγ14 (ref. 6) extending from the *Eco*RI(1,474) site to the *Pvu*II site on the vector DNA were ligated. The resulting ~2.0-kilobase-pair (kb) fragment was isolated, treated with *E. coli* DNA polymerase I (Klenow fragment) in the presence of dGTP, dCTP and dTTP and inserted into the *Sma*I site of the plasmid pSP64 (ref. 24) in the same orientation as the SP6 promoter to yield the plasmid pSPcε. The *Pst*I(−100)/*Xho*I(207) fragment from clone pcACRγ11 (ref. 9), the *Xho*I(207)/*Hin*dIII(1,181) fragment, the *Hin*dIII(1,181)/ *Acc*I(1,560) fragment and the ~180-bp fragment extending from the *Acc*I(1,560) site to the *Pvu*II site on the vector DNA, derived from clone pcACRγ5 (ref. 9), and the ~3.0-kb *Sma*I/*Pst*I fragment from pSP64 were ligated. The resulting plasmid pSPcγ, carrying the entire calf γ-subunit cDNA (except for a portion of the 5′-noncoding region) in the same orientation as the SP6 promoter, was identified by colony hybridization with both the above *Xho*I/*Hin*dIII and *Hin*dIII/*Acc*I fragments as probes and by restriction endonuclease analysis. The calf AChR ε- and γ-subunit-specific mRNAs were synthesized *in vitro*, using *Sac*I-cleaved pSPcε and *Eco*RI-cleaved pSPcγ, respectively, as templates and were then capped *in vitro* with vaccinia virus mRNA guanylyl transferase[23]. Each of these mRNAs, combined with the *T. californica* AChR α-, β- and δ-subunit-specific mRNAs[18] (molar ratio of the α-, β-, γ/ε- *and* δ-subunit-specific mRNAs, 2:1:2:1; total mRNA concentration, 120–340 ng μl^{-1}), was injected into *Xenopus laevis* oocytes (average volume injected per oocyte, ~20 nl). The oocytes were incubated for 3 days in conditions described previously[33] before being tested for response to ACh.

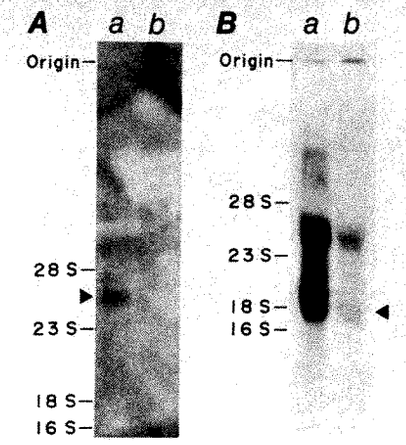


Fig. 4 Autoradiogram of blot hybridization analysis of calf muscle RNA with an AChR ε-subunit (*A*) or γ-subunit (*B*) cDNA probe. Samples of skeletal muscle poly(A)$^+$ RNA (40 μg each in *A* or 30 μg each in *B*) from a 4-month-old (lane *a*) or a 7-month-old (lane *b*) fetal calf were denatured with 1 M glyoxal and 50% dimethyl sulphoxide, electrophoresed on 1.5% agarose gels and transferred to a nitrocellulose filter[34] (*A*) or diazophenylthioether paper[35] (*B*). The hybridization probe used was the 1,535-bp fragment of clone pcACRε64 extending from the *Pst*I site flanking the 5′ end of the cDNA insert to the *Eco*RI(1,444) site (*A*) or an equimolar mixture of the 1,004-bp fragment extending from the *Pst*I site flanking the 5′ end of the cDNA insert to the *Acc*I(1,054) site and the *Acc*I(1,054)/*Acc*I(1,560) fragment, derived from clone pcACRγ5 (ref. 9) (*B*); the two probes, labelled by nick-translation with [α-^{32}P] dCTP, had nearly equal specific radioactivities (~4 × 10^8 c.p.m. per μg), but the photograph in *A* is more exposed than that in *B*, to intensify the hybridization signal. The size markers used were calf and *E. coli* ribosomal RNA. The positions of the ε-subunit mRNA (~4,200 nucleotides) (*A*) and the γ-subunit mRNA (~2,000 nucleotides[9]) (*B*) are indicated by arrowheads; the larger RNA species (~3,700 nucleotides) hybridizable with the γ-subunit cDNA probe probably represents incompletely spliced RNA as described previously[9].

component of a third type of AChR which appears at a particular stage of muscle development[26]. It is also possible that the ε-subunit (or the γ-subunit) is a constituent of the nicotinic AChR present in neuronal synapses[27].

Conti-Tronconi *et al.*[3] have determined the sequences of the amino-terminal 23–35 amino-acid residues of the four polypeptides derived from fetal calf muscle AChR. Three of these sequences agree reasonably well with the amino-terminal sequences of the mature α- (ref. 7), β- (ref. 8) and δ-subunits[10] deduced by us from the cDNA sequences, but the fourth sequence (designated x) matches neither the γ-subunit[9] nor the ε-subunit. This may imply the possible existence of still another subunit of the AChR.

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Expression of genes of the T-cell antigen receptor complex in precursor thymocytes

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The antigen receptor on T lymphocytes has recently been characterized as a heterodimeric, transmembrane glycoprotein consisting of disulphide-linked α (acidic) and β (basic) subunits of relative molecular mass (M_r) 40,000–45,000 each. The genes encoding these proteins have been cloned[1–4] and shown to resemble immunoglobulin genes in both overall structure and the requirement for DNA rearrangement before expression[5–11]. In humans, three additional proteins, termed the T3 complex, are found associated with the clonotypic receptor, and a role for T3 in receptor expression has been proposed[12–15]. Despite these recent advances in characterizing the antigen receptor complex, there is as yet little understanding of T-cell maturation, particularly the stage of T-cell ontogeny at which the genes encoding the antigen receptor and its associated structures are expressed and assembled. In the adult, stem cells destined to differentiate into T cells arise in the bone marrow and migrate to the thymus[16], where T-cell precursors proliferate, develop a preference for recognizing antigens in the context of self MHC molecules[17,18] and are released to the periphery. Recently, cells that have the properties of immature murine thymocytes have been isolated and described[19,20]. We have now analysed these cells with a series of molecular probes and we describe three distinct patterns of T-cell antigen receptor gene rearrangements in developing thymocytes.

Two-colour flow cytometric analysis of the Lyt2 and L3T4 T-cell-surface antigens on adult murine thymocytes and analysis of T8 and T4 markers on human thymocytes using monoclonal antibody plus complement-mediated lysis reveal four major subpopulations[19–22] (Fig. 1). In the mouse, cells bearing both Lyt2 and L3T4 make up most thymocytes (80–85%) and correspond to cells found predominantly in the cortex. Immunocompetent, mature thymocytes have one of two phenotypes, $Lyt2^+$, $L3T4^-$ or $Lyt2^-$, $L3T4^+$, which make up 3–5% and 8–15% of murine thymocytes, respectively[20,23]. The fourth subpopulation is negative for both Lyt2 and L3T4 markers and represents only 3–4% of thymocytes. Some of these cells bear low levels of the Ly1 surface antigen, a fact important for their initial isolation[20]. Thus their phenotype is $Ly2^-$, $L3T4^-$, $Ly1^{dull}$ and they have been called dull Ly1 (dLy1) cells. They are large, blast-like cells and may correspond to the blast cells with a similar phenotype that are found early in the developing fetal thymus[23]. dLy1 cells isolated with a purity of 97% have several properties indicating that they are T-cell precursors[19,20]. When cultured *in vitro*, 25–30% differentiate within 20 h to cells with the $Lyt2^+$, $L3T4^+$ phenotype. This experiment extends the observation of Ceredig *et al.* showing that $Lyt2^-$ precursors differentiate *in vitro* to $Lyt2^+$ thymocytes[24]. More importantly, adoptive transfer experiments indicate that these isolated cells re-enter and transiently repopulate the thymus of congenic irradiated recipient mice. Donor-derived cells with both the $Lyt2^-$, $L3T4^-$ and cortical ($Lyt2^+$, $L3T4^+$) phenotypes appear first in the thymus. Within 10–20 days after transfer, donor-derived cells have differentiated into thymocytes with the phenotypes of immunocompetent T cells ($Lyt2^+$, $L3T4^-$ and $Lyt2^-$, $L3T4^+$). However, cells in the dLy1 population have certain properties which distinguish them from

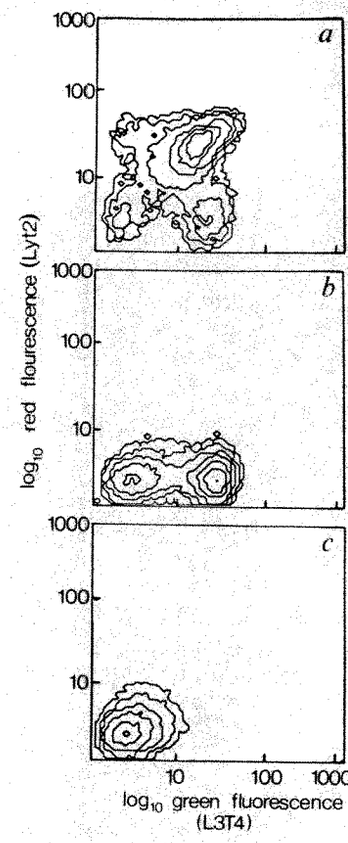


Fig. 1 Two-colour flow cytometric analysis of Lyt2 and L3T4 on B6 thymocytes during the isolation of the precursor thymocyte population. Thymocyte suspensions from C57BL/6 mice (6–8 weeks old) were prepared as described elsewhere[20]. Cells were incubated for 30 min at 4 °C with a saturating concentration of antibody, then washed twice in Hank's balanced salt solution with 0.1% bovine albumin and 0.1% sodium azide and resuspended for the next incubation. The sequence of reagent incubation for panel *a* was: (1) anti-L3T4 culture supernatant[25], (2) fluorescein isothiocyanate-labelled goat anti-rat immunoglobulin, (3) normal rat serum, (4) biotin-labelled rat anti-Lyt2[26], (5) Texas-red avidin. Flow cytometry was performed on a fluorescence activated cell sorter[19]. *a*, Unfractionated thymocytes. *b*, $Lyt2^-$ thymocytes, isolated as previously described[19]. Briefly, $20–30 \times 10^8$ thymocytes were incubated with anti-Lyt2.2 ascites fluid and rabbit complement. Viable cells were obtained by purification on Ficoll–Paque (Pharmacia). Cells were washed three times. *c*, Isolated $Lyt2^-$, $L3T4^-$, $Ly1^{dull}$ (dLy1) cells. $Lyt2^-$ cells were treated with anti-Ly1.2 ascites, purified anti-L3T4 antibody and complement, and again purified by centrifugation over Ficoll.

bone marrow-derived stem cells that can also repopulate the thymus, including a limited capacity for self-renewal, and the ability to give only a single wave of differentiation that generates the other three phenotypes in the thymus. Although studies on the functional properties of these dLy1 cells and their progeny remain to be performed, these data suggest that the dLy1 cells represent a population of precursor thymocytes at an intermediate developmental stage between marrow-derived stem cells and immunocompetent T cells.

The expression of RNA encoding T-cell antigen receptor proteins was assayed in the dLy1 precursor population to determine whether these early thymocytes were transcribing receptor genes. Total cytoplasmic RNA was analysed by Northern blotting with α-(TT11)[2] and β-(86T5) chain[26] cDNA probes (Fig. 2). These experiments reveal that the dLy1 cells as well as unfractionated thymocytes contain the 1.3- and 1.0-kilobase (kb) β-gene transcripts shown to represent *V-D-J* and *D-J* rearrangements respectively [9,10]. In this experiment the precursor (dLy1) cells contained β-chain-specific transcripts in a concentration approximately equal to the unfractionated cell. This indicates that within the highly purified dLy1 population, at

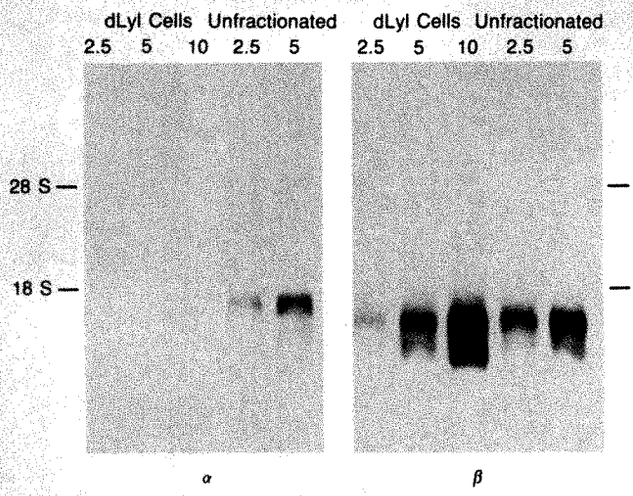


Fig. 2 Northern blot analysis of thymocyte and dLyl RNA to reveal α- and β-chain gene expression. Cells ($0.9–2.5 \times 10^8$) were homogenized in 4 M guanidinium thiocyanate and RNA was purified by centrifugation through CsCl[29]. The RNA was denatured in 2.2 M formaldehyde, and the indicated amount of RNA (μg) electrophoresed in a 1% agarose gel with 0.2 M formaldehyde, then transferred to nitrocellulose. The probe used to detect α-chain gene expression was the *Rsa*I to *Ava*II fragment comprising the constant region of the TT11 cDNA clone[2]. The 86T5 cDNA clone was used to detect β-chain gene expression[27]. Probes were labelled by nick-translation. Hybridization was in 5 × SSPE, 1 × Denhardt's solution, 10% dextran sulphate and 50% formamide at 42 °C. The blots were washed twice in 2 × SSPE, 0.1% SDS at 55° C and once in 0.2 × SSPE at 55 °C.

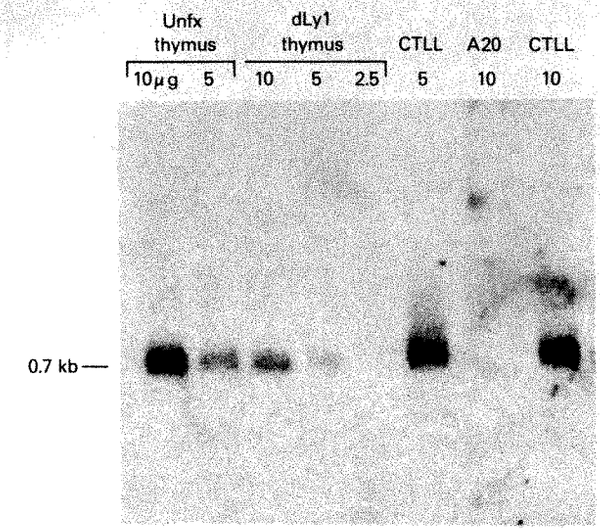


Fig. 3 Northern blot analysis with the T3 δ-chain probe. The RNA and blots were prepared as described in Fig. 2. The blots were prehybridized at 42 °C for 2 h in 50% deionized formamide, 5 × SSC, 5 × Denhardt's solution, 50 mM sodium phosphate, *p*H 6.5, 0.1% SDS with 250 mg ml^{-1} denatured salmon-sperm DNA. Hybridization was carried out in the same buffer for 16 h at 42 °C. The mouse T3 δ-chain cDNA insert of clone PEM-T3δ[28] was nick-translated and used as a hybridization probe. Following hybridization, blots were washed three times in 2 × SSC, 0.1% SDS at 25° C and twice in 0.1 × SSC, 0.1% SDS at 50° C.

least some cells transcribe RNA for the β-chain gene product. When the same blot was probed with the α-chain cDNA clone TT11, however, essentially no hybridizing RNA was observed in the dLyl population. Since the dLyl cells is an early thymocyte, these data suggest that α-chain RNA expression follows β-chain gene expression in intrathymic T-cell maturation.

The T-cell antigen receptor in mature T cells is associated with three molecules comprising the T3 complex. A cDNA insert of the clone PEM-T3δ, which encodes the murine T3 M_r 20,000 glycoprotein δ-chain[28], was used to probe the dLyl thymocyte population (Fig. 3). The probe hybridized to RNA from the purified dLyl population as well as to RNA from unfractionated thymus and the mature T-cell line CTLL, but not with A20 B-cell RNA. As RNA for this product is expressed in the dLyl population, this gene product is not likely to be limiting in the assembly of the T-cell antigen/receptor complex. Expression of RNA for the β-chain of the antigen receptor and the T3 δ-chain are thus both early events in T-cell maturation.

To analyse antigen receptor genes of early T cells at a clonal level, we fused purified precursor thymocytes with the drug-marked thymoma BW5147, reasoning that these large, proliferating immature cells would fuse readily with the thymoma line. They would thus behave like pre-B cells derived from fetal liver, which fuse efficiently to plasma cell lines[30]. Indeed, we observed a high frequency of fusions in such an experiment (1 in $5–10 \times 10^4$). Twelve of these lines were tested for the presence of the Thy 1.2 allele, a T-cell marker donated by the B6 dLyl cells and not by the AKR-derived Thy 1.1 BW5147 cells. Of these 12, 9 expressed the expected marker from the input cells, implying, though not proving, that they derived from fusions involving cells of the T lineage[31]. These cells were also stained with antibodies binding Lyt2 and L3T4 and were all negative for these markers, consistent with a dLyl ($Lyt2^-$, $L3T4^-$) origin.

DNA was isolated from these 12 lines and subjected to Southern blot analysis (Fig. 4*a*, *b*). Germline B6 mouse DNA digested with the restriction enzyme *Pvu*II gave three tightly migrating, though not always well-resolved, bands of 6.4, 6.2 and 6.0 kb hybridizing with the 86T5 β-chain probe. DNA from the BW5147 thymoma cell showed two non-germline bands at 6.5 and 5.8 kb, resulting from two separate rearrangements to the $C_\beta 2$ locus (R. Lechler and R.N.G., N. E. Lee and M.M.D., unpublished results). The hybrids have a combination of BW5147-derived bands, germline bands, and additional bands presumably representing rearrangements in the precursor population. Eight hybrids possess such additional rearrangements in their DNA, whereas four Thy 1.2^+ hybrids (1G8, 1G10, 2C9 and 2E5) contain non-BW5147 hybridizing bands of only germline pattern. To confirm the finding that these four hybrids had only germline bands of B6 origin, further analysis of these DNA preparations after digestion with the restriction enzymes *Hpa*I (Fig. 4*b*) and *Hin*dIII (data not shown) was performed. The additional experiments indicated that these four hybrids had all the expected germline and BW5147-derived restriction fragments without any additional bands. Thus an analysis with three restriction enzymes revealed that, while many hybridomas showed β-locus rearrangements consistent with the observed transcription of β-chain genes in the dLyl population, a substantial fraction of the hybrids retained nonrearranged β-chain genes.

The pattern of α- and β-chain gene expression in the dLyl and total thymocyte populations, together with these data on β-locus rearrangements in dLyl-derived T hybridomas, suggest three distinct patterns of T-cell antigen receptor gene rearrangement in developing thymocytes. Two of these subpopulations exist within the dLyl precursor population. The first, characterized on the basis of four hybrids, has germline β-chain and, because α transcripts are absent in dLyl cells, apparently unrearranged α-chain gene loci. The analysis of genomic DNA from eight additional hybrids defines a second subpopulation with rearranged β-chain, and by the same reasoning, germline α-chain genes. One can infer the existence of a third subpopulation, not present among dLyl cells, in which both gene loci are rearranged, as thymocytes bearing a mature α/β heterodimer[33] and an intact T3 complex[34] have been demonstrated with monoclonal antibodies. Although the T3 δ-chain gene is expressed in the dLyl subpopulation, no information yet exists to place expression of the other T3 components in this scheme.

The characterization of the first two of these subpopulations depends on certain assumptions about the fusion of dLyl cells

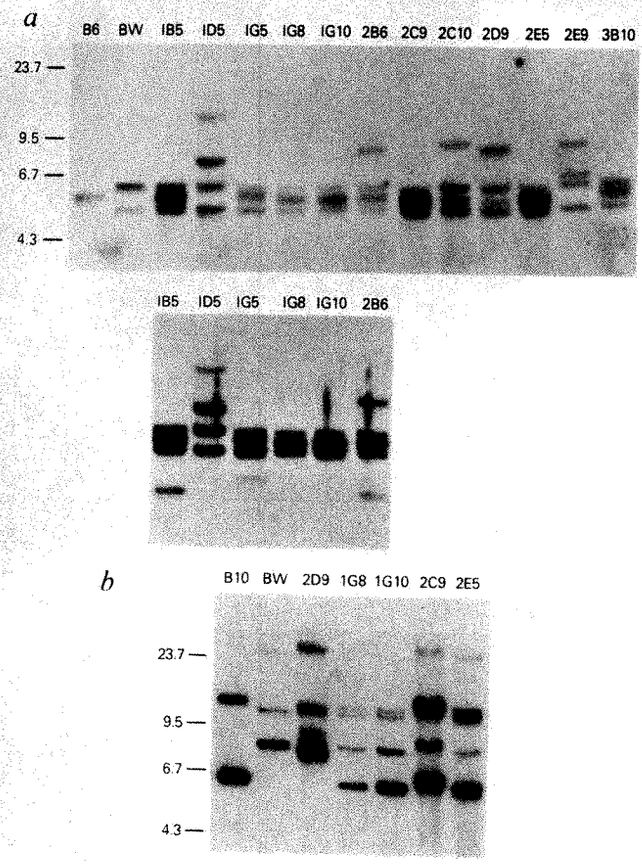


Fig. 4 Southern blot analysis of genomic DNA from dLyl hybridomas. dLyl cells were isolated as described in Fig. 1 and fused to the drug marked thymoma BW5147 with polyethylene glycol. Hybridomas were selected in hypoxanthine/aminopterin/thymidine medium. DNA was prepared from the cells by standard methods[32], digested with the restriction enzyme *Pvu*II (*a*) or *Hpa*I (*b*), electrophoresed through 0.7% agarose and blotted onto nitrocellulose. The 86T5 β-chain probe was used for hybridization and the blots were washed three times in 2 × SSC, 0.1% SDS at 25° C and three times in 0.2 × SSC, 0.1% SDS at 60° C. The inset represents a longer exposure of the blot in panel *a*. Germline DNA from liver is included in the first lane.

with the BW5147 thymoma line. First, that contaminating cells of other (non-dLy1) phentoypes did not fuse to the BW5147 thymoma. An analysis of those non-dLy1 cells showed that most had a mature phenotype and thus were likely to contain predominantly small resting cells that might not fuse without stimulation (Yeh *et al.*[35] and L.E.S., unpublished data). Furthermore, the 12 hybrids randomly chosen for DNA analysis bore the expected Lyt2$^-$, L3T4$^-$ phenotype of the dLy1 cell. Second, because the dLy1 population seems heterogeneous for a series of other cell-surface markers (B.J.F., unpublished data), it is possible that there was preferential fusion involving a subpopulation of dLy1 cells such as those with germline β-chain loci. The proportion of hybridomas that have germline β-chain genes may not accurately reflect the true fraction of dLy1 cells with this phenotype. We assume, however, that these cells are in the T-cell lineage because all bear the Thy 1.2 surface marker.

Because the relationships among the various thymocyte subpopulations remain disputed[16], it is not possible to make direct correlations between the various patterns of α- and β-chain gene expression described here, and a single, linear pathway of T-cell development. Nevertheless, the three distinct T-cell rearrangement patterns can be related to stages of the better-defined B-cell differentiation process[36]. In the B lineage, the heavy-chain genes first undergo rearrangement, followed by the appearance of intracellular μ proteins in the pre-B cell. Only after subsequent light-chain gene rearrangement and expression has occurred does the complete immunoglobulin molecule appear on the surface of the mature B cell. In the T lineage, at some point after commitment, cells with non-rearranged α- and β-chain genes can be found. The four hybrids with germline β-chain gene loci may be examples of such cells, although it remains to be shown that these cells have germline α-chain genes and are not cells of a lineage that do not use the β-chain gene locus (for example, suppressor T cells[37]). By analogy to B-cell development[38], we propose the name 'pro-T' cells for this committed precursor subpopulation. Cells of the second subpopulation are analogous to the pre-B cell. These pre-T cells have rearranged one of their receptor genes, β, and express RNAs corresponding to both *D–J* (1.0 kb) and *V–D–J* (1.3 kb) rearrangements. Although we have no evidence for *D–J* rearrangement preceding *V–D–J* as the 1.0- and 1.3-kb mRNAs are present in equal amounts, such a progression may occur[39] as in B cells[40,41]. At some later stage, the α-chain genes are rearranged and transcribed, giving rise to the complete receptor on the mature T cell. Such a sequence of rearrangements also seems to occur in the fetal thymus[42]. In the experiments of Raulet *et al.*, however, a preponderance of 1.0-kb β-chain RNA is seen in the fetal thymus, perhaps because during ontogeny the developmental steps are better synchronized. The complexity of events within this maturational scheme may account for the large number of β gene clones corresponding to *D–J* type rearrangements that were isolated from a thymocyte library[9]. A *V*-containing β-chain gene product may be needed before α-chain gene rearrangement, accounting for the relatively low level of α-chain transcripts in the thymus[2]. Although cells with characteristics of pro-T and pre-T stages can be found in the immature dLy1 population of thymocytes, and the thymus contains some T cells with mature phenotype and antigen receptors, further experiments are necessary to determine if differentiation through these stages takes place entirely or solely within the thymus. Repopulation experiments with isolated pro-T and pre-T cells should provide further insight into these issues.

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Molecular characterization and expression of the gene encoding human erythroid-potentiating activity

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Erythropoietin is the primary physiological regulator of erythropoiesis; however, *in vitro* studies have identified another class of mediators which appear to be important in stimulating erythroid progenitors. These factors have generally been referred to as burst-promoting activities (BPA), because they stimulate the growth of early erythroid progenitors referred to as burst-forming units-erythroid (BFU-E) which give rise to colonies of up to thousands of haemoglobinized cells[1,2]. We recently reported purification of a burst-promoting activity from medium conditioned by the Mo T-lymphoblast cell line infected with human T-cell lymphotropic virus type II (HTLV-II)[3,4]. This purified glycoprotein of relative molecular mass (M_r) 28,000 also stimulates colony formation by more mature erythroid precursors (CFU-E) and is therefore referred to as erythroid-potentiating activity (EPA)[5]. Purified EPA specifically stimulates human and murine cells of the erythroid lineage, unlike murine interleukin-3 (IL-3) which stimulates precursor cells from all haematopoietic lineages[6]. We report here the isolation of a complementary DNA molecular clone encoding EPA and its use in producing EPA in COS (monkey) cells and CHO (Chinese hamster ovary) cells. We also define the organization of the EPA gene in human DNA.

Purified EPA obtained from two 10-l preparations of medium conditioned by the Mo T-lymphoblast cell line was analysed in four separate samples using a gas phase sequenator. From a total of approximately 25 μg of purified protein, the following partial amino-acid sequence was obtained from the NH_2-terminus:

```
1          5          10         15         20         25
(A) T C V P P H ? Q T A F C N S D L V I R A K F V G T
```

Using this sequence, three different oligonucleotide probes were prepared. The first probe consisted of all 64 possible 12-mers encoding residues 9-12 above (QTAF). The second probe consisted of 64 14-mers predicted from residues 21-25 (AKFVG). The third probe was a pool of 16 53-mers predicted from residues 9-26 based on preferred codon usage. A cDNA library was prepared from Mo messenger RNA and enriched for sequences specific to mature T cells by hybridizing the single-stranded cDNA to mRNA from B cells (Daudi) and immature T cells (CEM). One set of replicate filters containing the enriched Mo cDNA library was hybridized with the 64 12-mers and the other set with the 64 14-mers described above. DNA from 10 clones which hybridized with both probes was prepared and analysed by digestion with *Pst*I and hybridization to each of the three oligonucleotide probes. Three clones, each of which had at least one internal *Pst*I site, hybridized to all three probes, and were further characterized.

A pool of eight strongly hybridizing 14-mers (corresponding to amino-acid residues 21-25) was used as a primer for sequencing the three putative EPA cDNA clones. The resulting DNA sequence was that expected from the amino-acid sequence of

```
        10         20         30         40                  56
GCCGCAGATC CAGCCCCCAG AGAGACACCA GAGAACCCAC C ATG GCC CCC TTT GAG CCC
                                              MET Ala Pro Phe Glu Pro

             71                 86                 101
CTG GCT TCT GGC ATC CTG TTG TTG CTG TGG CTG ATA GCC CCC AGC AGG GCC TGC
Leu Ala Ser Gly Ile Leu Leu Leu Leu Trp Leu Ile Ala Pro Ser Arg Ala Cys

116                131                146                161
ACC TGT GTC CCA CCC CAC GCA CAC ACG GCC TTC TGC AAT TCC GAC CTC GTC ATC
Thr Cys Val Pro Pro His Pro Gln Thr Ala Phe Cys Asn Ser Asp Leu Val Ile

        176                191                206                221
AGG GCC AAG TTC GTG GGG ACA CCA GAA GTC AAC CAG ACC ACC TTA TAC CAG CGT
Arg Ala Lys Phe Val Gly Thr Pro Glu Val Asn Gln Thr Thr Leu Tyr Gln Arg

             236                251                266
TAT GAG ATC AAG ATC ACC AAG ATG TAT AAA GGG TTC CAA GCC TTA GGG GAT GCC
Tyr Glu Ile Lys MET Thr Lys MET Tyr Lys Gly Phe Gln Ala Leu Gly Asp Ala

    281                296                311                326
CCT GAC ATC CGG TTC GTC TAC ACC CCC CCC ATG GAG AGT GTC TGC GGA TAC TTC
Ala Asp Ile Arg Phe Val Tyr Thr Pro Ala MET Glu Ser Val Cys Gly Tyr Phe

             341                356                371
CAC AGG TCC CAC AAC CGC AGC GAG GAG TTT CTC ATT GCT GGA AAA CTG CAG GAT
His Arg Ser His Asn Arg Ser Glu Glu Phe Leu Ile Ala Gly Lys Leu Gln Asp

386                401                416                431
GGA CTC TTG CAC ATC ACT ACC TGC AGT TTT GTG GCT CCC TGG AAC AGC CTG AGC
Gly Leu Leu His Ile Thr Thr Cys Ser Phe Val Ala Pro Trp Asn Ser Leu Ser

        446                461                476                491
TTA GCT CAG CGC CGG GGC TTC ACC AAG AGC TAC ACT GTT GGC TGT GAG GAA TGC
Leu Ala Gln Arg Arg Gly Phe Thr Lys Thr Tyr Thr Val Gly Cys Glu Glu Cys

             506                521                536
ACA GTG TTT CCC TGT TTA TCC ATC CCC TGC AAA CTG CAG ACT GCC ACT CAT TGC
Thr Val Phe Pro Cys Leu Ser Ile Pro Cys Lys Leu Gln Ser Gly Thr His Cys

    551                566                581                596
TTG TGG ACG GAC CAG CTC CTC CAA GGC TCT GAA AAC GGC TTC CAG TCC CGT GAC
Leu Trp Thr Asp Gln Leu Leu Gln Gly Ser Glu Lys Gly Phe Gln Ser Arg His

             611                626                641
CTT GCC TGC CTG CCT CGG GAC CCA GGG CTG TGC ACC TGG CAG TCC CTG CGG TCC
Leu Ala Cys Leu Pro Arg Glu Pro Gly Leu Cys Thr Trp Gln Ser Leu Arg Ser

656                675        685        695        705        715
CAG ATA GCC TGA ATCCTGCCGG GAGTGGAAGC TGAAGCCTGC ACAGTGTCCA CCCTGTTCCC
Gln Ile Ala

        725        735        745        755        765
ACTCCCATCT TTCTTCCGGA CAATCAAATA AAGAGTTACA CCAGCAAAAA AAAAA
```

Fig. 1 Nucleotide and deduced amino-acid sequence of the cDNA clone encoding EPA.

Methods. Standard gentle lysis procedures were used to prepare RNA from 2×10^9 lectin-stimulated Mo cells. Polyadenylated RNA was selected with oligo(dT)-cellulose and used as the template to synthesize the first strand of cDNA by reverse transcription. Approximately 1 μg of single-stranded cDNA, obtained from 10 μg of poly(A)$^+$ RNA, was enriched by hybridization to 10-fold excess concentrations of mRNA from a human B-cell line (Daudi) and immature T-cell line (CEM), and the unhybridized cDNA (~5% of input cDNA) was recovered by chromatography on hydroxylapatite. Standard techniques were used to synthesize the second cDNA strand, digest the single-stranded loop and add homopolymeric (dC) tails. The cDNAs were annealed with G-tailed pBR322 and used to transform *Escherichia coli*. A final yield of 40,000 colonies was obtained from double-stranded cDNA. EPA was purified from serum-free Mo-conditioned medium using the previously published method[3]. Two preparations of EPA containing ~25 μg were subjected to Edman degradation and analysed on a gas phase sequenator. Based on the amino-acid sequence obtained, three pools of oligonucleotide probes were constructed. The pools were used to probe replicate filters containing the recombinant plasmids described above. Potential EPA clones were identified by colony hybridization. DNA from positive clones was analysed by restriction digestion and hybridization to the oligonucleotide probes. EPA cDNA clones were verified by DNA sequence analysis using the dideoxynucleotide chain termination procedure primed with the oligonucleotides described in the text[19].

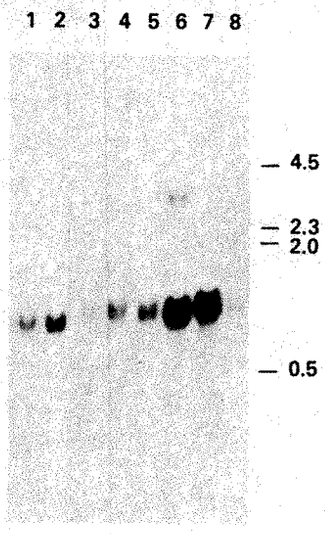


Fig. 2 Analysis of EPA mRNA from human cells and cell lines. Polyadenylated cytoplasmic RNA (5 μg) was isolated from U-937 cells cultured in the presence of 10 nM dexamethasone for 20 h (lane 1); U-937 cells (lane 2); Mo cells cultured in the presence of dexamethasone (lane 3); Mo cells (lane 4); Mo cells treated for 20 h with phytohaemagglutinin (0.3%) and phorbol myristate acetate (5 mg ml^{-1}) (lane 5); C10-MJ2 cells (lane 6); UCD-144-MLA cells (lane 7); and peripheral blood lymphocytes (lane 8). The mRNA was fractionated on a 1% agarose gel, transferred to nitrocellulose and hybridized with the EPA clone labelled with ^{32}P as described previously[12].

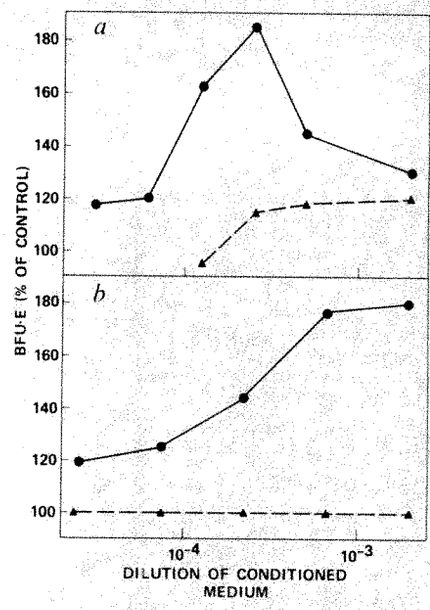


Fig. 3 Biological activity of EPA cDNA clones expressed in mammalian cells. *a*, Stimulation of human peripheral blood BFU-E by dilutions of supernatants containing recombinant EPA expressed transiently in COS cells (●) compared with mock-transfected COS cells (▲). *b*, Supernatant from CHO cells which constitutively produce high levels of recombinant EPA (●) compared with supernatant from a CHO line which does not express the EPA cDNA (▲).

Methods. The expression vector 91023(B) is described in detail elsewhere[17]. This plasmic contains eukaryotic regulatory elements from adenovirus, simian virus 40, and the mouse immunoglobulin and dihydrofolate reductase genes. COS 7 monkey cells were transfected with the EPA cDNA inserted into the 91023(B) vector using a previously described procedure[12]. Briefly, cells were incubated for 12 h at 37 °C in serum-free Dulbecco's modified Eagle's medium containing DEAE-dextran (M_r 5×10^6 Pharmacia, 250 mg ml^{-1}) with 0.1 M Tris *p*H 7.3 and 2 μg ml^{-1} plasmid DNA. After the incubation, cells were rinsed with serum-free medium and treated for 2 h at 37 °C with 1 mM chloroquine in medium containing serum. Cells were washed and medium was collected at varying intervals. Chinese hamster ovary cells (CHO) were transfected with the 91023(B) vector containing the EPA cDNA clone and a functional dihydrofolate reductase clone subcloned into the unique *Cla*I site. Stable transfectants were isolated by selection in growth medium without nucleosides for about 2 weeks. The transfected DNA containing the EPA sequence was amplified by repeated stepwise selection in methotrexate for ~2 months. To determine the levels of recombinant EPA protein synthesized, supernatants from transiently infected COS cells and stably infected CHO cells were analysed in a radioimmunoassay for EPA (J.C.G., J. Chan and D.W.G., in preparation). Samples which were positive for immunoreactive EPA were assayed for biological activity, by measuring stimulation of the growth of human peripheral blood BFU-E in methylcellulose culture[3]. Peripheral blood leukocytes were plated at a density of 3×10^5 cells ml^{-1} in 96-well tissue culture trays. Each well contained 0.1 ml of cells in methylcellulose and 0.01 ml of a dilution of the test supernatant. Control wells were those to which diluent alone was added, and they contained 20 colonies per well in panel *a* and 16 colonies per well in panel *b*.

residues 1-19, confirming that these cDNA clones encode the EPA protein.

The complete nucleotide sequence of all three clones was obtained by dideoxy sequencing of M13 subclones (Fig. 1). The cDNA encodes a protein of 207 amino acids. Based on the NH_2-terminal sequence of the mature protein, a signal peptide of 23 amino acids is predicted by the nucleotide sequence. Characteristic of other signal peptides, this region is rich in hydrophobic amino acids. The predicted relative molecular mass of the mature protein is in good agreement with the apparent 18,000 M_r observed following digestion of EPA with endoglycosidase F (ref. 4). Endoglycosidase F removes both high mannose and complex asparagine-linked carbohydrate moieties[7]; two possible sites for asparagine-linked glycosylation are amino acid 53 and 101. The presence of 12 cysteine residues in the deduced amino-acid sequence predicts a protein structure which could account for the marked heat stability previously described for EPA biological activity[5].

The EPA cDNA clone was used to screen a cDNA library prepared from the gibbon lymphosarcoma cell line UCD-144-MLA[8], a source of numerous lymphokines including burst-promoting activity. The resulting clones were sequenced and compared with the nucleotide sequence of human EPA. Seven nucleotides within the coding region differed between human and gibbon EPA, resulting in a single amino acid change from arginine to lysine at residue 43.

Further confirmation that these clones encode EPA was obtained by Northern analysis. mRNA was prepared from cells previously shown to produce burst-promoting activity. Figure 2 shows that Mo (lane 4), C10-MJ2[9] (lane 6) and UCD-144-MLA[8] (lane 7) mature T-lymphoblast cell lines, peripheral blood lymphocytes (lane 8) and the U-937[10] (lane 2) monocytic cell line produce a 0.9-kilobase (kb) transcript which hybridizes with the EPA cDNA probe. Erythroid-potentiating activity mRNA could not be detected in samples from the immature B-lymphoblastoid cell line Daudi (data not shown). These results are in agreement with previous reports of EPA activity, as well as the presence or absence of the EPA protein in cell supernatants as determined by immunoprecipitation and radioimmunoassay (unpublished results). Treatment of U-937 (Fig. 2, lane 1) and Mo (lane 3) cells with the glucocorticoid dexamethasone appears to decrease the level of EPA mRNA by approximately twofold. Similar results have been seen with other T-cell-derived growth factors including IL-2[11] and granulocyte-monocyte colony-stimulating factor (GM-CSF)[12]. Treatment of Mo cells with phytohaemagglutinin and phorbol myristate acetate (Fig. 2, lane 5) does not greatly increase the abundance of EPA mRNA over that produced constitutively. In similar conditions, GM-CSF mRNA levels are increased approximately two- to fourfold[12].

To provide definitive evidence that the cDNA clone encoded EPA, it was expressed in mammalian cells. The EPA cDNA

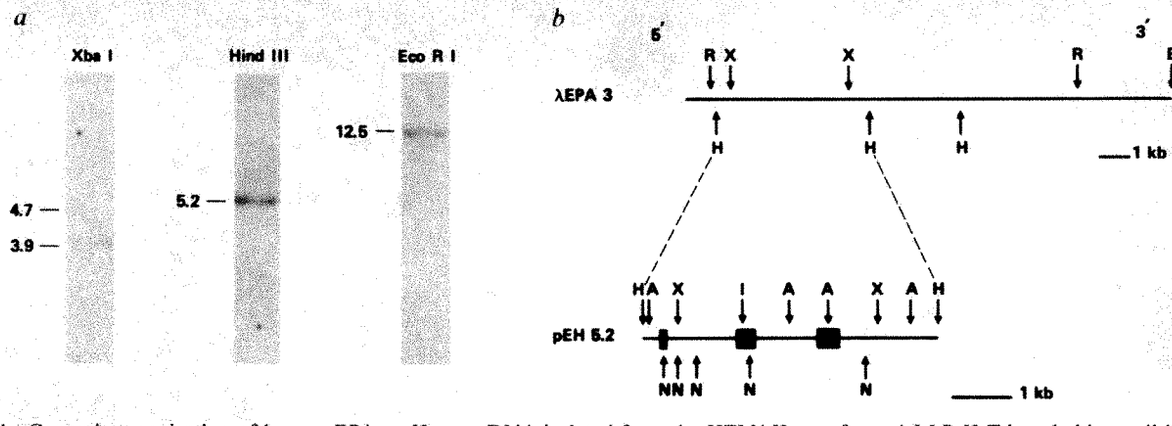


Fig. 4 Genomic organization of human EPA. *a*, Human DNA isolated from the HTLV-II-transformed J-LB-II T-lymphoblast cell line was digested with *Xba*I, *Hin*dIII and *Eco*RI and hybridized with the EPA cDNA. *b*, Restriction map of an EPA genomic clone in Charon 30 (λ EPA 3) and subcloned into pBR322 (pEH 5.2) are shown. Restriction sites are: R, *Eco*RI; X, *Xba*I; H, *Hin*dIII; A, *Ava*I; N, *Nco*I; I, *Hin*cII. **Methods.** High M_r DNA was prepared from a variety of cell lines, using standard procedures. The lengths of restriction fragments containing EPA sequences were determined by the method of Southern[12]. A λ phage recombinant library was constructed using Charon 30 and DNA from the J-LB-III human T-lymphoblast cell line[20]. The J-LB-III DNA was digested with the restriction enzyme *Sau*3A for various times and 10–25-kb fragments were isolated using a NaCl gradient[21]. The J-LB-III DNA was ligated to Charon 30 DNA which had been digested with *Bam*HI and purified on a NaCl gradient. Recombinant phage were recovered by *in vitro* packaging[22]. The recombinant phage were screened by hybridization to a probe consisting of a full-length EPA cDNA clone. Two smaller fragments of the EPA cDNA clone were isolated using *Pst*I, gel purified and used to generate detailed restriction maps of the EPA genomic clones.

clone was inserted into the 91023(B) mammalian expression vector and transiently expressed in COS monkey cells[13]. The concentration of EPA in the unfractionated COS cell supernatants was estimated, by radioimmunoassay, to be approximately 1 μg ml^{-1} 72 h after transfection, indicating efficient expression of the cDNA clone.

Supernatants from COS cells transiently infected with either the 91023(B) vector alone or the vector containing the EPA coding region were assayed for stimulation of human peripheral blood BFU-E. The results (Fig. 3*a*) demonstrate biological activity of the recombinant EPA transiently expressed in COS monkey cells. Good stimulation of erythroid bursts (>130% of control) is seen at concentrations as low as ~5 pM (based on the concentration of immunoreactive EPA). In every case, the supernatant from the mock-infected or uninfected COS cells had a slight stimulatory activity on human BFU-E, apparently due to some constitutive production by COS cells of a factor that stimulates human erythroid precursors.

In addition to transient expression of EPA in COS cells, it is useful to generate cell lines which constitutively produce high levels of EPA protein. Chinese hamster ovary cells with integrated copies of the 91023(B) vector containing the EPA coding region along with the dihydrofolate reductase gene were selected in methotrexate. After approximately 2 months of selection, levels of EPA protein in supernatants from 31 CHO cell clones were estimated by radioimmunoassay. One clone was found to constitutively produce levels of recombinant EPA similar to those obtained by transient expression in COS cells. The biological activity of recombinant EPA in the medium conditioned by this cell line was compared with medium conditioned by CHO cells which had been treated in the same way, but were not producing recombinant EPA. Figure 3*b* shows the stimulation of human peripheral blood BFU-E by recombinant EPA produced by this stable line of CHO cells. No endogenous burst-promoting activity is seen in the supernatant from CHO cells not producing recombinant EPA. The recombinant EPA is active in the picomolar range of concentrations, as is the purified protein from Mo-conditioned medium[3].

To study the molecular organization of the human EPA gene sequences, high-M_r DNA was prepared from human T-cell lines (J-LB-II, J-LB-III, J-WM-IV, Mo), B-cell lines (Wil), a myeloid line (HL-60) and a fibroblast line (143) and analysed by the method of Southern[14] for hybridization to the EPA cDNA clone. In all cases the same pattern was seen (Fig. 4*a*). The restriction enzyme *Xba*I generated a strongly hybridizing fragment of 3.9 kb and a faint band at 4.7 kb. A single fragment of 5.2 kb and 12.5 kb was detected following digestion with *Hin*dIII and *Eco*RI respectively.

To obtain a more detailed map of the structure of the EPA gene, a recombinant λ phage library (prepared from Charon 30[15] and J-LB-III DNA) was screened with the EPA cDNA clone as a probe. Three overlapping genomic EPA clones were obtained from approximately one million recombinant phage. The restriction map of one representative clone, λ EPA 3, is shown in Fig. 4*b*. This clone contains the 12.5-kb *Eco*RI fragment and the 5.2-kb *Hin*dIII fragment which was subcloned into pBR322 for further analysis (pEH 5.2). Restriction analysis of pEH 5.2 revealed that EPA appears to be encoded by a single gene approximately 3 kb in length, interrupted by at least two intervening sequences.

Human EPA specifically stimulates the growth of peripheral blood- and bone marrow-derived erythroid precursors from mouse and man. Il-3 is a 28,000-M_r glycoprotein isolated from the EL-4 mouse lymphoma cell line and WEHI-3 mouse myelomonocytic cells. Unlike EPA, IL-3 stimulates the growth of all haemotopoietic precursors, including BFU-E[6]. Murine IL-3 has been molecularly cloned and sequenced[16,17]. Comparison of the murine IL-3 sequence with the human EPA sequence, described here, reveals no homology. Thus EPA is not the human counterpart of murine IL-3, consistent with the biological observations that EPA does not stimulate pluripotent or myeloid precursor cells[3,4].

A computer search for protein homology did not reveal previously described proteins homologous to EPA (R. Doolittle, personal communication). A computer search for DNA homology (Genbank) did, however, reveal that a highly homologous partial murine cDNA clone had been isolated. This clone was obtained from virally induced C243 cells using hybrid selection of mRNAs whose translation products have antiviral interferon activity[18]. The cDNA clone pMIF 3/10 contains about 290 base pairs that could encode that 3′ 39 amino acids of a protein plus the 3′-untranslated region. This cDNA clone was reported to encode a murine β-interferon-like protein, but the relationship between this molecule and EPA is unclear. Erythroid-potentiating activity has no interferon activity (our unpublished observation), and full-length cDNA clones of pMIF 3/10 have not been isolated and expressed to provide direct evidence for interferon activity.

The physiological functions of EPA *in vivo* are unknown. Since EPA stimulates murine erythroid precursors *in vitro*, as well as human, it should be possible to study *in vivo* effects of this modulator in the mouse. The availability of adequate quan-

ties of recombinant protein will be critical in elucidating the role of EPA in normal haematopoiesis. Additionally, the availability of cDNA and genomic clones will make it possible to study the evolution and expression of this interesting erythroid haematopoietic growth factor.

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Myxococcus xanthus spore coat protein S may have a similar structure to vertebrate lens $\beta\gamma$-crystallins

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The Gram-negative bacterium *Myxococcus xanthus* has a complex life cycle[1] during which large amounts of a protein of relative molecular mass (M_r) 19,000, known as protein S, are assembled into a spore surface coat by a process that specifically requires calcium ions[2,3]. The gene for protein S has been cloned and the DNA sequence shows that the gene product is composed of four internally repeated homologous sequences, each 40 amino acids long[4]. Although protein S resembles calmodulin both in its internally duplicated structure and its ability to bind calcium[4], it apparently has a β-sheet secondary structure[5] rather than the helix–loop–helix motifs that characterize the calmodulin family[6–8]. We now show that protein S has a striking homology with the β- and γ-crystallins of the vertebrate eye lens[9–11] which are β-sheet proteins with internally duplicated structures. This implies that the β- and γ-crystallins evolved from already existing proteins, whose ancestors occurred in the prokaryotes. The biological function of protein S, as a closely packed, stable protein in a relatively dehydrated environment, has implications for the functions of crystallins, which are found closely packed in the lens fibre cells, where their stability is essential for maintenance of transparency.

γ-Crystallin has an internally repeated sequence of ~40 amino acids[9–11] arranged as two globular domains related by a pseudo-dyad. Each domain is formed from two Greek key motifs (strands *d a b c*) related by further pseudo-dyads and arranged as two antiparallel β-sheets, each sheet composed of strands *c' d a b* where strand *c'* is contributed by one motif and *d a b* by the second. In motif 1 the characteristic fold is made possible by the presence of a glycine at position 13 which allows the β-hairpin formed by strands *a* and *b* to fold back on to the sheet so that the peptide nitrogen of residue 11 hydrogen-bonds to the buried side chain of serine 34 which just precedes strand *d*. This leads to interactions between the aromatic amino acids at residues 6 and 11, which are protected from solvent by an arginine at residue 36. This arrangement is repeated in the other motifs with a small variation in sheet 3 only[10]. The amino-acid sequences of the β- and γ-crystallins from several species[12–19] have this arrangement of residues in common, suggesting an evolutionary relationship between the two crystallin classes and the existence of a protein structural superfamily, the $\beta\gamma$-crystallins[17].

Analysis of the repeated sequences of bovine γ-crystallin II(γ_{II}) shows that motifs γ1 and γ3, and motifs γ2 and γ4 have the strongest homologies. Motifs γ2 and γ4 are longer than γ1 and γ3, and appear to have several residues inserted in the loop region between strands *c* and *d* of the Greek key motifs. A similar analysis of protein S shows that S1 and S3, and S2 and S4 are the pairs with the highest homologies (60% and 45%), indicating a similar pattern of duplication, but greater similarity between the two halves than in $\beta\gamma$-crystallins. The optimum correspondence of repeated sequences between protein S and γ_{II} is nonlinear (Fig. 1). In terms of the number of residues, the S2/S4 pair corresponds most closely to motif γ1 of γ-crystallin II. Similarly, S1/S3 corresponds most closely to motifs γ2 and (not shown) γ4. At the level of sequence identity also, S2/S4 most closely resembles γ1, with 33% and 23% identity respectively, whereas S1/S3 matches γ2/γ4, with 26% identity between S1 and γ2 and 19% identity between S3 and γ4. The N-terminal domain of γ_{II} (γ1γ2) was used as the basis for a model for both proposed domains of protein S. Figure 2 shows a model structure for S1S2, the N-terminal domain of protein S. The structurally important residues are all present, allowing the main chain to follow the same path, forming the characteristic folded hairpin structure described for γ (refs 9, 10). The

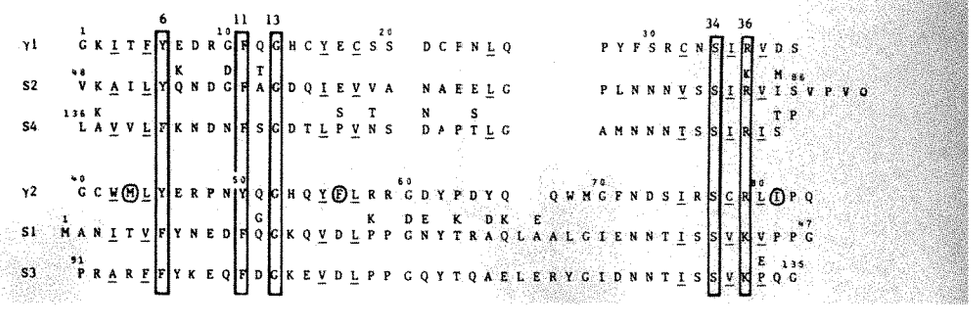


Fig. 1 Alignment of the four repeated sequences of protein S (S1–S4) with the first two motifs of γ-crystallin II (γ1, γ2). The protein S sequence is that defined for gene 2, with the residues for gene 1 given above; there is an inserted alanine between Ser 146 and Gly 147 in gene 1.

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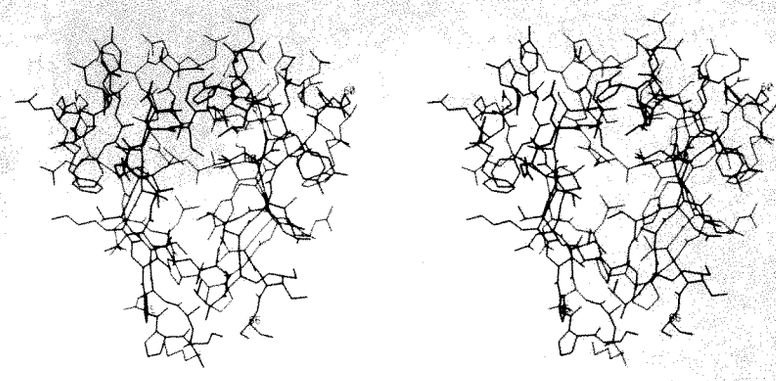


Fig. 2 A stereo view of the molecular model of one domain (S1 + S2) of protein S. The model was constructed by first aligning the sequences of protein S and bovine γ_{II} so that the structurally important glycine and serine residues coincide. The alignments are shown in Fig. 1. To simplify the comparison, only one motif of each pair in γ_{II} is shown. The two domains were modelled using the N-terminal domain of γ_{II} (comprising motifs $\gamma1\gamma2$) as a guide, with γ_{II} coordinates taken from the structure refined at 1.6 Å resolution[11]. The interactive graphics program FRODO (ref. 25, modified by T. A. Jones and I. J. Tickle) run on an Evans and Sutherland PS2 was used for model-building in the manner described previously for bovine βBp-crystallin[26] and murine β23-crystallin[18]. Main-chain atoms were not moved, except where two insertions were required in a surface loop of $\gamma2$ to model S1 and S3. The coordinates of invariant side chains were retained. For conservative changes, side-chain conformations were modelled closely on the crystallographic coordinates; remaining side chains were modelled to optimize favourable interactions and to avoid disallowed non-bonded contacts. The models have been subjected to energy minimization.

extensive hydrogen-bonding in the β-sheets and elsewhere[10] is also preserved.

Because of the non-sequential correspondence between related motifs, it was necessary to make and break inter-motif connections in the γ_{II} structure; this is easily achieved as each domain contains a pseudo 2-fold axis, the N-terminus of each strand *a* and the C-terminus of each opposing strand *d'* being disposed in space in a similar way (Fig. 3). We have attempted to build a model with sequential alignment of the two polypeptides, but it gives less good packing of the hydrophobic core.

The model shown in Fig. 2 indicates that the residues which occupy the space between the two sheets in each domain (S1S2 or S3S4) of protein S are conserved as hydrophobic, although there is a small overall decrease in their volume (see Fig. 1). For example, Tyr 16 of $\gamma1$ and the topologically equivalent Tyr 55 of $\gamma2$ which lie on strand *c* at the edge of the sheet are replaced by leucine, valine or isoleucine in protein S. More importantly, the buried Trp 42 is replaced by isoleucine in S1 and an alanine in S3. However, the inserted residues in the loop between strands *c* and *d* in S1 and S3 of protein S appear to contribute to the core packing, and Gly 70 of $\gamma2$ is replaced by isoleucine in protein S. Molecular mechanics calculations imply that the two β-sheets are packed more closely in protein S than in γ-crystallin II, while preserving the hydrophobicity of the contacts. Similar small relative shifts in β-sheet positions have been shown to occur in the azurin/plastocyanin family[20]. The exact relationship of the two sheets must await a detailed X-ray analysis, which is now underway[21].

In β-crystallins the two domains ($\gamma1\gamma2$ and $\gamma3\gamma4$) are related by a further pseudo-dyad which gives rise to closely packed hydrophobic interactions between residues of strands *a*, *b* and *d* of $\gamma2$ and $\gamma4$, involving residues which are conserved as hydrophobic in all $\beta\gamma$-crystallins. In motifs S1 and S3 of protein S, these are replaced by several charged groups, indicating hydrophilic contacts with solvent. However, inter-domain contacts may instead be formed between the sheets that are mainly composed of motifs S2 and S4, where hydrophobic residues do occur. Most of the surface residues of protein S are hydrophilic and many are charged, although the unusually large number of ion pairs found in γ-crystallin II, for example, Arg 36 to Asp 61 (refs 9, 10), are absent from protein S. Protein S is more acidic than γ-crystallin II, mainly because of the smaller number of arginines/lysines rather than an increase in the number of acidic residues. As in γ-crystallin II, there is a predominance of aspartates over glutamates.

Overall, the proposed structure for protein S is consistent with the data presented by Inouye *et al.*[4,5]. In particular, the inter-domain connecting peptide, as in bovine γ_{II}, would be relatively exposed and potentially vulnerable to tryptic cleavage (in the case of protein S). The so-called 'tryptic core structure'[4] would thus correspond to the first domain of protein S.

Calcium ions are thought to be specifically involved in binding protein S to spores. The existence of layers of protein S which are 300 Å thick[2] indicates that there may be intermolecular interactions between protein S molecules (maximum dimension ~80 Å) as well as with specific receptors on the spore. Because identical crystals of protein S may be obtained with or without calcium[5,21] and as calcium binding has no significant effects on the circular dichroism of protein S[5], protein S may be crosslinked to its receptors on the spore and possibly to other protein S molecules directly through surface Ca^{2+} sites which do not affect the conformation of the molecule.

As there is no direct evidence implicating any particular residues in calcium binding, we began by searching the model surfaces of the protein S gene 1 and 2 products for conserved arrangements of oxygen ligands, for example carboxylates of aspartate or glutamate, amides of asparagine or glutamine, O_γ of serine or threonine, and peptide carbonyl oxygens. Such calcium-binding ligands have been identified in a wide range of proteins. The most obvious candidate is a surface group of four acidic residues found close to the folded β-hairpin structure and including residues Glu 10, Glu 70, Glu 71 and Asp 11 (see Fig. 4); this would give only one binding site for calcium per protein S molecule and would be consistent with the ability of only ~45% of the 'tryptic core structures' to self-assemble on the protein S-deficient spores, as only one of the two domains would bind calcium. An alternative binding site occurs in each of the sequences S1 to S4 and therefore occurs twice in each domain. This binding site is composed of the conserved topological equivalent residues Glu 10, Glu 99, Asp 56 and Asp 144. The other ligands at each of these putative calcium-binding sites are derived from the conservatively varied sequence Glu 35-Asn 36-Asn 37-Thr 38-Ile 39-Ser 40 and equivalents of this, represent-

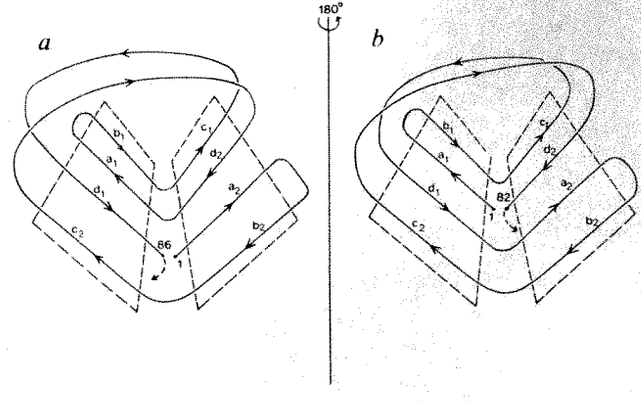


Fig. 3 The suggested arrangement of the strands in the first two repeated sequences (S1 and S2) of protein S (*a*) compared with that found in γ-crystallin II (*b*).

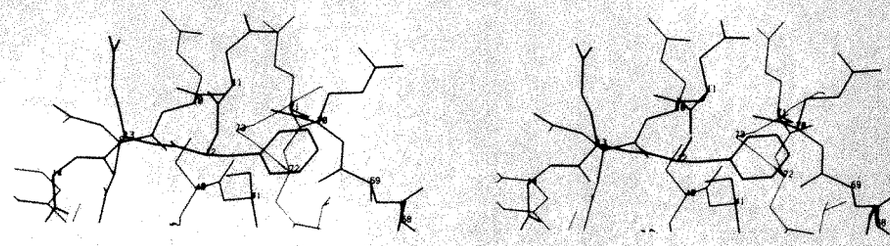


Fig. 4 A stereo view of a possible calcium-binding site involving Glu 10, Asp 11, Glu 70 and Glu 71.

ing part of the sequence Glu 35 to Lys 43 which has been identified by Inouye *et al.*[22] as homologous to the calcium-binding sequence of calmodulin. However, if our model is correct, the binding cannot involve Ser 41, as this residue is buried. There is no residue in protein S corresponding to the final glutamate which is conserved in the loop region of all members of the calmodulin family. These sites are the most obvious candidates for surface Ca^{2+} binding.

Finally, we must consider the implications of the observed structural similarity between protein S and $\beta\gamma$-crystallins for the function and evolution of vertebrate lens proteins. The putative calcium-binding sites described above are not generally conserved in bovine γ_{II} or in other $\beta\gamma$-crystallins[12-19]. However, calcium ions do seem to have an effect on the aggregation of crystallin in certain systems[23]. Lens proteins have a short-range order which leads to slowly changing protein concentrations and a smooth change of refractive index through the transparent lens; conceivably, this could be influenced by calcium, suggesting a specific role for ions in certain forms of cataract.

γ-Crystallins and protein S are highly stable molecules[24]; this may be due to the closely hydrogen-bonded structure of the repeated Greek key motifs, which lack the loose external loops found in many proteins, and to their symmetrical, tightly packed arrangements. Thermodynamic stability may be a general consequence of protein structures developed to survive for long periods in relatively dehydrated environments without turnover; this would apply to both spore proteins and lens crystallins.

γ-Crystallins have high proportion of cysteine and aromatic residues which are not found in protein S. None of the six cysteines found in $\gamma1\gamma2$ is retained in protein S. The two tryptophans in γ-crystallin are replaced by aliphatic residues and the loss of 10 phenylalanines and tyrosines is compensated for by only three at other positions. It has been argued[10,11] that the cysteines and associated aromatic amino acids are important in protecting the lens proteins from the effects of ultraviolet radiation and other abuses which lead to free radical formation. These aspects of the $\beta\gamma$-crystallin structure may have evolved to meet the special requirements of the lens.

The intramolecular homology of protein S indicates that a pattern of gene duplication similar to that proposed for $\beta\gamma$-crystallins has occurred. The fact that S2 resembles $\gamma1$ and S1 resembles $\gamma2$ suggests that a common ancestor could have been an asymmetrical dimer of two 40-residue units. In the crystallin line of descent, the genes for these molecules were fused in the order $\gamma1$–$\gamma2$ while the reverse order occurred for protein S. In both cases, further duplication and fusion led to the present two-domain structures.

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The nucleotide sequences of *copia* and *copia*-related RNA in *Drosophila* virus-like particles

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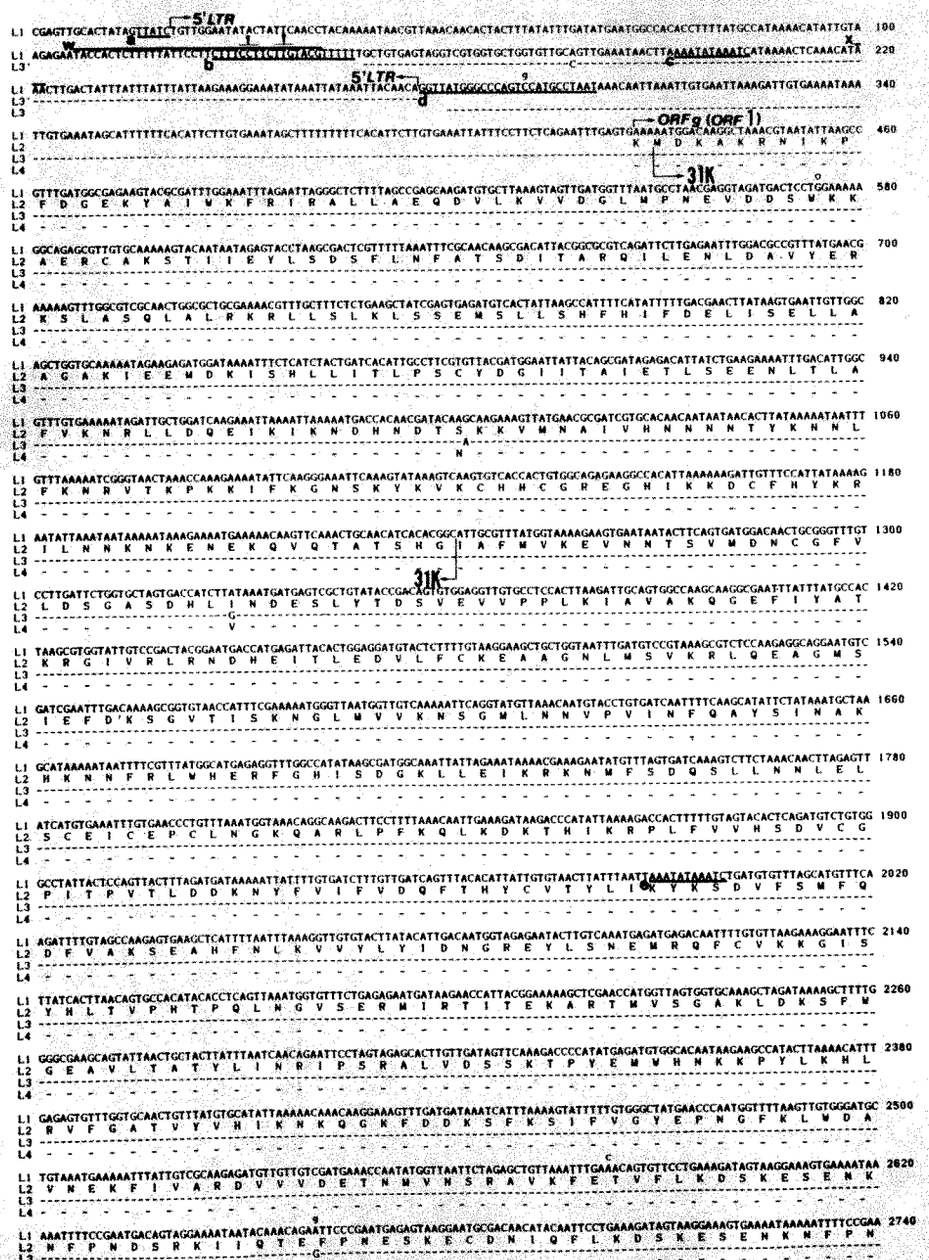
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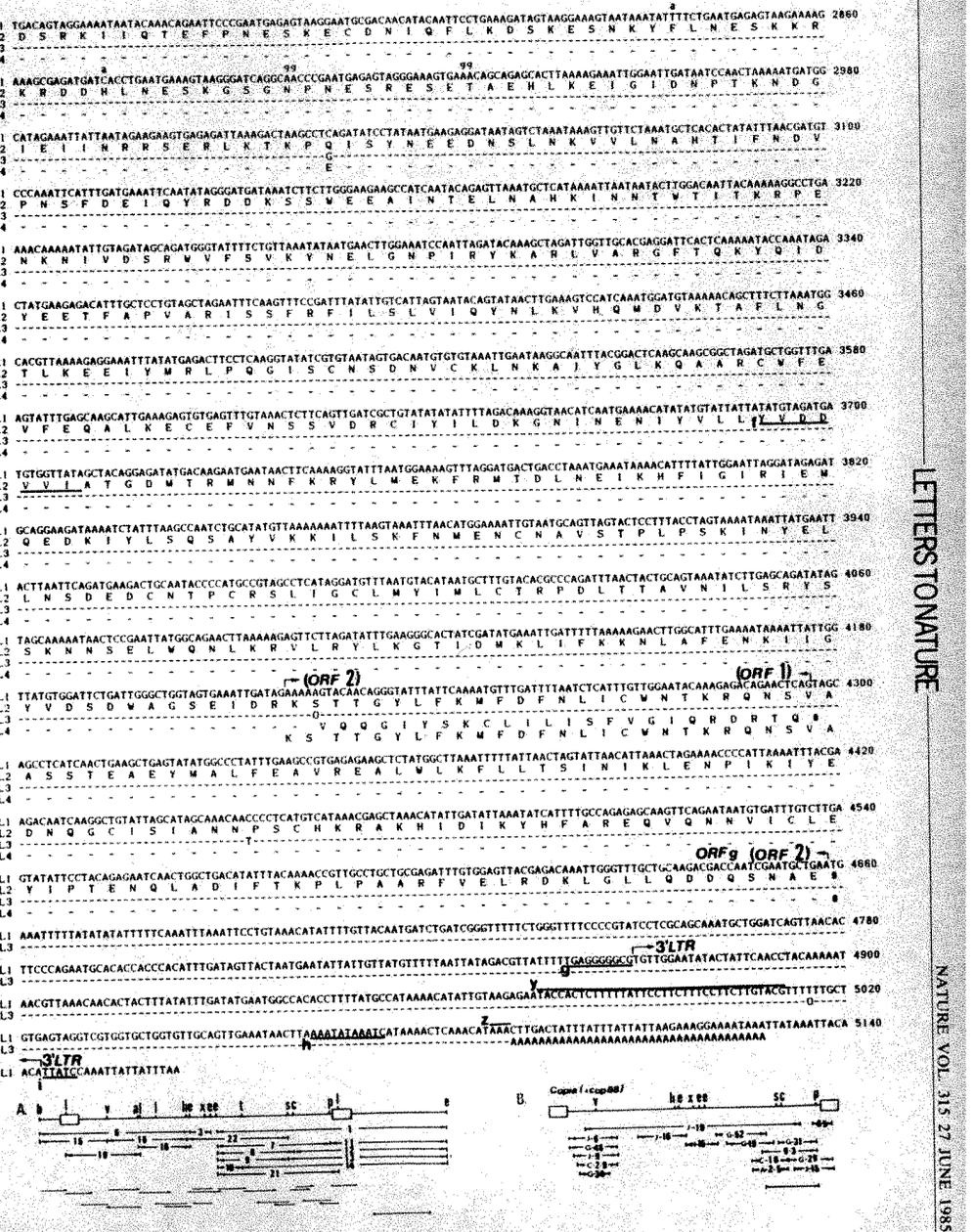
We have shown previously that *Drosophila* cells contain virus-like particles (VLPs) containing 5-kilobase (kb) RNA that hybridizes to a transposable element, termed *copia*. We have suggested that VLPs and *copia* are derivatives of viral particles and proviral forms, respectively, of '*copia*' retrovirus, a putative *Drosophila* retrovirus[1]. To further clarify the relationship between *copia* and *copia*-related RNA in VLPs (VLP H-RNA), we determined and compared their nucleotide sequences. VLP H-RNA was found to be an unspliced, genome-sized transcript of *copia*, and, like retroviral genome RNA, VLP H-RNA is terminally redundant with termini localized in the long terminal repeats (LTRs) of *copia*. VLP H-RNA contains two long open reading frames (ORFs), one of which includes the coding sequence for a predominant VLP protein of relative molecular mass (M_r) 31,000 (31K). Here we show that, in contrast to *17.6* ORF2 (ref. 2), ORFs of *copia* have no extensive amino-acid sequence homology to the RT region[2] of the reverse transcriptase of retrovirus in vertebrates. Because of a one-base insertion/deletion, the two ORFs in VLP H-RNA are fused and become a single, longer ORF in a genomic *copia*.

Using cDm2055 DNA[3] as a probe, we isolated 20 recombinant phage containing *copia* from a cloned library of *Drosophila melanogaster* DNA[4] and made restriction maps (data not

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Figure 1


5'LTR
5'LTR
ORFg (ORF 1)
3'K
3'K
3'LTR
3'LTR


(ORF 2)
(ORF 1)
ORFg (ORF 2)
3'LTR
A.
B.

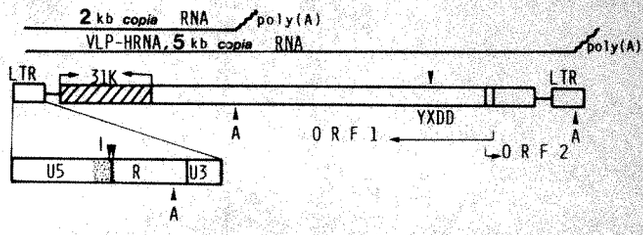


Fig. 2 Genome organization of *copia* and its gene products shown schematically. The 5′ LTR has been enlarged to make the internal structure of LTR more easily discernible. The ambiguity in the 5′ end of U3 results from the three consecutive A residues at the poly(A) attachment site, whereas that of R results from the real heterogeneity in the 5′ end of VLP H-RNA. Two downward arrows labelled I show the positions of the 5′ ends of the two major VLP H-RNA. The locations of the putative polyadenylation signal of *copia*, AAATAATAAATC, are shown by the arrows labelled A. Note that one of the polyadenylation signals is near the region corresponding to the 3′ end of 2-kb *copia* RNA. The structure of genomic *copia* is based on the sequence of VLP H-RNA, although a *copia* in λcop88 contains a single ORF covering the entire regions of both ORFs 1 and 2 (see Fig. 1). A polypurine tract similar to those of other *Drosophila* transposons and vertebrate retroviruses[12] can be seen in the 3′-untranslated region. The expected coding sequence of the predominant 31K VLP protein is shown by a shaded box and appears to be included in both 2- and 5-kb *copia* RNA (VLP H-RNA). The location of the most conservative amino-acid sequence among RNA dependent polymerases, Y(M, L, V or G)DD(hydrophobic amino acid)$_3$, is shown by the arrow at YXDD. Association of 31K VLP protein and VLP H-RNA is considered to result in the production of VLP.

Fig. 1 (Opposite) Nucleotide sequences of *copia* and cDNA of *copia*-related RNA in VLP (VLP H-RNA). The complete nucleotide sequence of *copia* in λcop88 is shown as the putative sense strand in L1, whereas L3 and L3′, respectively, show the composite cDNA nucleotide sequence of VLP H-RNA and the DNA sequence complementary to the 5′ terminal sequence of VLP H-RNA determined by the dideoxy-chain termination method using VLP H-RNA as a template. The ranges of long ORFs found in both *copia* and VLP H-RNA are shown by L-shaped arrows labelled ORF, with amino-acid sequences shown below the corresponding nucleotide sequences (L2 and L4). Dashes, nucleotide or amino-acid sequences identical to those of *copia* in L1 and L2. 0s in L3 indicate one-nucleotide deletions in VLP H-RNA, whereas # denotes the termination codon of each ORF. Underlines, nucleotide sequences corresponding to the duplicated target DNA; *a* and *i*, 5′ start sites of cellular 2- and 5-kb RNA[6]; *b*, expected polyadenylation signal; *c*, *e*, *h*, nucleotide sequence complementary to the 26-nucleotide long synthetic oligodeoxynucleotide for primer extension experiments; *d*, the amino-acid sequence best conserved among various polymerases[10,11]; *f*, polypurine sequence corresponding to the counterparts of other *Drosophila* transposons and vertebrate retroviruses (*g*)[12]. Overlines labelled *w* and *y*, respectively, show the 5′ terminal region of VLP H-RNA in the 5′ LTR and its equivalent in the 3′ LTR. Two vertical arrows indicate 5′ ends of two major VLP H-RNAs. Thin lines associated with the arrows show the degree of inaccuracy (±1 nucleotide) for the determination of 5′ ends. Overlines *z* and *x* exhibit the poly(A) attachment site of VLP H-RNA in the 3′ LTR and its equivalent in the 5′ LTR, respectively. The ranges of 5′ LTR, 3′ LTR and expected coding region of the predominant M_r 31,000 VLP protein (31K) are shown by L-shaped arrows. The end of the 31K region was tentatively determined based on the relative molecular mass and amino-acid composition of the 31K VLP protein. The base substitutions and deletion found in the nucleotide sequences of *copia* regions 1–1,143 and 2,302–2,931 (described in refs 6, 7) are shown by small letters and *o*, respectively, above the *copia* sequence (L1).

Methods. The nucleotide sequence of *copia* was determined by digesting DNA extracted from λcop88 by various restriction enzymes and recloning the resultant DNA segments containing *copia* pieces using plasmid pUC9 (ref. 13) as a cloning vector. Sizes and locations of the cloned DNA segments are shown by thick lines with arrows and numbers in scheme *A*. After *Escherichia coli* cells (JM83 (ref. 13) harbouring a desired plasmid) were cultured in 5–10 ml L-broth for 12–15 h at 37 °C with shaking, plasmid DNA was prepared by the alkali method[14]. Contaminated RNA was removed by a treatment of 10 μg ml^{-1} of RNase for 30 min at 37 °C and final concentration of 7.5% polyethylene glycol in 0.94 M NaCl for 1 h at 0 °C. Nucleotide sequence was determined by the chain termination method[15] modified by M. Hattori (unpublished) using the DNA cloned in pUC9. Two different kinds of synthetic oligonucleotides [M13 single-stranded primer (5′ GTAAAACGACGGCCAGT3′) and M13 reverse sequence primer (5′ CAGGAAACAGCTATGAC3′) (P-L Biochemicals)] were used as primers for sequencing. The overall strategy for sequencing is depicted by thin underlines in the lower margin illustration (*A*). Scale bar; 1,000 bp; the two boxes show the LTRs. Restriction enzyme cutting sites used are as follows: a, *Acc*I; b, *Bam*HI; c, *Cla*I; e, *Eco*RI; h, *Hin*dIII; l, *Bal*I; p, *Hpa*I; s, *Pst*I; t, *Aat*I; v, *Pvu*II; x, *Xba*I. The nucleotide sequence of VLP H-RNA was determined as follows. As described previously[1], VLP was purified from a 5 l culture of GM_2 cells and total VLP RNA extracted. After the VLP H-RNA was purified by gel electrophoresis, cDNA was constructed and cloned into pBR322 using the procedures described by Maniatis *et al.*[16]. Restriction mapping and blot hybridization allowed overlapping of the cDNA inserts obtained and a composite restriction map of the cDNA of VLP H-RNA to be made. The restriction map obtained was found to be very similar to that of *copia*. Thus, in scheme *B*, their relative positions are depicted using the restriction map of the *copia* in λcop88 as a reference. Plasmid DNA was purified essentially as described elsewhere[2]. The complete or partial nucleotide sequences of A-25, G-19, G-29, G-34, G-46, J-15, J-16, J-19, H-15 and 3-5 were determined by the procedures of Maxam and Gilbert[17]. We found no discrepancy in the nucleotide sequence in the overlapped region. The position of poly(A) attachment site in G-29 was identical to that in 3-5. Thus, in L3, the composite sequence is shown as the nucleotide sequence of the cDNA of VLP H-RNA. We consider the sequence shown in L3 to be identical to that of VLP H-RNA, apart from the DNA–RNA difference, because (1) at its 3′ end, it contains a long poly(A) tract, a typical terminal structure of eukaryotic mRNA and retroviral genome RNA; and (2) a separate experiment (not shown) indicated that only the DNA strand of *copia*, complementary to the one whose sequence is shown in L1, is hybridizable to VLP H-RNA. The nucleotide sequence near the 5′ terminus of VLP H-RNA was determined by the chain termination method[15]. After 3 μg VLP RNA and 65 ng of ^{32}P-labelled oligodeoxynucleotide (see Fig. 4*a*) in 40 μl 0.1 M NaCl, 1 mM EDTA and 10 mM Tris-HCl (*p*H 8.3) was preincubated at 85 °C for 5 min, then at 60 °C for 10 min. 10 μl 500 mM Tris-HCl (*p*H 8.3) containing 50 mM dithiothreitol and 80 mM $MgCl_2$, 1 μl RNasin, 1 μl reverse transcriptase and 8 μl 25 μM dCTP were added. To each of four 14-μl aliquots, 8 μl of the dNTP-ddNTP mixture of M13 sequencing kits (Takara Shuzo Ltd, Japan) was added. The resultant mixture was then incubated at 40 °C for 20 min. After four dNTPs (1 mM each) in 4 μl was added, the incubation was continued for further 20 min. The reaction products were precipitated by adding 2.5 μl 3 M sodium acetate (*p*H 5.2) and 2 vol ethyl alcohol, and subjected to gel electrophoresis for sequencing.

shown). As the restriction map of the *copia* in λcop88 was very similar to that of most of the *copias* that we cloned, the complete nucleotide sequence of the *copia* in λcop88 was determined as representative of *copia* sequences (Fig. 1, L1). The *copia* in λcop88 is 5,143 base pairs (bp) in length and terminates with 276-bp LTRs whose nucleotide sequences are identical or very similar to the published LTR sequences of other *copias*[5]. Partial nucleotide sequences of the region flanked by LTRs have been reported elsewhere[6,7]. The sequences in these studies are essentially identical to the counterparts described here (Fig. 1). Translation of the nucleotide sequence showed that *copia* contains a long ORF, ORFg (Fig. 1, L2), residing on a DNA strand corresponding to the major cellular *copia* RNAs[6].

We purified VLP H-RNA by gel electrophoresis and made complementary DNA clones. We found no clones that completely covered the entire region of the VLP H-RNA; the information from the 5′ end of the RNA could not be recovered because S_1 nuclease was used. Nevertheless, the overlapping of several cDNA clones (Fig. 1) allowed us to determine 97% of the total sequence of VLP H-RNA from its 3′ end (Fig. 1, L3). The nucleotide sequence of the major portion of the residual 3% was determined by the chain termination method using VLP H-RNA as a template (Fig. 1, L3′). These results show that the nucleotide sequence of VLP H-RNA corresponds to the *copia* sequence shown in Fig. 1 (L1) except for some minor base changes and a terminal poly(A) tract. Because of a deletion of an A residue located somewhere between residues 4,220 and 4,224, the ORF of *copia* (ORFg) is split in two, slightly overlapping ORFs 1 and 2 in VLP H-RNA (Fig. 1). The 3′ end of VLP H-RNA that lacks poly(A) is located in the 3′ LTR of *copia*. However, we found no AATAAA, a typical signal for polyadenylation, in this LTR. The sequence AAATATAAATC is present ~20 nucleotides upstream from the poly(A) attachment site of the 5-kb RNA and in the region expected for the 3′ terminus of the 2-kb RNA (ref. 6; Figs 1, 2). This sequence, or part of it, may function as a polyadenylation signal in *copia*.

To determine the location of the 5′ end of VLP H-RNA, we carried out a primer extension experiment using total VLP RNA and ^{32}P-labelled, 26 nucleotide long synthetic oligodeoxynucleo-

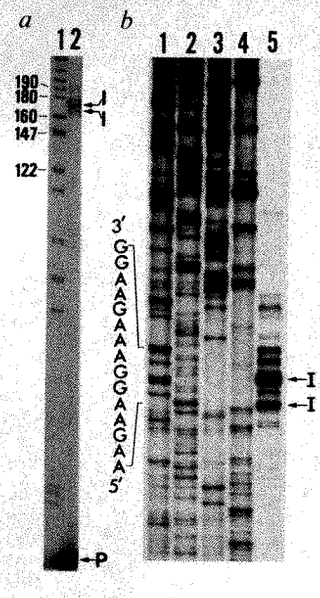


Fig. 3 Determination of the 5′ end of VLP H-RNA by primer extension. The expected locations of the 5′ ends of two major VLP H-RNAs are shown by arrows labelled I, whereas the arrow labelled P shows that of the 26-nucleotide-long primer used. *a*, pBR322 DNA was digested with *Hpa*II, labelled with ^{32}P by kinase treatment, denatured and used as a marker. Molecular sizes in the left margin are in bp. Using total VLP RNA (3 μg) and 26-nucleotide-long synthetic DNA (65 ng) (see Fig. 4*a*) as a template and primer, a primer extension experiment was carried out essentially according to Treisman *et al.*[18]. After the reaction, samples were denatured, applied to 45-cm-long 8% polyacrylamide gel in the presence of 7 M urea, and electrophoresed. Lane 1, pBR322 marker; lane 2, products of primer extension of VLP RNA with primer. *b*, Numbers in lanes 1–4 show the nucleotide sequence pattern obtained by the modified dideoxy-chain termination method when the DNA of plasmid 15 (see scheme *a* in Fig. 1) and the 26-nucleotide-long synthetic DNA identical to that used for primer extention of VLP RNA (Fig. 4*a*) were used as a template and primer. Lanes 1, 2, 3 and 4, respectively, show the locations of G, A, T and C residues. The sample in lane 5 is an aliquot of the reaction products shown in lane 2 in *a*.

tide (see Fig. 4*a*). Two intensive bands, 166 and 172 nucleotides in length, along with ~20 less intensive bands of similar size, were observed (Fig. 3), suggesting that VLP H-RNA is heterogeneous in the location of its 5′ end. By comparing the positions of these bands (Fig. 3*b*, lane 5) with the dideoxy-chain termination pattern of the 5′ terminal region of *copia* (lanes 1–4), we determined the range of the 5′ ends of VLP H-RNA (Fig. 1, overline *w*). According to Flavell *et al.*[6], the 5′ ends of cellular *copia* RNA are similarly heterogeneous in sequence and localized in an almost identical region in the 5′ LTR of *copia* (see Fig. 1). Taking together their findings and the results described above, we conclude that VLP H-RNA is an unspliced genome-sized transcript of *copia* (see Fig. 2). As with vertebrate retrovirus, VLP H-RNA has terminal redundancy, 77–116 nucleotides in length, and the *copia* LTR can be divided into three regions, 106–142 bp (U5), 77–116 bp (R) and 54–57 bp (U3) (Fig. 2). These findings provide additional evidence for the notion that *copia* RNA may be reverse-transcribable[1,8].

VLP H-RNA has weak messenger RNA activity and can be translated into polypeptides immunologically related to VLP proteins[1]. To identify the coding region for the predominant 31K VLP protein[1], the partial amino-acid sequence of one of the polypeptides obtained from the 31K protein was determined and found to be identical to that of the amino-terminal region of the polypeptide expected to be derived from ORFg of *copia* (or ORF1 of VLP H-RNA) (Fig. 4*b*). Furthermore, the calculated amino-acid composition of the M_r 31,000 amino-terminal

a 5′ ATTAGGCATGGACTGGGCCCATAACC 3′

b 31K VLP protein: Asp-Lys-Ala-Lys-(X)-Asn-Ile-Lys-Pro-
copia ORF1 protein (Amino terminus): Met-Asp-Lys-Ala-Lys-Arg-Asn-Ile-Lys-Pro-

Fig. 4 *a*, Nucleotide sequence of a 26-nucleotide-long synthetic oligonucleotide used as the primer. It is complementary to a portion of *copia* sequence (see underline *d* in Fig. 1, L1) and was synthesized using a Model 380A synthesizer (Applied Biosystems). *b*, Comparison of the partial amino-acid sequence of the predominant 31K protein of VLP and that of the amino-terminal region of the putative ORFg (or ORF1) protein of *copia*. X, an unidentified amino acid.

Methods. VLP was purified from a 12-l culture as described previously[1] and the predominant 31K protein purified by large-scale SDS-polyacrylamide gel electrophoresis. The 31K VLP protein was treated with cyanogen bromide[19] and the resultant polypeptides separated and purified by reverse-phase liquid chromatography (RP300 (4.6×250 mm), Brown Lee). The partial amino-acid sequence of the major polypeptide was determined by stepwise Edman degradation using a gas-phase automated sequencer (Model 470 A, Applied Biosystems). The recovery of other polypeptides was low because of self-aggregation.

portion of the putative *copia* ORFg (ORF1) polypeptide was found to be almost identical to that observed for the 31K VLP protein (data not shown). Thus, we suggest that the coding region for the 31K VLP protein is located in the 5′ terminal region of the ORFg (ORF1) (Figs 1, 2*b*) and the 31K VLP protein is one of the cleavage products of the putative polypeptide(s) expected to be produced from ORFg (or ORF1).

To determine whether *copia* has the coding sequence for the reverse-transcriptase-like enzyme associated with VLP[1], we compared the amino-acid sequences of ORFs 1 and 2 with those of the reverse transcriptases of retroviruses[9] and equivalent polypeptides of *Drosophila* transposons such as *17.6* (ref. 2). However, no appreciable homology could be seen except for Tyr-(Met, Leu, Val or Gly)-Asp-Asp(hydrophobic amino acid)$_3$, the most conservative sequence among various polymerases[10,11], near the carboxy-terminus of the ORFg (ORF1) polypeptide(s) (Figs 1, 2). This suggests that *copia* contains the coding region for the reverse-transcriptase-like enzyme, which has a different amino-acid sequence from the counterparts of other *copia*-like elements[2] and retroviruses[9]. Furthermore, this reduced homology partly results from the extensive rearrangement within the putative *pol* region of *copia* (the region between nucleotides 1,242 and 4,658 in Fig. 1; K.S. *et al.*, unpublished).

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Phosphorylation of the glucose transporter *in vitro* and *in vivo* by protein kinase C

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The Ca^{2+}- and phospholipid-dependent protein kinase (protein kinase C) is present in many mammalian tissues[1], and its important physiological protein substrates are only now beginning to be identified. A useful advance in identifying these intracellular substrates has been the recognition that the kinase is the receptor for phorbol esters, which stimulate phosphotransferase activity[2–4]. Phorbol ester-induced changes in protein phosphorylation in intact cells may thus be taken, in part, as a probable indication of protein kinase C activation. The many cellular effects of phorbol esters include the stimulation of glucose uptake[5–8], although the response of glucose uptake to phorbol esters appears to be complex, apparently varying in response time and requirement for protein synthesis[6,7]. Such observations prompted us to explore one possible explanation for the alteration of glucose uptake, namely, phosphorylation of the glucose transporter by protein kinase C. We report here that incubation of purified human erythrocyte glucose transporter with rat brain protein kinase C results in the phosphorylation of a protein of relative molecular mass (M_r) 50,000–60,000 which has subsequently been identified as the glucose transporter by specific immunoprecipitation with a monoclonal antibody. Immunoprecipitation of membrane proteins from ^{32}P-labelled human erythrocytes revealed a phorbol ester-stimulated phosphorylation of the transporter. This covalent modification of the glucose transporter may thus, in part, underlie the ability of phorbol esters and certain hormones to stimulate glucose uptake.

Incubation of a preparation of purified human erythrocyte glucose transporter with protein kinase C resulted in the phosphorylation of a protein that migrated on SDS-gel electrophoresis as a broad band of apparent M_r 50,000-60,000 (50-60K), together with higher-M_r species, some of which did not enter the SDS gel (Fig. 1*a*). The phosphorylation was absolutely dependent on the addition of Ca^{2+}; because the transporter preparation contained erythrocyte membrane phospholipids, added phosphatidylserine caused only a slight increase in phosphorylation. It has been shown previously that the transporter is a glycoprotein that migrates as a broad band of apparent M_r 50-60K on SDS gels, due to heterogeneity in its oligosaccharides[9,10]. In addition, on incubation with detergents, the protein has a tendency to form dimers and higher-M_r aggregates which is not prevented by reducing agents[10,11]. No phosphorylation of these bands was observed on incubation with casein kinase I, casein kinase II or the catalytic subunit of the cyclic AMP-dependent protein kinase (not shown). Because the preparation of glucose transporter used in these experiments was only 70–80% pure[11], the identity of the ^{32}P-labelled protein as the transporter was established unequivocally by specific immunoprecipitation with a monoclonal antibody directed against the erythrocyte transporter (antibody G3)[12] (Fig. 1*b*). The bands at M_r ~100,000 and at the top of the gel seen in Fig. 1*b* (and in Fig. 3*b*) are due to the dimer and the higher-M_r aggregates of the transporter[10,11].

Nearly 0.5 mol of ^{32}P-phosphate per mol cytochalasin B binding was incorporated into the transporter on prolonged incubation with protein kinase C (Fig. 2*a*). Although the purified transporter is contained in largely unsealed membranes of erythrocyte lipids[11,13], it is possible that not all of the potential phosphorylation sites are exposed to the kinase or that the preparation already contains phosphorylated amino acids. Limited acid hydrolysis of ^{32}P-labelled transporter and subsequent thin-layer electrophoresis revealed only ^{32}P-phosphoserine (not shown). HPLC separation of ^{32}P-phosphopeptides, generated by prolonged tryptic digestion, yielded only one ^{32}P-peptide (Fig. 2*b*) which also contained only ^{32}P-phosphoserine. These data support the conclusion that the phosphorylation is confined to a single protein in the transporter preparation and probably to a limited number of phosphorylation sites.

To determine whether protein kinase C phosphorylates the glucose transporter *in vivo*, human erythrocytes were labelled with $^{32}P_i$ and exposed for 20 min to phorbol 12-myristate 13-acetate (PMA), 4α-phorbol didecanoate (inactive phorbol; 4αPDD) or dimethyl sulphoxide (DMSO), the solvent used. We found that PMA, but not 4αPDD, stimulated the phosphorylation of two easily identifiable proteins in ^{32}P-labelled membranes (Fig. 3*a*). These seem to correspond to band 4.1 (M_r 80K), as reported previously in rabbit erythrocytes[14], and a minor ^{32}P-phosphoprotein of M_r 125K. ^{32}P-labelled membrane protein was solubilized and immunoprecipitated with the monoclonal antibody. Specific immunoprecipitation of ^{32}P-labelled transporter indicated that the transporter was phosphorylated in intact cells in the basal state and that the phosphorylation increased substantially in response to PMA (Fig. 3*b*).

These data suggest that phorbol ester-stimulated glucose uptake may be, in part, a consequence of protein kinase C-

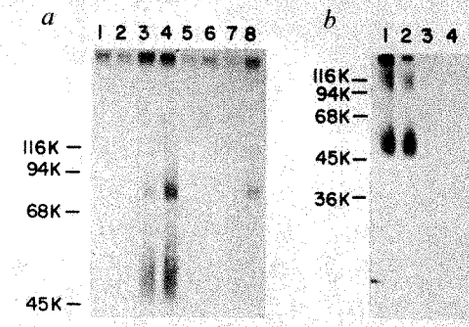


Fig. 1 *In vitro* phosphorylation of glucose transporter by protein kinase C (*a*) and ^{32}P-protein identification by immunoprecipitation (*b*). In *a*, human erythrocyte glucose transporter (55 μg ml^{-1}; lanes 1–4) or buffer (lanes 5–8) was incubated for 30 min with [γ-^{32}P]ATP (100 μM), $MgCl_2$ (10 mM) and protein kinase C (4,000 pmol units ml^{-1}) in the presence of EGTA (1 mM; lanes 1, 5), EGTA plus phosphatidylserine (PS) (40 μg ml^{-1}; lanes 2, 6), $CaCl_2$ (1 mM; lanes 3, 7), and $CaCl_2$ plus PS (lanes 4, 8). No phosphorylation of any protein was observed in the absence of added kinase. In *b*, aliquots of the incubations of the transporter with added kinase were immunoprecipitated with monoclonal antibody to the transporter (lanes 1, 2) or with control monoclonal antibody (lanes 3, 4), and the immunoprecipitates separated by SDS-gel electrophoresis before autoradiography. Lanes 1, 3, kinase reaction with $CaCl_2$ and PS; lanes 2, 4, kinase reaction with $CaCl_2$ alone. M_r markers are indicated on the left of *a* and *b*.

Methods. The glucose transporter from human erythrocytes was purified by the method of Baldwin *et al.*[11]. Protein kinase C was partially purified from rat brain through the gel filtration step by the method of Kikkawa *et al.*[1]; the kinase used in these experiments had a specific activity of 200 nmol per min per mg protein against histone III-S. Kinase assays were performed by the method of Parker *et al.*[4]. Immunoprecipitation was carried out by solubilization of the transporter (1 μg) in 1.5% octyl glucoside, followed by incubation of the samples on ice with 1.5% octyl glucoside/protein A-Sepharose to which 10 μg of the specific or control monoclonal IgG was complexed. The immune complexes were washed and then solubilized with 1.0% SDS in the absence of reducing agents and without heating. SDS gels were run according to Laemmli[24].

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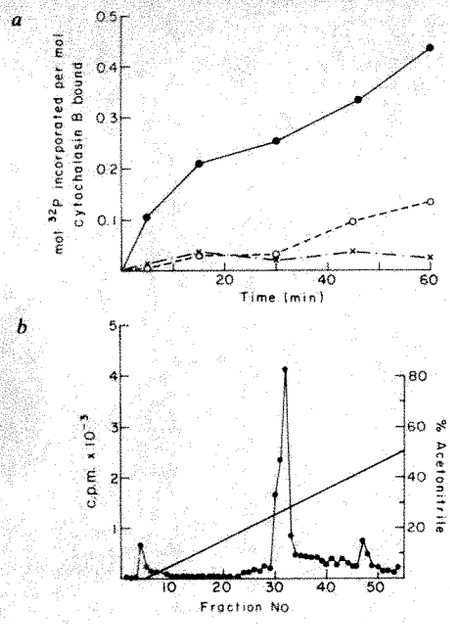


Fig. 2 Time course and stoichiometry of glucose transporter phosphorylation (*a*) and HPLC separation of tryptic ^{32}P-phosphopeptides (*b*). In *a*, the glucose transporter preparation was phosphorylated with protein kinase C in the presence (●) and absence (○) of added $CaCl_2$ and in the absence of added kinase (×), as in Fig. 1, and ^{32}P incorporation quantitated by trichloroacetic acid (TCA) precipitation in the presence of carrier bovine serum albumin. Control values for kinase autophosphorylation have been subtracted from total ^{32}P incorporation. Results are expressed as mol ^{32}P incorporated per mol cytochalasin B binding (taken as 1 binding site per mol functional transporter). Cytochalasin B is a high-affinity ligand for the transporter. Its binding provides a measure of the amount of functional transporter in a preparation, which is typically ~60% of the protein weight[11]. In *b*, phosphorylated transporter was precipitated with TCA, washed and digested with 20% trypsin (w/w) at 37 °C for 18 h. The solubilized ^{32}P-peptides were separated by HPLC with C_{18} reverse-phase chromatography using an ascending gradient of acetonitrile in H_2O/0.1% trifluoroacetic acid. When the same procedure was applied to a control reaction mixture containing only protein kinase C, no significant peak of ^{32}P-peptide was found.

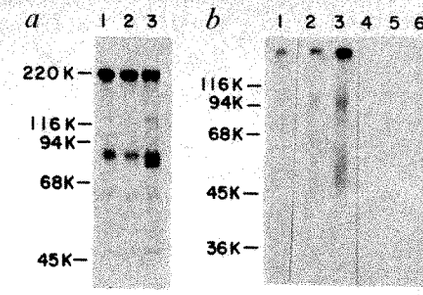


Fig. 3 Phorbol ester-stimulated protein phosphorylation of human erythrocyte membrane proteins (*a*) and glucose transporter, as determined by immunoprecipitation (*b*). In *a*, human erythrocytes were labelled with $^{32}P_i$, and membranes prepared by the method of Patel and Fairbanks[25]. Labelled cells were exposed for 20 min to phorbol 4α-didecanoate (1 μM, lane 1), DMSO (0.1%, lane 2) and phorbol 12-myristate 13-acetate (lane 3), before cell lysis. Membrane protein (20 μg) from each sample was separated on SDS gels before autoradiography. In *b*, membrane protein from the same samples was solubilized with 2% octyl glucoside, centrifuged to remove insoluble cytoskeletal aggregates and the soluble ^{32}P-proteins were immunoprecipitated with anti-transporter monoclonal antibody (lanes 1–3) or control monoclonal antibody (lanes 4–6). Lanes 1, 4, 4αPDD; lanes 2, 5, DMSO; lanes 3, 6, PMA. Preliminary experiments had established the presence of protein kinase C in human erythrocytes; although not detectable as a Ca^{2+}- and PS-stimulated kinase in red blood cell lysates, it was readily recognized following DEAE-cellulose separation of lysate proteins (not shown).

mediated phosphorylation of the glucose transporter. We are now investigating whether the phosphorylation of the erythrocyte transporter *in vivo* in response to PMA alters its activity and whether the stimulation of glucose uptake by PMA in other cell types is correlated with phosphorylation of the transporter *in vivo*. In addition to the possibility that such phosphorylation may directly affect the kinetic parameters of the transporter, in some cell types it may also lead to the redistribution of the transporter between the plasma membrane and intracellular membranes, such that a larger fraction of the transporter lies within the plasma membrane[15].

Evidence has been accumulating that epidermal growth factor and platelet-derived growth factor may lead to the activation of protein kinase C in intact cells[16–20]; this is often accompanied by an increase in phosphatidylinositol turnover, with the generation of diacylglycerol, an activator of the kinase[21]. Epidermal growth factor has also been shown to increase glucose uptake[22], while phorbol esters mimic the insulin-stimulated phosphorylation of the insulin receptor β-subunit on serine residues in IM-9 lymphocytes, suggesting that the kinase may be activated by insulin[23]. The present study suggests that the diverse effects of several hormones and phorbol esters on glucose transport may in part be linked through a common mechanism, phosphorylation of the glucose transporter by protein kinase C.

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Unfashionable continua

Michael Berry

An Idiot's Fugitive Essays on Science: Methods, Criticism, Training, Circumstances.
By C. Truesdell.
Springer-Verlag 1985. Pp.654. DM 158, $45 20.

TO DESCRIBE Clifford Truesdell as a theoretician specializing in the mechanics of continuous distributions of matter would, although strictly correct, be misleading. It would fail to convey what this collection of reviews, prefaces and lectures abundantly does convey, the broad scale of Truesdell's researches in mechanics and its history and his concern for and lack of sympathy with the way science is practised today, all so intensely felt and expressed with such mastery of language that my attention was gripped and held.

It is important to study continuum mechanics because

Matter is commonly found in bodies consisting of materials. Analytical mechanics turned its back on this fact, creating the centrally useful but abstract concepts of the mass-point and the rigid body... "modern" physics likewise turns its back, since it concerns solely the small particles of matter, declining to face the problem of how a specimen made up of such particles will behave in the typical circumstances in which we meet it. Materials, nonetheless, continue to furnish the masses of matter we see and use from day to day: air, water, earth, flesh, wood, stone, steel, concrete, glass, rubber, All are *deformable*. A theory aiming to describe their mechanical behavior must take heed of their deformability and represent the general principles it obeys.

That theory is continuum mechanics. In renovating its foundations during recent decades, Truesdell and his colleagues have reversed the customary approach:

From the time of NEWTON until recently, many natural scientists considered the mass-point the fundamental quantity of nature, or at least of mechanics. They believed that matter was composed of many small particles obeying the laws of classical mechanics, and that, consequently, the behavior of gross matter could be predicted, in principle, to any desired accuracy, from a knowledge of the intermolecular forces Thus continuum mechanics appears as an approximate or at best secondary theory within classical mechanics. While this tradition clings on in physics teaching today, it defies reality.... The smallest units of matter are no longer believed to obey Newtonian mechanics.... In fact it is almost the rule that *Newtonian mechanics, while not appropriate to the corpuscles making up a body, agrees with experience when applied to the body as a whole...* . Only paedagogical custom has hindered the realization that *as a physical theory, continuum mechanics is better than mass-point mechanics.*

This has been an unfashionable view:

... "physics", by definition, is become exclusively the study of the structure of matter, while anyone who considers physical phenomena on a supermolecular scale is kicked aside as not being a "real" physicist. Often "real" physicists let it be known that all gross phenomena easily *could* be described and predicted perfectly well by structural theories; that, aside from the lack of "fundamental" (i.e structural) interest in all things concerning ordinary materials such as water, air, and wood, the blocks to a truly "physical" (i e. structural) treatment are "only mathematical".

It is curious that these same persons are often sympathetic to non-structural theories on the borders of physics. They do not suggest that a mathematical physiologist investigating models for behavior would do better to try to integrate the equations of motion for the elementary particles making up a rat

That was written in 1962. Although the point is still worth making, its thrust is blunted by the increasing readiness of physicists to apply theories where they are appropriate, with less fundamentalist prejudice against the macroscopic. One of several reasons for this is the renewed appreciation and extensive development of Poincaré's discovery of great complexity (including chaotic behaviour) in the solutions of the equations of mechanics, even when, as with mass-points, the equations themselves are simple. I would have liked to learn Truesdell's opinion of this new branch of mechanics, but was disappointed to find no mention of it.

Penetrating reviews of books about the Bernoullis, Euler and Newton illustrate Truesdell's insistence that criticisms should be based on detailed study of original sources rather than the writings of commentators. It is a pity that he fails to live up to these standards when attacking "applied catastrophe theory" as a misuse of mathematical modelling; apart from a brief quotation (without reference) from Zeeman, his remarks are all based on articles by other critics of the theory, and he fails to mention those cases (even in continuum mechanics) where the mathematics has found valid application.

More generally, Truesdell laments the decline of scholarship:

The world of "learning" has become a federation of hives of frightened bees, who so as to maintain their little waxen prisms sting all strangers, be they lions or mice or only bees from the next-door office.

He detests the way science has become a large-scale organized enterprise, that is:

.. science for, by, and of the demos, in a word, *plebiscience*. Plebiscience demands what POST has called "inevitable research: . research which is bound to yield *some* results"...Plebiscience is *big science*. Small science was done by a few great men. Big science calls for many little men.

Worse might be to come:

Plebiscience is an intermediate stage. The next and last is *prolescience* In it not only is all research inevitable research, but also only outcomes previously known and accepted are allowed The function of prolescience will be to confirm and comfort the proletariat in all that it will by then have been ordered to believe. Of course that will be mainly social science.

But after pages of splendid raging, with sour prejudice alternating with flashing insight, he is not without hope:

Even now, I think, there are men who live for science, men who strive to bake not witches' mess but giants' bread What they deliver may be as tedious to the plebs as a lute, but let us not forget that while a cure for cancer may raise the mean age of the population to ninety, a good lute may last to make beautiful music under the hands of men and women yet unborn.

Amen. □

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Celebration of mass on the back of a whale, from an engraving dated 1621 The picture is reproduced from a reprint of the original, 1925 edition of Whale Ships and Whaling. A Pictorial History, *by George Francis Dow Publisher is Dover, New York, price is $8.95.*

Fifty years of the special senses

F. W. Campbell

Foundations of Sensory Science.
Edited by W. W. Dawson and J. M Enoch.
Springer-Verlag 1984. Pp.577. DM 288, $82.30.

THIS IS a collection of essays written by scientists who have accumulated age and wisdom and achieved success in their respective fields of neurosensory research The editors have encouraged them to clothe their accounts with personal details of how, and why, they chose a particular research direction. The result is a unique and fascinating volume, which it is unwise to start reading too late at night.

Today custom dictates that scientists report their findings in a cold impersonal style. Worse, most of us know that we often put our last experiment at the beginning of our papers, and the false-starts at the end, thereby giving a spurious impression of undue intelligence and rationality. As Edwin Land (of the Polaroid Corporation) once said, "An original idea only takes a few seconds cessation of stupidity". Many such seconds are brought to life in the book, in a relaxed manner which is much more illuminating than the formal prose of the scientific paper.

The 15 contributors are of international repute and they cover the entire range of special sense neurophysiology, including the relevant psychophysics.* Each describes their chosen problem as it was when they started as a young worker, and then unravels each twist of serendipity that put them back onto the successful track. The excitement and pleasure of the hunt are captured vividly, as are the dull and dispiriting periods which most researchers know all too well

The young, computer-orientated scientist of today may be surprised to learn that so many of the experiments described were done with simple, home-made apparatus; indeed, the most useful hobby seems to have been the building of short-wave radio and radar devices. However, it would be a mistake to conclude that research was straightforward in those times and, furthermore, that all the "easy" experiments have now been done. The picture that emerges is that the secret of research is to ask the right question at the right time and never to be discouraged by failure.

The authors are drawn from many different countries, and for almost all of them the Second World War had a dramatic impact on their workplace, personal life and future. Many of them were deflected into applied research, which they appeared to be equally good at, and in many cases their eventual field of study seems to have been determined simply by chance.

This elegant book provides a snapshot (with an exposure time of 50 years) of the foundations of the techniques which have enabled us to begin to probe the great, unsolved mystery of how animals sense their ambient environment. It is very well illustrated and included are most of the classical experiments up to the present time. The names index contains some 1,300 entries, and a newcomer to work on any of the special-senses would do well to begin here where they will be directed to the relevant seminal research. Moreover, the book brings together the sensory physiology of a very wide variety of species. One of the best starting points is Fig. 1 on page 382, where an illustration of the bombykol receptor cell of the moth will immediately lead you into the pheromone quest.

The editors, W. W. Dawson and J. M. Enoch, must have had a difficult time blending together the writings of such a variety of personalities. This they have done with an affection for experimental neurosensory science which runs through every page. □

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* "Comparative Physiology of Invertebrates. Hearing and Vision", H. Autrum, "The Development of Auditory Neurophysiology", H. Davis; "Microstructure of the Inner Ear", H. Engstrom; "Biophysics of the Mammalian Ear", J J Zwislocki, "Neurophysiology of the Retina", T. Tomita; "Recollections of Early Laboratory Experiments on Vision", L R Riggs; "The Perception of Light and Colour", W D. Wright; "Binocular Vision" and "Optics and Vision", G A Fry; "A Personal Vision", R. Granit; "Taste Electrophysiology, Sensory Coding, and Behavior", C Pfaffmann, "A Personalized History of Taste Biophysics", L. M. Beidler; "Insect Olfaction — Our Research Endeavour", D. Schneider; "Cutaneous Temperature Sensitivity", D. R. Kenshalo; "The Sensory Detection of Vibrations", W D. Keidel, "Fifty Years of Vestibular Science", O. Lowenstein.

More of the unseen Universe

Reuven Ramaty

Gamma Ray Astronomy.
By Rodney Hillier.
Oxford University Press: 1984. Pp. 202. £19, $29.95.

IN THIS rather brief volume, Dr Hillier has attempted to present the theoretical concepts, the principal observational results and the detection techniques of gamma-ray astronomy. After an introductory chapter and an account of astrophysical gamma-ray production, five chapters are devoted to detection techniques and four to observations. Thus most of the important aspects of gamma-ray astronomy are covered, but to me the book seems to lack the depth that would be expected from a modern work on the subject. Moreover many of the topics covered have been previously treated in Stecker's *Cosmic Gamma Rays*, published in 1971, and in Chupp's *Gamma Ray Astronomy* of 1976.

The book is not entirely up to date and contains some errors. The Crab nebula is powered by the Crab pulsar and not by ^{249}Cf (p.11). The energies of the line photons resulting from positron annihilation are equal to the electron rest mass energy and not to half of this energy (p.12). And the lifetime of the 4.43 MeV level in ^{12}C is less than 5×10^{-14} s, and not $\sim10^{-12}$ s (p.6) This difference has important astrophysical consequences regarding the width of the line, as was pointed out by Lingenfelter and Ramaty in connection with gamma-ray line emission from interstellar grains (*Astrophys. J.* **211**, L19; 1977).

There are also some omissions and some topics are covered rather sketchily, for example optical flashes from gamma-ray bursters. Even though optical observations technically do not belong to the realm of gamma-ray astronomy, Schaefer's discovery (*Nature* **294**, 722; 1981) of a historical optical flash in the error box of a gamma-ray burster has been one of the most important observational advances in this area in the past few years. As such, it should have been included in the book. Similarly, because of their close relationship to gamma-ray observations, neutron observations from solar flares (Chupp *et al. Astrophys. J.* **263**, L95; 1982) should also have been mentioned. Another topic not fully covered is the time variability of the 0.511 MeV annihilation line from the direction of the galactic centre. This variability, first reported by Riegler *et al. (Astrophys. J.* **248**, L13; 1981) effectively excludes multiple or extended positron sources, for example pulsars, supernovae, primordial black holes or cosmic rays. The reference to the work of Paciesas *et al.* (p.161) relates only to the variability of the continuum emission from the direction of the galactic centre, but not to that of the annihilation line.

Overall, this is a book to which students wishing to become acquainted with the subject can be directed (though some mathematical ability is needed to get the best out of it). On the other hand, because of the failings outlined above, I cannot recommend Hillier's *Gamma Ray Astronomy* to advanced graduate students or active researchers. □

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Doing the rounds of the Solar System

Carl D. Murray

Rings: Discoveries from Galileo to Voyager.
By James Elliot and Richard Kerr
MIT Press·1985. Pp.209.
$17.50, £19.50.

OBSERVATIONS of the occultation of star SOA 158687 by the planet Uranus on 10 March, 1977, were intended to provide information on the shape and atmosphere of Uranus An unexpected bonus for Jim Elliot and his team in the Kuiper Airborne Observatory was the first discovery of a ring system since the time of Galileo After more occultations, the spectacular successes of the Pioneer and Voyager spacecraft and the increased use of charge coupled devices (CCDs), astronomers now have the luxury of studying planetary rings on a comparative basis.

Even to document the recent discoveries and theories is a difficult task after such an influx of ground-based and spacecraft results. But the authors of this book do not merely present the observations together with the latest well-polished theories, they also treat the reader to a revealing and honest portrayal of scientific discovery, complete with rejected grant applications, rivalry and occasional good luck

In particular, the account of the spacecraft flybys captures the unique atmosphere of "instant science", when making discoveries "is just a matter of who's around". Explaining them can take a little longer. Theorists have had to turn their attention to such phenomena as narrow rings, eccentric rings, multiple rings and "spokes". The book relates how, during the Voyager 1 encounter with Saturn, some of the best theorists were unable to explain the "braided" appearance of Saturn's F-ring Although there were doubts that gravitational forces alone could account for such a feature, one of the faithful remarked, "Don't worry about Newton's equations, most people don't realize how many solutions they have" Current theories of the F-ring have shown that his faith in gravity was not misplaced.

Accounting for some features can lead to more fundamental problems Studies of satellite–ring interactions and the identification of spiral density waves have provided an explanation for most of the structure in Saturn's A-ring. However, the same studies reveal the possibility that unless some locking mechanism can be found we may have to come to terms with the fact that the rings are relatively short-lived phenomena.

The combination of first-hand experience and good journalism has resulted in an immensely readable account of how ring studies have evolved over the past three centuries Glimpses of the personalities involved and clear explanations of the various physical processes and ring theories help to convey the excitement of science This golden age of discovery is not over In January 1986 we can expect images of the Uranian rings from Voyager 2 Before the end of the decade we will have further information on the peculiar rings or arcs of material around Neptune and a look at Jupiter's ring from the Galileo spacecraft. There will be more discoveries, more puzzled astronomers, more theories and, we must hope, an equally fascinating sequel to this splendid book □

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Levels of dispute

Paul Hoyningen-Huene

Science and Values: The Aims of Science and Their Role in Scientific Debate.
By Larry Laudan
University of California Press·1984.
Pp.149. $15, £14.25.

SCIENCE, viewed as a social enterprise, has two complementary features: the formation of agreement from disagreement and the formation of disagreement from agreement. The task of explaining these features was made prominent more than 20 years ago by Thomas S. Kuhn in his *Structure of Scientific Revolutions*. Laudan takes up the subject once more, thus putting himself in competition with Kuhn and many other writers. Before addressing the resulting quarrels, we should look at the model that Laudan proposes.

Laudan distinguishes three levels of scientific commitment on which agreement or disagreement occurs: a "factual level", concerning claims about the world, a "methodological level", concerning claims about the correct way of doing science, and an "axiological level", concerning the basic cognitive aims of science (that is, those properties of theories that are constitutive of *good* theories). Traditionally, the interaction between these levels is seen as follows: disagreement on the factual level can be rationally resolved by recourse to the methodological level, and disagreement on the methodological level by recourse to the axiological level. The second of these steps is possible since methodological rules are instruments for realizing cognitive goals. The obvious difficulty which this hierarchical dependence of levels leaves unresolved is that disagreement on the axiological level cannot be eliminated.

Laudan adds more interactions to the traditional hierarchical dependence of the levels, resulting in what he calls the "reticulated model". In this model, the methodological level is also influenced by the factual level. Scientific methods can and must, he says, be judged also with respect to the factual level. what is already known about a subject matter obviously influences the choice of subsequent scientific methods. Second, the axiological level is connected to the other levels by feedback loops that sometimes allow for rational resolution of axiological disagreement. Explicit cognitive values — value statements — may, according to Laudan, rationally be criticized for being "utopian", that is for being incapable of application, or for not being consonant with shared exemplary scientific work. (At this point, however, Laudan appears to confound explicit value *declarations* with values displayed in actual scientific *practice*) The interdependence of the different levels in the reticulated model permits rational transitions between many of the eight combinatorially possible states of three-level agreement–disagreement.

Laudan's development of the model is accompanied by attacks on other writers in the field. Correspondingly, he declares his book to be a contribution to the "decanonization of a discipline's patron saints" (xiii), and his principal target throughout is T.S. Kuhn. But the creature decanonized by Laudan is largely a product of his own imagination For example, a central sin that Laudan identifies in Kuhn's work is what he calls "the covariance fallacy", the assertion of a rigid bond between changes on the factual and on the axiological level (43ff.) But Kuhn, in an article that Laudan repeatedly cites, has described at length a very different sort of association, one that invokes just the sort of delayed feedback loop that Laudan takes to be his own. Elsewhere, documenting Kuhn's distrust of rationality, Laudan attributes to him the view "that it was *perfectly reasonable* for Priestley to hold onto phlogiston theory" (pp.72–73, my italics). But what Kuhn said in the relevant passage is the reverse: "the historian can always find men — Priestley, for instance — who were *unreasonable* to resist for as long as they did" (*Structure*, p 159, my italics). These examples stand for a host of others. Scholarly debate should not be conducted in this way. □

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●The most recent volume to appear in Pergamon's *Key Environments* series is *Antarctica*, edited by W N Bonner and D W H Walton. Price is £14 95, $23.95 For review of the first three volumes see *Nature* 312, 205 (1984)

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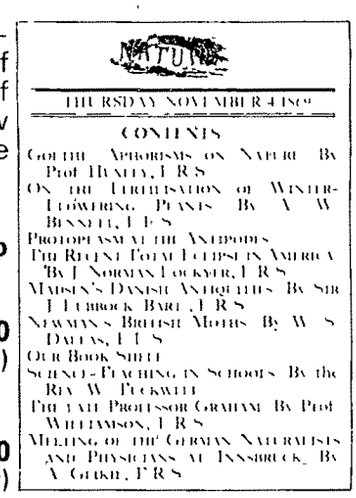
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COLLEGE OF MEDICINE

DEPARTMENT OF MEDICAL MICROBIOLOGY

RESEARCH OFFICER

required for work on a one-year project on the production of monoclonal antibodies commencing September, 1985.

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ROTHAMSTED
EXPERIMENTAL STATION
Harpenden, Herts AL5 2JQ

HIGHER SCIENTIFIC OFFICER

required by the Insecticides & Fungicides Department to work on techniques to delay insecticide resistance in the white fly. The post is funded by Ciba-Geigy AG for a period of two years.

Qualifications: First or Upper Second Class honours degree in either Entomology, Agricultural Science or Applied Biology with at least two years relevant post-graduate experience. A PhD would be an advantage.

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Applications giving full cv, the names of two referees and quoting Ref: 596 should be sent as soon as possible to The Secretary. Closing date 19 July 1985. Further particulars on request. (6948)A

THE UNIVERSITY OF LEEDS
FACULTY OF MEDICINE

MANAGER, LABORATORY ANIMAL SERVICES

Applications are invited for the above post available from 1 October 1985. Applicants should have not less than 12 years' experience in animal technology and appropriate management experience. The successful candidate will be responsible for the provision of laboratory animal services to the departments of the Faculty of Medicine. The service has a total technical staff of 26 and is responsible for a wide range of laboratory animals.

Salary on Grade II of the salary for Other Related Staff (£11,675 — £14,925) (under review).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no. 93/9.

Closing date for applications 24 July 1985. (6920)A

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(2) to work on projects involving molecular genetics of plant-microbial interactions —

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BIOTECHNOLOGY

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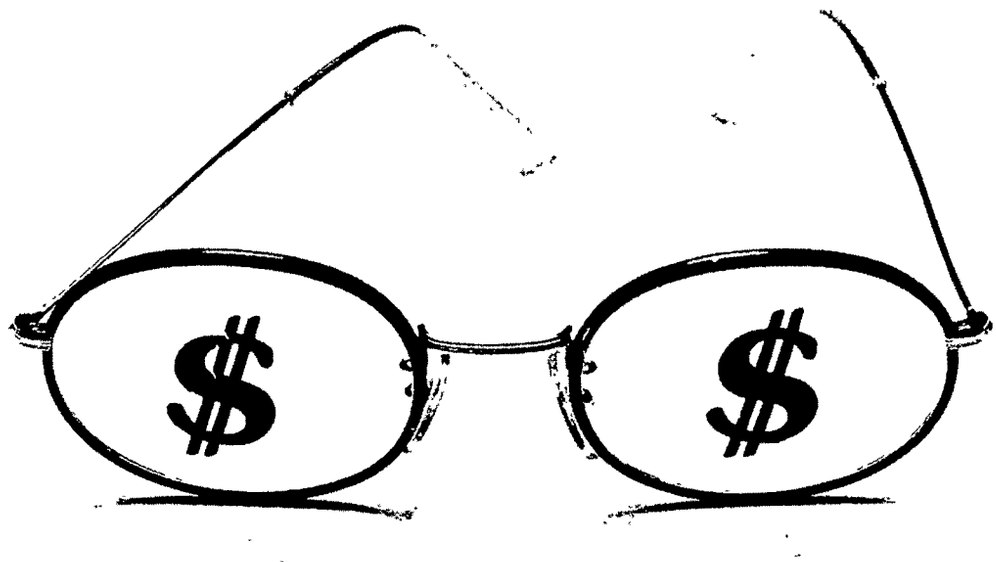
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A neuroscientist to help in the building up of a team to research into new antipsychotic drugs, several years post-doctoral experience, not necessarily gained in the pharmaceutical industry, is essential.

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UNIVERSITY OF NATAL
Department of Biochemistry
PIETERMARITZBURG

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The appointee will be expected to participate at all levels in the teaching programme including his/her own specialisation. In addition the appointee will be encouraged to become involved in active research. It is essential therefore that applicants have an interest in both teaching and research.

The minimum qualification for the post is a M.Sc. degree.

The appointment carries an attractive salary package, details of which are obtainable from the Staffing Section on request (telephone 0331-63320). The salary offered will be determined according to the qaulifications and/or experience of the successful applicant.

Application forms, further particulars of the post, and conditions of service, are obtainable from the Registrar, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, with whom applications on the prescribed form must be lodged no later than 31 July 1985, quoting reference PMB 43/85.

Application forms for overseas applicants are available from the Secretary, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. (W1968)A

BIBRA

DIRECTOR

The British Industrial Biological Research Association wishes to appoint a Director, as Chief Executive, in succession to Professor D.M. Conning, recently appointed as Director-General of the British Nutrition Foundation.

Founded in 1960 to carry out toxicological research, the Association currently is funded mainly by Government and the food and allied industries. The Association conducts multi-disciplinary research to develop methods of safety evaluation, supply information and prepare toxicological assessments for use by industry, government departments and regulatory authorities internationally.

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Applications, which will be treated in the strictest confidence, should be addressed to the Chairman of Council, Mr E.A. Flack at Grindsted Products Limited, Northern Way, Bury St. Edmunds, Suffolk IP32 6NP, England. (6914)A

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DEPARTMENT OF BIOLOGY
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Further details of the project and of research in the Department are available from the Postgraduate Tutor.

Applicants should possess a good honours degree in an appropriate subject. They should provide a curriculum vitae including full details of any experience relevant to the project and the names of two academic referees.

Applications should be sent to: The Postgraduate Tutor, Department of Biology, Oxford Polytechnic, Heading Oxford OX3 0BP.

CLOSINF DATE 12th July 1985.

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DEPARTMENT OF RESEARCH AND SPECIALIST SERVICES

MINISTRY OF AGRICULTURE

ZIMBABWE

As part of its plans for strengthening the research and extension services in agriculture, the Department of Research and Specialist Services requires scientists and technologists to work on three year assignments in the positions indicated below.

Since independence, the high level of services provided by the Department to the established commercial farmers has been maintained but special attention is being given to the needs of the small farmers and the resettlement and communal areas. The Department has responsibilities for the furtherance of the welfare and technical progress of Zimbabwe agriculture, for conducting research in agricultural science and crop and livestock production and for the provision of services to the agricultural industry. It is also entrusted with regulatory services. The Department employs a staff of over 350 professional and technical officers and some 1500 supporting personnel.

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— **IRRIGATION AGRONOMIST for LOW COST IRRIGATION SYSTEMS**
(To establish minimum requirements for a variety of crops, particularly wheat, cotton, vegetables and a wide range of tree crops)

LIVESTOCK AND PASTURES DIVISION

— **PLANT BREEDER FOR GRASSES**
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— **RANGE ECOLOGIST**
(To design trials for reclamation of degraded sandweld rangeland in communal areas)

— **RUMINANT NUTRITIONIST**
(To design research trials on nutrition of cattle and small livestock and review current research into fattening and intensified production systems)

— **MEAT TECHNOLOGIST**
(To develop training programmes for meat graders)

RESEARCH SERVICES DIVISION

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(To provide analytical service in soil physical properties, support soil survey staff by research on soil suitability for irrigation and other development and in rapid field assessment of permeability and moisture release)

— **SOIL MICROBIOLOGIST**
(To conduct research on microbiological aspects of rhizobial innoculants and coordinate work of the Inoculant Factory and Microbiological Laboratory)

— **PLANT ECOLOGIST**
(To provide vegetation inputs for natural resources survey of communal lands, involving aerial photograph & satellite imagery interpretation, computerised analysis of vegetation data and sampling techniques and to conduct research into use of vegetation in reclamation of degraded lands)

— **SENIOR AGRONOMIST/PHYSIOLOGIST**
(To devise technical packages for communal area research trials covering appropriate varieties, populations and fertiliser requirements for maize, field beans, sunflower, groundnuts, sorghum, soyabeans, pearl millet and cowpeas and an agronomy programme covering proper tillage methods, moisture conservation and resistance to waterstress of major field crops)

The training of local staff through in-service lecture courses, practical demonstrations and examinations, on the job training and supervision of post graduates and diplomate research studies is an essential and integral part of all assignments.

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Remuneration package includes US dollar salaries and allowances net of local tax, furnished housing, annual leave passages and assistance with education expenses. These appointments are funded by the World Bank and successful applicants will be contracted to AMEX Consultancy Ltd, who have been retained by the Government of Zimbabwe to support this important programme.

Applications in writing with full CV's and references to AMEX Consultancy Ltd, 28 Old Church Street, London SW3 5BY.

AMEX

Consultants in Agricultural Development Management and Marketing

(W1980)A

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Horsham, West Sussex

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To be responsible for, and to operate a flow cytometer within a broad range of applications in the areas of cell biology and molecular biology.

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Suitable candidates should have an interest in modern analytical and instrumental techniques, a basic knowledge of computing, and a sound background knowledge of immunology and cell biology.

Practical experience in the use of flow cytometry, although not essential, would be an advantage.

The salary and benefits package for this position is very attractive, and will include relocation expenses where appropriate.

Please send a letter of application, together with a full c.v. to:-

Miss S. J. Byrne, Personnel Officer, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex RH12 4AB.

(6981)A

CIBA–GEIGY



ANIMAL NUTRITIONISTS (2) VACANCY INT/001/85

The International Livestock Centre for Africa (ILCA) seeks two Animal Nutritionists to be based at Headquarters in Addis Ababa. One will be required to participate in ongoing research programmes on the evaluation of crop residues and the nutrition of draught animals. The other, who will be expected to travel extensively in various parts of Africa, will be concerned with an ongoing programme in the nutrition of trypanotolerant livestock and those exposed to trypanosomiasis.

Successful applicants will have PhD degrees in ruminant nutrition as well as several years experience in the field. Knowledge of French would be a considerable advantage for applicants for both posts.

Salary and related benefits will be in line with comparable international organisations.

Applicants should airmail personal history and names of three referees. Applications citing the above reference number should arrive to the Head of the Personnel Department, International Livestock Centre for Africa, P.O. Box 5689, Addis Ababa, Ethiopia before August 31, 1985.

(W1982)A

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

VIROLOGY DIVISION

Applications are invited for a postdoctoral position in initiate an independent programme to study microtubule mechanisms in the spatial organisation of cells. The candidates should have several years experience in relevant areas of cell biology, and be interested in collaborating with scientists with related research programmes. The Institute offers excellent facilities and opportunities for collaboration in this area at both molecular and cellular levels.

The appointment will be for five years and the salary will be at an appropriate point on the scales for University non-clinical academic staff, plus £1,233 London allowance and superannuation provision.

Applications, giving full curriculum vitae, research experience and the names of two professional references, should be sent to the Director, National Institute for Medical Research, Mill Hill, NW7 1AA before 26th July 1985, quoting ref: V/7/M. (6950)A

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Applications are being accepted for the position of Associate Scientist for a PhD in Immunology and at least 3 years of experience. The candidates selected will become an integral part of the research team involved with the study of cell-mediated immunity and viral diseases. The candidate should also have extensive knowledge in the study of coranaviruses, orthomyxoviruses, and cellular immunology.

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Forward résumé to: Patricia Menei, Personnel Dept., THE WISTAR INSTITUTE, 36th & Spruce Sts., Philadelphia, PA 19104. EOE. (NW1912)A

POSTDOCTORAL POSITION

available in a clinical immunology laboratory to study the ontogeny of the immune response with special emphasis on the relationship between differentiation and cancer. Minimum qualifications are a Ph.D. degree with a background in either immunology, biochemistry, cell or molecular biology. Funding starts 1 Sept. 1985.

Please send résumé, statement of research interests, and names of three references to: Dr. Florence Hofman, Department of Pathology, University of Southern California, Los Angeles, CA. 90033.

(NW1917)A

Microbiologists

Antibacterial Chemotherapy

The Wellcome Research Laboratories is the main centre for the research activities of The Wellcome Foundation Ltd., a major group of pharmaceutical and chemical companies with headquarters in the UK and a turnover close to £800m.

The major objective of the Department of Biochemical Parasitology is the discovery and development of novel drugs for the microbial and parasitic diseases of man, including tropical diseases.

We now have two challenging opportunities for experienced Ph.D. Microbiologists to join a multidisciplinary team committed to the rational design and development of new antibacterial agents.

Senior Microbiologist £11,400–£15,800

You will play a key role in the overall development and operation of the evaluative component of the team's effort and will also undertake personal research work pertinent to that area. Five years' post-doctoral experience in antibacterial chemotherapy, preferably in an industrial or hospital environment, is essential for this appointment. The ability to integrate effectively with scientists at all levels and from other disciplines will also be a prime requirement. Ref: WRL/347.

Microbiologist £9,400–£12,500

You will be one of a small group of microbiologists engaged in the evaluation of known and newly designed antibacterial agents at the whole organism level, both *in vitro* and *in vivo*. Flair for innovation, especially in the development of novel assays, plus 1 or 2 years' post-doctoral experience are essential for this position. This appointment will initially be for one year in the first instance, with probable renewal for at least 2 more years. There may be opportunity for transfer to an established position in the longer term. Ref: WRL/348.

We offer an attractive starting salary, dependant on experience, and our excellent benefits package includes five week's holiday, pension fund, sick pay scheme, subsidised restaurant and assistance with relocation expenses where appropriate.

Please write with full details, quoting the appropriate reference, to Dr H D Avshu, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS. (6983)A

Wellcome

X-ray Crystallography
Protein Chemistry
Protein Modelling

Cold Spring Harbor Laboratory is committed to a major new thrust in the study of protein structure. We are seeking to appoint either junior or senior staff members with expertise in the areas of X-ray crystallography of proteins (2 positions), protein chemistry (1 position) and protein modelling (1 position). The successful candidates will have demonstrated creativity and productivity in one of these areas. Applicants with interests in the areas of DNA-protein interactions, oncogenes, hormone receptors, growth factors or protein kinases will be especially welcomed. Interested persons should send curriculum vitae, statement of future research plans and names of referees to

Dr RJ Roberts
Cold Spring Harbor Laboratory
PO Box 100
Cold Spring Harbor, NY 11724

Cold Spring Harbor Laboratory

An equal opportunity employer

(NW1896)A

Structural Molecular Biologist

BROOKHAVEN NATIONAL LABORATORY

A staff position is open in the Biology Department for a structural molecular biologist with experience in the field of small angle X-ray scattering. This is an opportunity for a scientist to develop an independent program at the small angle scattering station of the National Synchrotron Light Source (NSLS). The scientist will also be responsible for the operation and development of the small angle scattering facility.

Candidates should submit a curriculum vitae that includes a list of publications and the names of three references, together with a statement of research interests, to: H.Z. Kondratuk, Biology Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, Long Island, NY 11973. Equal Opportunity Employer m/f.

BROOKHAVEN NATIONAL LABORATORY
ASSOCIATED UNIVERSITIES INC

(NW1904)A

The European Molecular Biology Laboratory, an international research institute situated in Heidelberg, West Germany, invites applicants for the following vacancy at the Laboratory's Outstation at the Deutsches Elektronensynchrotron (DESY) site in Hamburg

BIOCHEMISTRY LABORATORY ASSISTANT

The Outstation at the storage ring DORIS is dedicated to the use of synchrotron radiation in biological structure research The successful candidate will be responsible for maintaining the biochemical facilities used by visiting groups carrying out research projects at the Oustation for limited periods of time Besides this, there is an opportunity to participate in a limited in-house project on assembly properties of components of the cytoskeleton or on the structure of chromatin

Applicants should have an appropriate degree and advanced technical skills A knowledge of more than one of the official languages of the Laboratory (English, French, German) would be an asset

The net monthly salary will be between DM 2770, - and DM 3170, - Certain allowances are payable in addition, depending on personal circumstances An initial contract of 3 years' duration, which can be renewed, will be offered

Please write briefly for an application form, quoting reference 85/16 to
EMBL,
Personnel Section,
Postfach 10 2209,
D-6900 Heidelberg

EMBL

(W1978)A

Bristol Polytechnic

Science Department

Researcher A (2 Posts)

Applications are invited from candidates with or expecting to gain a first or upper second class honours degree in a relevant discipline. Both posts, funded by NAB, are tenable for three years from 1 October 1985 and the successful candidates will be expected to register for a higher degree with the CNAA.

Researcher A in Biochemistry

— Ref No R/59

To work on membrane bound enzymes of red cells thought to be involved in the molecular basis of the Tb syndrome. It will be carried out under the joint supervision of Dr K Cattell and Mr. T Williamson, Science Department, Bristol Polytechnic and Dr D Anstee, South West Regional Transfusion Centre, Southmead, Bristol.

Researcher A in Molecular Genetics

— Ref No R/60

To undertake genetic and molecular studies with the nitrogen-fixing actinomycete, **Frankia** sp under the supervision of Dr D Veal.

Salary Scale: £5,667 — £6,405 per annum.

For further details and an application form, to be returned by 12 July 1985, please contact the Personnel Office, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol or ring Bristol 656261, Ext 216 or 217.

Please quote appropriate Reference Numbers in all communications.

Avon County Council is an Equal Opportunities Employer.

(6925)A

About us
We belong to the foremost names of the research in the pharmaceutical industry.

Our Biochemical Research Centre is located in Tutzing on the Starnberg Lake, just south of Munich in an area of exceeding natural beauty.

In view of our expanding research activities in Biotechnology we now have some

Postdoctoral Positions

open – limited for two years

We are looking for Biochemists to work on a project involving the isolation of immunoglobulin genes and the construction of recombinant antibodies.

Applicants should have a strong background either in enzymology and/or protein chemistry or in recombinant DNA technology

Applications with curriculum vitae, description of research background and interests should be sent to

mannheim boehringer

Boehringer Mannheim GmbH
Biochemica Werk Tutzing
– Personalabteilung P-B –
Bahnhofstr. 9–15, 8132 Tutzing

(W1971)A

VISITING PROFESSORSHIPS IN FLORISTIC BOTANY

Under the terms of an endowment from Dr. and Mrs. L.R. Hesler, the Botany Department, The University of Tennessee, Knoxville, is able to support a limited number of visiting professors of floristic botany. Stipends will be determined by research proposals, and may be used to support salary and expenses in floristic, revisionary of monographic plant systematics.

Letters of inquiry may be sent to: Hesler Endowment Fund, Botany Department, University of Tennessee, Knoxville, TN 37916, USA. (NW1899)A

THE UNIVERSITY OF BRITISH COLUMBIA
DEPARTMENT OF MICROBIOLOGY
CELLULAR IMMUNOLOGIST

Applications are invited for a postdoctoral fellow to join a group working on the role of antigen specific immunoregulatory molecules in the immune response. The initial work will involve the establishment of T-cell lines and hybrids which produce or respond to these factors, the development of rapid assay systems and studies on the biology of these factors. This position offers an excellent opportunity to gain training in the application of molecular biological techniques to immunological problems.

Application with CV and names and addresses of two referees should be sent to Dr D.G. Kilburn, Department of Microbiology, The University of British Columbia, #300, 6174 University Boulevard, Vancouver, B.C. V6T 1W5. (NW1911)A

PLANT BIOLOGIST/BIOCHEMIST

Bristol £11,500–£12,700

An opportunity has arisen in our Research function in Bristol for a scientist to study the growth and physiology of tobacco cell cultures.

Candidates should have a degree in biochemistry, biology or a related subject, a sound knowledge of plant metabolic pathways and some experience of cell culturing techniques. The job demands a self motivated approach and considerable creative and innovative ability.

We offer a progressive salary scale starting at £11,500 and rising to £12,700, together with pension and sickness benefit schemes. Working hours are based on flexitime and we have superb sport and social facilities at our Athletic Club, as well as excellent on site staff facilities.

Please send full CV or write/telephone for an application form to Mr. A. W. L. Mortimore, Personnel Services, Imperial Tobacco Limited, P.O. Box 244, Hartcliffe, Bristol BS99 7UJ. Tel. Bristol (0272) 781111 Ext. 2206.

(6955)A

IMPERIAL TOBACCO LIMITED

Imperial Tobacco Limited

Imperial Tobacco is an equal opportunity employer.

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS (ICRISAT) near Hyderabad, India

invites applications for

PRINCIPAL PLANT PATHOLOGIST (PULSES)

To lead the pulses (chickpea and pigeonpea) pathology team in the development, execution, and reporting of results of plant disease research projects that meet the needs of the interdisciplinary, international Pulses Improvement Program, and participate in activities that contribute to the achievement of the Institute's research and training objectives. The major emphasis for disease control research is on the identification and utilization of stable and durable host plant resistance. A Ph.D. in Plant Pathology, and at least 7 years relevant research experience are required. Proficiency in English and a willingness to travel frequently in the chickpea — and pigeonpea — growing regions of the world are essential. Tropical agricultural research experience is desirable. The appointee will be based at the ICRISAT Center, Patancheru, 30 km NW of Hyderabad. Hyderabad offers good access by air to international airports and has good living conditions, recreational and medical facilities. Educational facilities exist for children to the age of eleven or twelve.

Salary at principal staff level, according to qualifications and experience. Send résumé, including present salary level, and names of three referees, via air mail, to:

Director General, ICRISAT Patancheru P.O., A.P. 502, 324, India

within 60 days from the date of advertisement.

(W1974)A

BIOGEN

is a biotechnology company with laboratories in Switzerland and the United States, developing commercial applications of molecular biology in the areas of agriculture, chemicals, pharmaceuticals and energy.

In Geneva a vacancy now exists for a

SCIENTIST

to work in the area of FERMENTATION TECHNOLOGY

The ideal candidate should:

- hold at least an M.SC. or equivalent;
- have experience of bacteria, yeast and fungi;
- have experience in laboratory and pilot plant fermentation techniques;
- have extensive experience in the use of computers for bacterial fermentation;
- be fluent in English and have good knowledge of French.

Please send your résumé accompanied by the names and addresses of at least three references whom we may contact on your behalf to

BIOGEN S.A.
Personnel Manager
46, Route des Acacias
1227 Carouge — Geneva
Switzerland

(W1967)A

Department of Agriculture and Rural Affairs

ANIMAL RESEARCH INSTITUTE, WERRIBEE, VICTORIA, AUSTRALIA 3030

AGRICULTURAL RESEARCH OFFICERS
(3 years) Three Positions

Salary Range: Science-3, $30,598 — $30,755 (2 positions)
Science-2, $26,697 — $29,167 (1 position)

We are seeking to appoint 3 people to undertake research into the management of livestock production systems using systems research techniques.

(1) SCIENCE-2, Position No. Ex.1039
(2) SCIENCE-3, Position No. Ex.1040

(Appointment is based on qualifications and experience)

More specifically, the successful applicants for these two positions will be required to develop computer programs for use by extension officers in formulating farm management decisions for either prime lamb or beef producers.

(3) SCIENCE-3, Position No. Ex.1041

More specifically, the successful applicant for this position will be required to identify opportunities for improving the profitability of wool production.

Applicants for all positions must have completed a degree or diploma in Science, Agricultural Science or an equivalent qualification.

It is desirable that applicants have an extensive knowledge of computing systems and simulation modelling; broad knowledge of plant and animal physiology and farm management practices; experience and expertise in computer systems and simulation studies, preferably in relation to agriculture, knowledge of Victorian or other prime lamb or beef production systems would be an advantage for positions nos. Ex.1039 and 1040; knowledge of Victorian or other wool production systems would be an advantage for position no. Ex.1041.

The successful applicants will be joining a small experienced team based at Werribee, with Statewide responsibilities for implementing the program

Employment in these positions is initially authorised for 12 months but is expected to be extended to 3 years.

Written applications are invited from men and women quoting the relevant position nos. and giving details of qualifications and experience and a contact telephone number. Applications should be forwarded to the Director of Personnel, Department of Agriculture and Rural Affairs, 166 Wellington Parade, East Melbourne, Victoria 3002, Australia, by the close of business on July 12, 1985.

For further information contact Dr. D. A. White on (03) 7411300.

(W1979)A

NEUROENDOCRINOLOGIST
Department of Physiology
QUEEN'S UNIVERSITY

A vacancy exists for an experienced neuroendocrinologist. The preferred applicant should have the Ph D and/or the M.D degree, several years teaching experience and a record of independent research of high quality. The applicant will be expected to lead an active research program and to participate in undergraduate and graduate teaching. The facilities for research are outstanding and the appointment is a regular one under tenure regulations. The appointment will be made at a level appropriate to the candidate's qualifications

Applications are encouraged from candidates of either sex. Applicants should submit a curriculum vitae, a brief statement of their research interests and objectives, selected reprints, and the names of three referees from whom a confidential assessment of research and teaching abilities may be obtained to: Dr. V. C. Abrahams, Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6. (Second Advertisement).

(NW1870)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions:

Research School of Biological Sciences

DEPARTMENT OF DEVELOPMENTAL BIOLOGY
POSTDOCTORAL FELLOW GRADE I/RESEARCH FELLOW

A post is available from 1 January 1986 for studies of the nature and function of cytoskeletal components of plant cells. Experience of techniques of protein biochemistry is required Postdoctoral experience is a prerequisite for appointment as Research Fellow. The appointee will be expected to develop independent and/or collaborative research programmes with other members of the Department who are using immunological and microscopical techniques to study the plant cell cytoskeleton. Knowledge of plant systems is desirable but applications from animal cell biologists will be welcome

Closing Date **1 September 1985** Ref: BS 6 6.1

DEPARTMENT OF NEUROBIOLOGY
POSTDOCTORAL FELLOW GRADE I/RESEARCH FELLOW

A position is available from late 1985 for analysis of the invertebrate nervous system by any of the modern single-cell techniques such as microelectrode recording, monoclonal techniques, neurochemistry or on-line data analysis of neuron activity. The appointee will be expected to develop independent and/or collaborative research programmes with other members of the Department. Two positions may be available.

Closing date. **1 August 1985** Ref: BS 6 6.2

VIRUS ECOLOGY RESEARCH GROUP
POSTDOCTORAL FELLOW GRADE I/RESEARCH FELLOW

A position is available from 1 January 1986 for studies of the molecular evolution of viruses. Experience of techniques of protein and nucleic acid biochemistry, especially nucleic acid sequencing, is required. Experience with viruses would be helpful but is not essential. The appointee will be expected to develop a collaborative research programme with other members of the Group, this might involve studies of viruses from one or other of the poxvirus, orthomyxovirus, tymovirus or tobamavirus groups.

Closing date. **1 September 1985** Ref: BS 6.6 3

CONDITIONS OF APPOINTMENT: Salary will be in accordance with qualifications and experience within the range. Research Fellow $A26,236-$34,467 p.a.; Postdoctoral Fellow Grade I $A22,614-$25,905 p.a. Current exchange rate $A1=UK 50p

Appointment, unless otherwise stated, will be: Senior Research Fellow/Research Fellow normally up to three years with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years.

Grants are provided towards travel and removal, though special conditions may apply to persons appointed to externally funded positions Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be sent to the Acting Registrar of the University, GPO Box 4, Canberra, ACT 2601, Australia. Further information also available from the Secretary General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W1983)A

PLANT BREEDING INSTITUTE
PLANT PHYSIOLOGIST

required for a period of two years, supported by EEC funding, for research on the drought resistance of dryland rice. The work will involve the testing of breeding lines of rice which differ in their capacity to accumulate abscisic acid and to measure the consequences for growth, tissue water relations and water use efficiency under conditions of limited water supply. Most of the work will be done at Cambridge but a working visit overseas will also be necessary.

Candidates should have a first or upper second class honours degree in an appropriate biological subject, with at least two years' postgraduate experience and a good knowledge of plant water relations.

Appointment will be at Higher Scientific Officer (£7,788 to £10,541 pa). Applications with c.v. and names and addresses of three referees should be sent to the Establishment Officer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, quoting **reference PHY/219** by 18th July 1985.

Further particulars available on request.

The Institute is an Equal Opportunities Employer. (6919)A

DIRECTOR

Herzberg Institute of Astrophysics
Ottawa, Ontario

The Institute

The National Research Council of Canada invites applications for the position of Director of its Herzberg Institute of Astrophysics whose headquarters are located in Ottawa, Canada. The Institute, which was created in 1975, is engaged in basic research in astrophysics and related topics. It carries out programs in optical and radio astronomy, optical and microwave spectroscopy, and space science.

In addition to its Ottawa laboratories, the Institute operates:
the Dominion Astrophysical Observatory in Victoria, British Columbia
the Dominion Radio Astrophysical Observatory at Penticton, British Columbia
and the **Algonquin Radio Observatory at Lake Traverse, Ontario** and it is a major participant in the support and use of **the Canada-France-Hawaii telescope.**

The Position

Reporting to the Vice-President (Physical Sciences and Engineering), the Director will be responsible for planning, organizing, implementing and controlling the Institute's programs and will advise management on long term research goals. The Institute has a personnel complement of approximately 200, of whom about 50% are professional scientists and engineers, and an annual operating budget of approximately four million dollars excluding salaries.

The Candidate

The person appointed will have an established reputation for outstanding leadership in research and the ability to lead, manage and coordinate the activities of the Institute. Candidates will have a doctorate in astronomy or related physical science and a distinguished record of achievement in science, as well as demonstrated ability in research management.

SALARY will be commensurate with qualifications and is supplemented by a competitive benefits package including relocation allowance.

PLEASE SENT DETAILED RÉSUMÉ IN CONFIDENCE TO:
General Manager
Personnel Branch
National Research Council of Canada
Ottawa, (Ontario), Canada, K1A OR6. (NW1897)A

National Research Council Canada | Conseil national de recherches Canada | Canada

INTERNATIONAL RICE RESEARCH INSTITUTE
POSITION ANNOUNCEMENT — ENTOMOLOGIST

The International Rice Research Institute announces a vacant position for Entomologist at its headquarters in Los Banos, Philippines. The candidate will provide leadership for research and training in host plant resistance. Duties include development of research methodologies using modern instrumentation; insect rearing techniques; research on various aspects of screening rice germplasm for resistance to insects; study of the nature and causes of resistance and emergence of insect biotypes.

The scientist should work closely with plant breeders on the development of insect resistant rice cultivars and in the study of the genetics of resistance to insects in plants; and should direct research programs of PhD, MS, and nondegree scholars.

Candidates should be preferably under 40 years of age, possess a PhD in agricultural entomology with training and experience in host plant resistance. The candidate must be a teamworker and be able to work closely with plant breeders and an inter-disciplinary group of scientists in an international setting. The candidate must have an appreciation of the role of host plant resistance in Integrated Pest Management and have a genuine interest in working with weed scientists, plant pathologists and other entomologists in the development of integrated pest control programs.

IRRI is dedicated to assist developing countries improve the productivity and stability of their rice farming systems. It has well equipped and well staffed laboratories and field research facilities.

Interested persons should apply by 25th July 1985 with a copy of their biodata, a summary of their research accomplishments, a list of their publications and names of three referees to:

Dr. M.S. Swaminathan
Director General
International Rice Research Institute
P.O. Box 933
Manilla, Philippines

(W1975)A

Applications are invited for a position in neurophysiology at the Assistant Professor level in the Department of Physiology at the University of Manitoba Faculty of Medicine. Candidates should have a PhD or equivalent, with two or more years of post-doctoral training. Preference will be given to individuals with experience and a continuing interest in electrophysiological analysis of neural networks and/or neurotransmitter actions using *in vitro* or *in vivo* systems Send curriculum vitae, an outline of future research plans, and arrange for three letters of reference to be forwarded to: **Dr. Henry G. Friesen, Professor and Head, Department of Physiology, The University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0WE Canada.** Deadline for receipt of applications is 1 September 1985 In accordance with Canadian immigration requirements this advertisement is directed to Canadian citizens and permanent residents.

(NW1857)A

THE DEPARTMENT OF GENERAL MICROBIOLOGY
of the
UNIVERSITY OF BERN
SEEKS A
PROFESSOR OF MICROBIOLOGY

with particular interests in molecular biology Applicants should be experienced in the area of genetics of eukaryotic microorganisms.

For further information contact Prof R. Braun, Department of General Microbiology, Baltzer-Strasse 4, 3012 Bern.

Applications including CV, list of publications, information of teaching experience, research activity and possible references should be sent by September 15, 1985, to Erziehungsdirektion des Kantons Bern, Abteilung Hochschulwesen, Sulgeneckstr. 70, 3005 Bern, Switzerland. (W1966)A

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS (ICRISAT) near Hyderabad, India

invites applications for

PRINCIPAL GROUNDNUT BREEDER

To assume the leadership of an ongoing international research program in groundnut breeding that aims to produce high-yielding cultivars with resistance on fungal and virus diseases, insects and other pests, and drought, and breeding for earliness, high yield, and quality factors To work in close collaboration with a multidisciplinary team to ensure adequate evaluation of breeding stocks for resistance traits and field performance To develop and evaluate international trials and cooperative projects with national and regional programs. A Ph.D. in Plant Breeding with at least 7 years research experience, an excellent command of written and spoken English, and a willingness and ability to travel frequently are essential. Experience in tropical agricultural and multidisciplinary team research and an ability to speak French are desirable. The appointee will be based at the ICRISAT Center, Patancheru, 30 km NW of Hyderabad Hyderabad offers good access by air to international airports and has good living conditions, and recreational and medical facilities. Educational facilities exist for children to the age of eleven or twelve.

Salary at principal staff level, according to qualifications and experience. Send résumé, including present salary level, and names of three referees, to.

Director General, ICRISAT Patancheru
P.O., A.P. 502 324, India

within 60 days from the date of advertisement.
(W1973)A

Director of In Vitro Fertilization Laboratory

ASSISTANT/ASSOCIATE PROFESSOR OF CLINICAL OB/GYN

Ph.D. with post doctoral training, experienced in in-vitro fertilization to develop new program. Interested persons are invited to submit vitae by September 15, 1985, to:

J.W. Roddick, M.D.,
Chairman
Department of OB/GYN
Southern Illinois University
School of Medicine
P.O. Box 3926
Springfield, IL 62708

SIU is an affirmative action equal opportunity employer. (NW1919)A

SIU

UNIVERSITY OF KEELE

'NEW BLOOD' LECTURER IN BIOCHEMISTRY (CELL BIOLOGY)

(re-advertisement)

Applications are invited for a 'New Blood' Lectureship in Biochemistry, tenable from October 1st 1985. Candidates should have experience in mammalian cell biology, preferably in areas related to those of Professor Lloyd's research group, which include pinocytosis, lysosome function and membrane transport. Salary in the range £7,520 — £14,925 pa. Applicants should be 35 or under at the commencement of appointment, and should not currently hold a permanent University appointment in the United Kingdom.

Further particulars and application forms are available from The Registrar, University of Keele, Staffordshire, ST5 5BG. Closing date for applications: 12 July, 1985.

Previous applications will be reconsidered, and re-application is unnecessary. (6918)A

UNIVERSITY OF ABERDEEN
Department of Bio-Medical Physics and Bio-Engineering

LECTURESHIPS (2) IN ELECTRONIC ENGINEERING

Applications are invited for two posts of LECTURER IN ELECTRONIC ENGINEERING applied to Medicine, each with active hospital involvement, to join team of international renown in the Department to design, build and teach modern microelectronic systems. One of the posts is responsible for technical staff on a preventive maintenance service for hospital electronic equipment. Practical people are required who like to roll up their sleeves and make it work.

Salary on Lecturer Scale: £7,520 — £14,925 per annum, with appropriate placing, according to qualification and experience (scale under review).

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 22 July 1985. (6921)A

ORGANIC GEOCHEMIST

STANFORD UNIVERSITY

The School of Earth Sciences is intent on adding a new program in organic geochemistry and is seeking an individual with a solid geologic perspective to lead this effort Current research in all four departments in the School including Applied Earth Sciences, Geology, Geophysics, and Petroleum Engineering involves work on sedimentary systems and we view organic geochemistry as an emerging tool of fundamental importance to these studies We also think that organic geochemistry constitutes a field of especially exciting potential for the future studies of the origin and fate of organic matter in modern and ancient sedimentary environments, chemical fossils, sedimentary hosted mineral deposits, petroleum geochemistry, as well as applications to study of the tectonic and sedimentary evolution of continental margins

Applicants for this tenure-track position should have an established research record involving application of organic geochemistry to geological questions, an enthusiasm for teaching, and a clear ability to garner and maintain support for their research program We invite applications from both junior and senior scientists but we are most interested in finding a mid-career scientist of international reputation who is stimulated by the challenge of establishing a new program within the unusually diverse intellectual and geological environment offered by the San Francisco Bay region, Stanford University, and the School of Earth Sciences

Applicants should send a letter outlining their research and teaching interests along with vita including the names of three individuals familiar with their work to

Prof. James C. Ingle, Chairman, Search Committee,
Department of Geology, School of Earth Sciences,
Stanford University, Stanford CA 94305

The level of this appointment is open and may be at Assistant, Associate, or Full Professor depending upon the individual selected Application deadline is September 30, 1985

Stanford University is an Equal Opportunity Employer and we especially encourage applications from women and minority students (NW1882)A

Southampton THE UNIVERSITY

PROFESSOR OF HUMAN MORPHOLOGY

Applications are invited from graduates in medicine or science for an established Chair of Human Morphology. Further details may be obtained from the Secretary and Registrar, the University, Southampton, SO9 5NH, to whom applications (10 copies from persons in the UK) should be sent before 23 August 1985. (6930)A

TWO POSTDOCTORAL POSITIONS

are available immediately for a Ph.D. biochemist and a molecular biologist to participate in studies of gene regulation by steroid receptors. Projects include the purification and characterization of human estrogen and progesterone receptors, cloning of the corresponding genes and preparation of monoclonal antibodies. Experience with steroid receptors, protein purification and analysis, monoclonal antibody production and/or recombinant DNA techniques is desired. Salary is negotiable.

Send curriculum vitae and three letters of references to: Dr Geoffrey L. Greene, The University of Chicago, Ben May Laboratory for Cancer Research, 5841 S. Maryland, Chicago, Illinois 60637. (NW1903)A

Section Leader

Molecular Biology Development

attractive salary + car : Rural South Bucks

Amersham International, a highly successful British company is a market leader producing a wide range of high technology products for life science research, medicine and industry.

We now have an interesting opportunity for an innovative scientist to join our Molecular Biology Development section and lead a team of about ten dedicated professionals in the development of a wide range of novel products to facilitate advances in this field.

To meet the demands of Biotechnology, the section has developed a number of products for DNA sequencing, nick translation, in vitro RNA synthesis, in vitro packaging, in vitro protein synthesis, vectors and hybridisation membranes, as well as its renowned high quality radiochemicals. The section plays an increasingly important role in the Division's business strategy and is currently developing an important range of new products.

Reporting to the head of Molecular Biology Development, you will be responsible for helping to identify new product opportunities and for developing these through your team to the successful launch of new products.

Probably a post-doctoral scientist with several years' experience in recombinant DNA technology you must be able to demonstrate a blend of managerial skills and appropriate technical knowledge vital for this position.

Salary will reflect both qualifications and experience and will be enhanced by a benefits package which includes 25 days' holiday, a generous pension scheme, and relocation assistance, where appropriate.

Please write, detailing your career to date, to Simon Voake (SV) at the address below or telephone Little Chalfont (02404) 4488, ext. 3569 for an application form.

Amersham International plc (6985)A

Amersham Laboratories
White Lion Road, Amersham
Buckinghamshire

Amersham

UNIVERSITY OF MUNICH
Department of Physiology
NEUROPHYSIOLOGIST

16 month postdoctoral position available 9/85 to study neuronal plasticity and epileptogenesis in mammalian brain slices.

Curriculum vitae and names of 2 referees to:

Dr. N. Galvan, Physiologisches Institut, Pettenkoferstr. 12. 8000 Munich 2, W. Germany. Tel: 0049-89-5996250. (W1970)A

POSTDOCTORAL POSITION

available Sept. 1, 1985 for analysis of genes of the chicken major histocompatibility complex (MHC). Background in immunology, genetics and/or biochemistry required.

Send curriculum vitae and names of three references to: Dr. Carol M. Warner, Department of Biochemistry, Iowa State University, Ames, IA 50011. (NW1916)A

POSTDOCTORAL POSITIONS
HOWARD HUGHES MEDICAL INSTITUTE/ PHILADELPHIA

Fully funded postdoctoral positions in molecular biology and molecular genetics are now available at the Howard Hughes Medical Institute of the University of Pennsylvania. Active research projects include: transcriptional and translational control of eukaryotic gene expression, mechanisms of cellular transformation and gene amplification, molecular mechanisms of chromatin inactivation and the application of molecular genetics to studies of inherited disease.

For infomation please contact:
Dr. Steve Liebhaber, Coordinator
Howard Hughes Medical Institute
Department of Human Genetics
Medical Labs Building/G3
University of Pennsylvania School of Medicine
Philadelphia, PA 19104 (NW1869)A

HEAD OF DEPARTMENT OF GEOLOGY & PHYSICAL SCIENCES

Oxford Polytechnic invites applications from people qualified to provide academic leadership in this key department which offers courses in geology, physical sciences, physics and chemistry. Applicants should have a substantial record in research. Appropriate industrial experience would be an advantage.

The post is vacant from 1 September 1985. Salary will be in the range £17397 — £19170 per annum (under review from 1 April 1985)

Further particulars and application forms may be obtained from the Staffing Office, ext 404

Closing date for applications 12 JULY, 1985

oxford polytechnic

Gipsy Lane, Headington, Oxford, OX3 0BP
Telephone: Oxford 64777

AN EQUAL OPPORTUNITIES EMPLOYER

SUSSEX CLINICAL RESEARCH CONSULTANTS LIMITED

has vacancies for

CLINICAL RESEARCH ASSOCIATES

to assist with contract clinical research projects in both Phase III and Phase IV. Previous experience is essential. These posts would suit graduates in the life sciences, and will make demands on their management abilities.

Salary £9,500 — £10,000 + car.

Application with full curriculum vitae to:
NICK PLATT, SCRC LTD, LOXFIELD CHAMBERS
GRANGE ROAD, UCKFIELD, EAST SUSSEX TN22 1QU
Telephone: 0825-5025. Telex: 878110G
(6937)A

FACULTY POSITIONS IN ELECTRICAL ENGINEERING (SOLID STATE DEVICE AND MICROELECTRONICS AREA)

The Pennsylvania State University at University Park, Pennsylvania invites applications for tenure track faculty positions at the Assistant Associate and Full Professor ranks in Electrical Engineering in the area of Solid State Device and Microelectronics Applicants should have an earned doctorate in electrical engineering, engineering physics or related fields, and have demonstrated the ability to conduct research and teaching in the field of Solid State Device and Microelectronics

The successful candidates are expected to join the existing effort in the solid state device research and help develop it into one of prominence. The College of Engineering has made substantial commitments to support this program by hiring of an endowed professor in this area

Currently, research in the solid state device and related materials area includes research in silicon device processing, such as plasma and reactive ion etching, thermal nitridation of silicon and various oxide research including ultra-thin oxides, and defect control. In addition, research in III-V device area includes material defects, electrical noise and performance improvement of micron and sub-micron stuctures

Combined research in the solid state device area typically amounts to about one million dollars annually Collaborative research opportunities exist with the Materials Science department, Engineering Science and Mechanics department, the Chemistry and the Physics departments and the Materials Research Laboratory at Penn State.

Résumés, or inquiries should be directed to the following address by no later than September 15, 1985, or until suitable candidates are identified

Advisory Committee
Department of Electrical Engineering
Box H, 129 Electrical Engineering East
University Park, PA 16802

An Equal Opportunity/Affirmative Action Employer (NW1909)A

UNIVERSITY OF DUNDEE

Ninewells Hospital and Medical School

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

POST-DOCTORAL SCIENTIST (IN VITRO FERTILIZATION)

A Post-Doctoral Scientist, with a PhD in some area of biological or medical science, is required to undertake research and supervise a laboratory for in vitro fertilisation in the treatment of human fertility within the Division of Obstetrics and Gynaecology in a newly funded Unit of Reproductive Medicine. Equal emphasis is placed on the research potential of IVF, cryopreservation, and other areas relevant to infertility

A cytogeneticist as well as supporting technical and clerical/secretarial staff are also to be appointed and the on-site facilities, which are excellent, include an endocrine laboratory.

The appointment is available at this stage for three years and will be made within the Research and Analogous Staff Range IA scale (currently £7,520 — £12,150 but due for revision) with initial placing dependent of qualifications and experience

Enquiries about the post may be made to Dr John Mills at Ninewells Hospital and Medical School (telephone Dundee (0382) 60111)

Applications (2 copies) containing full career details and the names of 3 referees should be sent by 11th July 1985 to the Personnel Officer, The University, Dundee DD1 4HN. Please quote reference EST/72/85J (6922)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS Centre for Applied Microbiology and Research

VACCINE RESEARCH AND PRODUCTION LABORATORY

Basic Grade Microbiologist

A research programme has been initiated to develop a live attenuated vaccine against Rift Valley Fever. The programme is a collaborative project between VRPL in CAMR and the NERC Institute of Virology at Oxford The work at CAMR will fall into two categories Firstly the location of the genetic material coding for the attenuated virus phenotype will be determined. Secondly, in vivo model systems for Rift Valley Fever will be used to compare the pathology of virulent and attenuated virus strains.

Applicants should have a good degree in Microbiology and/or Biochemistry. Preference will be given to candidates with previous research experience in animal virology

The post will be for 3 years and further details can be obtained from Dr J R Stephenson (Tel. (0980) 610391)

Salary: £6,559 rising to £7,063 by annual increments

National Health Service terms and conditions will apply.

Closing Date: 31 July 1985

Application forms are obtainable from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG. Telephone Idmiston 610391.

Please quote Post No 72 when applying (6924)A

UNIVERSITY COLLEGE CORK

RESEARCH POSITIONS IN MICROBIOLOGY/ BIOTECHNOLOGY

POSTDOCTORAL RESEARCH ASSOCIATES (2)

To work on projects involving molecular genetics of plant-microbial interactions —

(a) To investigate genes involved in carbon metabolism in rhizobium (Genetic Engineering)

(b) To investigate the regulation of carbon metabolism in Rhizobium and plant growth promoting pseudomonas SPP. (Biochemical-Genetics/ Physiology)

Positions are available from 1 July 1985 and salary will be in the range - IR£8,596 — IR£11,173 (less PRSI contribution) according to experience.

Applications including a cv and the names of two referees should be sent as soon as possible to Dr F O'Gara, Microbiology Department University College, Cork, Ireland, from whom further particulars are available. (6885)A

UNIVERSITY OF DURHAM

LECTURESHIP IN THEORETICAL PHYSICS

Applications are invited for a 'New Blood' Lectureship in Theoretical Particle Physics to be held jointly in the Departments of Mathematical Sciences and Physics and tenable from 1 October 1985 or as soon as possible thereafter. The successful applicant may work on any aspect of theoretical particle physics.

Salary on the scale £7,520 — £14,925 with USS (under review)

Applications (3 copies) naming three referees should be sent by 19 July 1985 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (6927)A

RESEARCH ASSOCIATE

Position available as of 1 October 1985 for the duration of up to 5 years (the salary will be in the range of DM 65.000, — to 70 000, — according to BAT II/III tariff) The project involves investigation of primary processes of water cleavage in photosynthesis Method: pulse spectroscopy, biochemistry, ESR, etc.

Applicant should have a very good degree in chemistry or physics Knowledge of German is desired Possibility for further qualification (ie PhD) is given

Applications with two references should be addressed to: Prof Dr H T Witt, Max-Volmer-Institut for Biophysical and Physical Chemistry, Technische Universitat Berlin, Strasse des 17 Juni 135, 1000 Berlin 12 (W1972)A

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF BIOCHEMISTRY

PROTEIN ENGINEERING OF GLUTAMATE DEHYDROGENASE

A postdoctoral research assistant, supported by SERC is required from 1 October 1985 to join a multi disciplinary team involved in a programme of site directed mutagenesis on the enzyme Glutamate Dehydrogenase The appointee would be expected to work primarily in the area of molecular stucture determination with the aim of predicting and analysing the effects of mutations on enzyme structure and function Applicants should have experience in crystallography, preferably protein crystallography.

The appointment will be for a fixed term of three years with the initial salary in the range £7,520 — £8,920 on scale 1A for Research and Analogous staff Applications including a copy of a curriculum vitae and the names of three referees should be sent to Dr D W Rice at the Department of Biochemistry, the University, Sheffield S10 2TN, not later than 19 July 1985 Quote ref. R295/G (6933)A

THE UNIVERSITY OF SUSSEX

LECTURER IN PLANT PHYSIOLOGY

in the School of Biological Sciences, from 1st October 1985 to 30th September 1986. Applications are invited from qualified men and women with a primary interest in Environmental Physiology

Salary on the Lecturer scale £7,520 to £14,925 pa.

Send self-addressed envelope (6" × 9") for further particulars and an application form to Mrs L Vivian, Personnel Office, The University of Sussex, Falmer, Brighton, BN1 9RH. Closing date for applications Friday 19th July 1985 (6979)A

UNIVERSITY OF LONDON

British Postgraduate Medical School

THE CARDIOTORACIC INSTITUTE at the BROMPTON HOSPITAL

CYSTIC FIBROSIS DEPARTMENT

MLSO

Required for routine and research work involving contact with patients. Experience in immunological techniques desirable Salary depending on age and experience, range £5,738 — £6,726 + £1,084 London Weighting. The post is for one year initially.

Applications, together with curriculum vitae and names and addresses of two referees to The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP by 8 August 1985 (6977)A

THE ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen, AB2 9SB

MICROBIAL GENETICIST/ BIOCHEMIST HSO/SSO GRADE

Applications are invited for this post in the Microbial Biochemistry Department

The Rowett Research Institute is the foremost agricultural institute in the UK carrying out fundamental research into the nutrition of man and farm animals. The Microbial Biochemistry Department contributes to this programme in studying the microbiology of the alimentary tract as it relates to the nutrition of the host animal A highly innovative young scientist is required to initiate studies which will examine the rumen from a new angle, that of a genetic ecosystem Areas likely to be explored in the first instance are the spread of resistance factors to feedlot antibiotics in vivo and in mixed cultures in vitro, and the regulation of expression of enzymes involved in the breakdown of plant fibre and protein

The successful applicant will probably be a PhD graduate with some postdoctoral experience. A genetics background might be helpful but is not essential More importance is attached to the scientific ability of the candidate Scientists with a more general microbiology, biochemistry and molecular biology experience are therefore encouraged to apply. Training in rumen microbiology will be given within the Institute, and it is envisaged that a proportion of the work in the first year will be done at a leading microbial genetics laboratory in the UK.

Qualifications— First or upper second class honours degree in Microbiology, Biochemistry or related subject with at least 2 years relevant post-graduate experience for HSO grading and 4 years for SSO

Salary Scales.— HSO £7,788 to £10,541 pa, SSO £9,772 to £12,653 pa, with placing according to experience.

The position is Superannuable and the Institute is an Equal Opportunities Employer.

Applications in writing together with full CV and the names/ addresses of 2 referees should be sent to the Secretary of the Institute from whom further particulars are available. Tel. 0224 - 712751 (Ext 338).

Closing date 18th July 1985. (6917)A

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
DEPARTMENT OF PSYCHOLOGY
Brain, Behaviour and Psychiatry Research Group

POSTDOCTORAL RESEARCH WORKER AND RESEARCH STUDENT

to work on the application of electrochemical methods (in vivo intracerebral voltammetry) to the study of behaviour in the rat Applicants should have training in either the biological aspects of psychology or biochemistry, electrochemistry or pharmacology

Salary for the two-year postdoctoral appointment (MRC-supported) will be £7,520 — £9,860 pa plus £1,233 London Allowance on the 1A scale for research workers, the Research Studentship will be on the same terms as an MRC Studentship.

Application forms for the Research Worker are available from the Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF quoting Ref JG/N.

Applications for the Research Student, with the names of two referees, should be sent to Professor J A Gray, Department of Psychology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF

Closing date for applications 12 July 1985. (6940)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL
(University of London)
DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH FELLOW and RESEARCH TECHNICIAN

Applications are invited for the above posts for a 3-year project on the action of heparin and heparin sulphate and their derivatives on vascular smooth muscle cell matrix synthesis. Starting salary up to £8,920 pa plus £1,233 pa London Allowance for the Post-doctoral post and up to £6,420 pa plus £1,084 pa London Allowance for the Technician depending on age and experience

Applicants should have a degree, or equivalent, in biochemistry, cell biology or related discipline Previous experience in tissue culture and/or extracellular matrix biochemistry would be an advantage. Further details from Dr R M Mason, Tel: 01-741 1958.

Applications including a full curriculum vitae and the names of two academic referees should be sent to The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London, W6 8RP, as soon as possible after the appearance of this advertisement (6936)A

THE UNIVERSITY OF NEW ENGLAND
Department of Biochemistry, Microbiology & Nutrition

RESEARCH INTO MODIFICATION ON RUMEN FERMENTATION

SENIOR POSITION — MOLECULAR BIOLOGIST

Equal Opportunity is University Policy

A Molecular Biologist is required to establish a research group to study manipulation of rumen microbes using recombinant DNA techniques with a view to increasing fermentation of lignified forages.

This new group will join an existing group of nutritional biochemists and microbiologists led by Dr J. Nolan and Professor R. Leng. Dr T. Bauchop recently accepted appointment as Senior Microbiologist with the group.

The appointment is available immediately. It will be for three years initially, when the whole programme will be reviewed by the Australian Meat Research Committee which is funding the project.

The appointee will be expected to attract and supervise postgraduate students as part of his/her duties

Applicants must have extensive experience, research ability and a publication record appropriate to senior levels of appointment.

Informal enquiries to Professor Leng 067 73-2707.

Appointment will be dependent on experience, qualifications, and ability in one of the ranges:

Professorial Fellow	**A$54,948 pa**
University Fellow	**A$46,397 pa**
Senior Research Fellow	**A$35,203 — A$41,029 pa**

Assistance with travel and removal expenses available.

CLOSING DATE: 1st August 1985
POSITION NO: 502

Applications including the names and addresses of three referees and stating the position number should be sent to the Staff Officer, University of New England, Armidale N.S.W. 2351, prior to the closing date. Applicants should forward a copy of this advertisement to their referees and ask them to send their reports direct to the Staff Officer, Mr B.G. Turner, marking their envelopes 'Private and Confidential' and quoting the position number, before the above closing date so as to expedite the appointment process. (W1981)A

THE NATURE CONSERVANCY COUNCIL

HIGHER SCIENTIFIC OFFICER (Freshwater Botanist)

The Scottish field unit and the Chief Scientist's Directorate intend to appoint a botanist to carry out a survey of freshwater loch on Speyside and in the N of Scotland The contract, which will be for approximately two years in the first place, but is likely to be rewarded for at least a further year, will be based at Edinburgh

Qualifications and Experience: Applicants should have a good degree in botany or another appropriate biological subject, and at least two years postgraduate experience of freshwater survey work They must have a good working knowledge of water-plants and be able to drive.

Salary — Depending on qualifications and relevant experience, £7,435 rising to £10,039.

For further details and an application form please contact Mr S Woodfield, Personnel 1, Nature Conservancy Council, Northminster House, Peterborough, PE1 1UA to whom completed application forms should be returned by the 18 July 1985.

The Nature Conservancy Council is an equal opportunity employer. (6935)A

MEDICAL RESEARCH COUNCIL
Dunn Nutrition Unit Cambridge

SCIENTIST

Applications are invited from Post Doctoral Scientists with experience in human energy metabolism, to join a team studying nutrition and health in our field station in rural Gambia. The proposed studies involve the use of a whole-body indirect calorimeter to investigate physiological adaptations to low energy intakes with particular emphasis on pregnancy and lactation The successful candidate will be expected to take joint charge of these studies and to co-ordinate them with our programme of stable isotope research.

The appointment will initially be for two years of which 2-3 months will be spent in Cambridge and Lausanne, Switzerland, undergoing training in calorimetery techniques The remaining time will be spent in our Gambia field station with six weeks home leave after one year. Depending on progress there should be a possibility of extension for a further two years The appointment will start in October 1985. Salary in the range of £8,920 — £11,205 plus overseas allowances

Please send applications with a full cv and the names of three referees to Dr R G Whitehead, MRC, Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ

Closing date 15 July 1985 (6971)A

Please mention
nature
when replying to these advertisements

LEONHARD PROFESSORSHIP IN SOLID STATE DEVICE ENGINEERING

Applications and nominations are invited for a recently endowed professorship in Solid State Device Engineering within the Department of Electrical Engineering at The Pennsylvania State University. Applicants should have an earned doctorate in electrical engineering, engineering physics, or a related field, and have demonstrated the ability to conduct research and teaching in the field of Solid State Device and Microelectronics Strong leadership qualities are also emphasized

The successful applicant is expected to join the existing effort in solid state device research and help expand and develop it into one of prominence in both research and instruction. The College of Engineering has made substantial commitment to support this program including updating of the laboratory facilities and new faculty hiring.

Currently, research in the solid state device and related materials area includes research in silicon device processing, such as plasma and reactive ion etching, thermal nitridation of silicon and various oxide research including ultra-thin oxides, and defect control. In addition, research in III-V device area includes material defects, electrical noise and performance improvement of micron and sub-micron structures.

Combined research in the solid state device area typically amounts to about one million dollars annually. Collaborative research opportunities exist with the Materials Science department, Engineering Science and Mechanics department, the Chemistry and the Physics departments and the Materials Research Laboratory at Penn State.

Résumés, nominations, or inquiries should be directed to the following address by no later than September 15, 1985, or until a suitable candidate is selected.

Chairperson, Leonhard Professor Search Committee
Department of Electrical Engineering
Box J, 129 Electrical Engineering East
The Pennsylvania State University
University Park, PA 16802

An Equal Opportunity/Affirmative Action Employer. (NW1910)A

IGNEOUS PETROLOGY

Université de Montréal, Faculté des arts et des sciences, Département de géologie

A TENURE-TRACK POSITION IN INGEOUS PETROLOGY

Fonction Duties: To carry out active research in his/her field of interest and to teach and advise students at the graduate levels.

Qualifications: Ph.D. or equivalent is required.

Salary: In accordance with the collective agreement.

Application deadline: September 3, 1985.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada

Applications, together with curriculum vitae and names of three referees, should be sent to: M. Bernard Mamet, directeur, Département de géologie, Faculté des arts et des sciences, Université de Montréal, C.P. 6128, succursale A. Montréal (Québec) H3C 3J7, CANADA. (NW1905)A

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

DIVISION OF PARASITOLOGY

Applications are invited for a postdoctoral position to work with Dr C.J. Sanderson for a period of three years. The project is to work on a recently identified lymphokine controlling eosinophil differentiation. An interest in cell differentiation or molecular biology would be an advantage.

The salary will be in the range £8,920 — £10,720 per annum plus £1,233 London allowance, with superannuation provision.

Applications, together with curriculum vitae, research experience and interests and the names of two professional referees should be sent to the Director, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA before 26th July 1985, quoting Ref: P/9/WH0/S. (6949)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for an SERC funded post to work on the mechanisms by which globular proteins fold into their active state. Spectroscopic scattering, hydrodynamic and monoclonal antibody techniques will be used to study naturally occurring, mutant and recombinant proteins. The appointment is for three years tenable from September 1985 or soon after

Salary will be up to £7,980 per annum on the Range 1A salary scale: £7,520—£12,150 per annum according to age, qualifications and experience

Candidates with PhD or expecting to have gained a PhD by the autumn should send applications with curriculum vitae and the names and addresses of three referees to Professor R H Pain, Department of Biochemistry, University of Newcastle upon Tyne, NE1 7RU by July 18th. (Telephone 0632 328511 Ext 3437) (6929)A

THE UNIVERSITY OF MANCHESTER

POST-DOCTORAL RESEARCH POST

Applications are invited for this post, tenable in the Department of Medicine at Hope Hospital, Salford (Head of Department Professor L A Turnberg). The successful applicant will work on the electrophysiology of the colon in health and disease. Experience of intracellular microelectrode techniques would be an advantage. The post is for 2 years commencing from a date to be arranged Initial salary pa. £7,520 — £8,920 depending on experience Superannuation.

Further details may be obtained from Dr G I Sandle, Department of Medicine, Clinical Sciences Building, Hope Hospital, Salford M6 8HD (tel 061-789-7373 ext 236), to whom applications should be returned by July 22nd. (6960)A

LEUKAEMIA RESEARCH FUND CENTER AT THE INSTITUTE OF CANCER RESEARCH

A TECHNICIAN

is required for a post in the above laboratory based at Fulham Road, London SW7. The work will involve cell culture and cloning of human and mouse haemopoietic cell lines required for studies on genetic alterations in leukaemia. Experience in tissue culture is essential.

Salary in the range of £5,738 — £6,924 pa plus £1,042 London Weighting Allowance dependent on age and experience.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited. Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL quoting reference 301/B/89/N. (6969)A

THE UNIVERSITY COLLEGE WALES ABERYSTHWYTH

DEPARTMENT OF AGRICULTURE

Applications are invited for the post

RESEARCH ASSISTANT

to work on the effects of environment and cultural practices on the crisping quality in early harvested potato crops The project is supported by a grant from the Potato Marketing Board and be tanable for three years

Candidates should possess a good Honours degree in Agriculture or a relevant Agricultural or Biological Science, the person appointed may register for a higher degree Salary on the IB Range for Research and Analogous Staff £6,600—£8,920 per annum.

Applications forms and further particulars can be obtained from the Registrar (Staffing Officer), The University College Wales, Old College Kings Street, Aberysthwyth, Dyfed SY23 2AX (Tel No: 0970 — 3177 Ext. 207). Closing date for applications Friday 19 July 1985 (6984)A

YALE UNIVERSITY The Department of Chemistry invites applications for two tenure track Assistant Professor positions, effective academic year 1986

We seek outstanding individuals in any field of chemistry Applications from those whose research interests overlap with the areas of organic or inorganic chemistry and the biological sciences are particularly welcome

Applicants should send a curriculum vitae, a statement of research plans, and should arrange for the submission of three letters of recommendation All materials should be received by December 1, 1985

Send application to Prof. S.L. Schreiber, Chairman, Search Committee, Department of Chemistry, Yale University, P.O. Box 6666, New Haven, CT 06511. Yale University is an EO/AA employer. (NW1895)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral position to work with Dr Colin W Jones (in collaboration with Dr John R Linton, Shell Research) on the physiology and energetics of metabolite production in bacteria

Applicants should have experience in microbial physiology or biochemistry.

The position is for three years and the initial salary will be in the range £7,520 — £12,150 per annum, plus superannuation

Applications, including a curriculum vitae and the names and addresses of three referees, should be sent by July 19th to Mrs J Dean, Department of Biochemistry Secretary, University of Leicester, Leicester LE1 7RH (6923)A

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts AL5 2JQ

ORGANIC CHEMIST

with good practical ability required by the Insecticides and Fungicides Department to work in a team synthesizing powerful insecticides related to the pyrethrins. Excellent instrumental facilities available. This temporary two year post is funded by the British Technology Group to exploit promising previous work The appointment should start as soon as possible.

Qualifications: First or Upper Second Class honours degree and at least two years post-graduate experience A PhD in a relevant field of organic chemistry would be an advantage.

Appointment in the grade of Higher Scientific Officer on scale £7,788—£10,541. Non-contributory superannuation

Apply in writing to the Secretary, naming two referees and quoting Ref. 595 by 17 July 1985. Further particulars on request. (6947)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work on a project supported by ICI to study the growth and development of winter barley. The appointment will be made for a fixed period of up to three years, beginning in September

Applications should have, or shortly expect to receive, a PhD in agricultural science or crop physiology. Experience in field studies of growth and development in cereals is desirable.

Initial salary up to £9,860 a year (under review) on the 1A Range for Research and Analogous Staff (£7,520 — £12,150) (under review) according to age, qualifications and experience

Informal enquiries may be made to Professor J Elston (Tel: 0532 431751 ext 377).

Application forms and further particulars may be obtained from the Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference no 53/27 Closing date for applications 18 July 1985. (6963)A

OXFORD POLYTECHNIC
DEPARTMENT OF GEOLOGY AND PHYSICAL SCIENCES
LECTURER II/ SENIOR LECTURER IN GEOPHYSICS
POST NO.

The Department of Geology and Physical Sciences invites applications for the permanent post of Lecturer II/Senior Lecturer in Geophysics, the successful applicant to take up the post on 1 September 1985, or as soon thereafter as possible.
The appointee will be expected to teach Geophysics are related aspects of Geology to Honours Degree level Earth Scientists and to give a course in Geophysics as part of the Oxford Polytechnic Postgraduate Diploma in Petroleum Exploration Geology. In addition, there is a strong possibility that an opportunity will exist for collaboration with the National School of Mines in Rabat, Morocco. Any such collaboration may lead to salary enhancement. The successful applicant will be joining a strong team of Geologists at Oxford Polytechnic and will be expected to undertake research and/or consultancy.
Applications are invited from suitable qualified candidates holding an Honours Degree in Geology or related science and with postgraduate experience in Geophysics. A background which includes Geophysical research and/or industrial experience will be an advantage and preference may be given to those with experience in seismic interpretation
Basic Salaries.
Senior Lecturer:
£11,175 — £14,061 (under review)
Lecturer II
£7,548 — £12,099 (under review)
Closing date for completed application forms
For further details and application forms, pleasy apply to the Staffing Office, Oxford Polytechnic, Gipsy Lane, Headington, Oxford OX3 0BP. Telephone Oxford 64777, Extension 364. Closing date July 15th 1985.
An Equal Opportunities Employer
(6966)A

MEDICAL RESEARCH COUNCIL
Dunn Nutrition Unit, Cambridge
SCIENTIST

Applications are invited from Post Doctoral Scientists with experience in human energy metabolism, to join a team studying nutrition and health in our field station in rural Gambia. The proposed studies involve the use of a whole-body indirect colorimeter to investigate physiological adaptations to low energy intakes with particular emphasis on pregnancy and lactation. The successful candidate will be expected to take joint charge of these studies and to co-ordinate them with our programme of stable isotope research

The appointment will initially be for 2 years of which 2 to 3 months will be spent in Cambridge and Lausanne, Switzerland undergoing training in calorimetry techniques The remaining time will be spent in our Gambia field station with 6 weeks home leave after one year. Depending on progress there should be a possibility of extension for a further two years. The appointment will start in October 1985

Salary in the range £8,920 — £11,205 plus overseas allowances

Please send applications with a full cv and the names of three professional referees to Dr R G Whitehead, MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ.

Closing date for applications is 15 July 1985. (6982)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF MEDICINE
MRC Clinical Immunology Research Group
CHIEF TECHNICIAN

Applications are invited for the post of Chief Technician in this Unit which is vacant at present. The group is headed by Professor D K Peters with its main interests in the clinical immunology of autoimmunity, nephritis and persistent virus infections, it comprises approximately 12 scientists and research fellows and 10 Technicians. The successful applicant would be responsible for the administrative running (including ordering and supervision of equipment) of the laboratories, and training of technicians However they would also be expected to have a keen interest and to participate in experimental work and should have a background in immunological research

Interested applicants are invited to visit the Unit by contacting Dr J G P Sissons (01-743 2030 ext 345)

Salary on scale £9,458 to £10,975 plus £1,084 London Allowance a year.

This is a readvertisement, previous applicants need not apply.

Application forms and further particulars from the Personnel Office Royal Postgraduate Medical School, 150 Ducane Rd, London W12 0HS quoting ref: 2/110/N

Closing Date: 18 July 1985.
(6973)A

LH FERMENTATION

Due to continued expansion, LH Fermentation who are leaders in the manufacture and distribution of Fermentation and Biological Culture equipment on a world-wide basis, have a requirement for Sales Personnel in both their Home and Export Sales Departments. The successful applicant will have experience of medium to large scale microbial or mammalian cell culture.

EXPORT SALES EXECUTIVE

Based in our Stoke Poges office the Export Sales Executive will be responsible for the technical support of our Scandinavian and Western European markets The successful candidate will be prepared to travel extensively within these markets and have the personal drive and ambition to succeed in this important environment
Although previous Export Sales experience would be an advantage, full Sales and Documentation training will be given to the successful applicant
A company car will be provided Salary dependent on experience
Please send full C V in confidence to
Mr. S M Asbridge, Export Sales Manager, LH Fermentation Ltd, Bells Hill, Stoke Poges, Bucks SL2 4EG Tel.02814 5100.

UK TECHNICAL SALES REPRESENTATIVE

Reporting to our Stoke Poges office, the successful candidate will cover the London and South East area Previous sales experience is not required as full professional sales training will be given
A company car will be provided Salary dependent on experience
Please send full C V in confidence to
Mr. R. Foster, UK Sales Manager, LH Fermentation Ltd, Bells Hill, Stoke Poges, Bucks SL2 4EG. Tel.02814 5100.

(6987)A

LH
THE WORLD STANDARD IN BIOTECHNOLOGY
LH Fermentation Ltd is a Porton International Group Company

HOUGHTON POULTRY RESEARCH STATION
Grant-Aided by the Agricultural & Food Research Council
TEMPORARY MOLECULAR BIOLOGIST

Applications are invited for the above post to work on molecular aspects of pathogenesis of Marek's disease Areas of interest are in situ hybridization and characterization of RNA transcripts and their translated products in infected cells.

Candidates should have recently completed a PhD degree or be graduates with experience in molecular biology of nucleic acids A background in virology would be an advantage but is not essential

The post is for a period of 2 years and is supported by the Leukaemia Research Fund Informal enquiries to Dr N Ross (Tel 0480–64101).

The appointment will be made to the Higher Scientific Officer grade (salary £7,788 × 6 — £10,541) or the Senior Scientific Officer grade (salary £9,772 × 7 — £12,653) depending on experience and qualification.

An application form and further particulars may be obtained from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs The closing date for applications is 25th July 1985. (6972)A

THE ANIMAL VIRUS RESEARCH INSTITUTE
Ash Road, Pirbright, Woking, Surrey GU24 0NF
HIGHER SCIENTIFIC OFFICER

Following the restructuring of the Animal Virus Research Institute, applications are invited for a new post of Higher Scientific Officer to work in the Division of Molecular Biology, Director's Group. The appointment to commence as soon as possible The successful candidate will work on the mechanisms of replication of fowl plague virus RNA.

Candidates should have a First or Upper Second Class Honours degree with at least two years post graduate qualifying relevant research experience.

Salary scale £7,788 — £10,541, sick leave and non-contributory superannuation schemes, 22 days annual holiday. House may be available for suitable married applicant

Applications to include a curriculum vitae and the names of two referees should be sent as soon as possible to. The Secretary, The Animal Virus Research Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF. Tel: 0483 232441/8.

The Animals Virus Research Institute is an Equal Opportunities Employer (6945)A

UNIVERSITY OF UTAH

As part of a cooperative program with the Department of Biology, the Department of Chemistry is inviting applications for a tenure-track Assistant Professorship for individuals whose research interests are in the area of structure and function of biological macromolecules. The selected candidate is expected to demonstrate excellence and creativity in research and to play an active role in teaching. Preference will be given to individuals who apply modern chemical methods to the study of proteins or nucleic acids in areas which complement existing programs.

Send résumé including a description of research plans and three letters of reference by 10/1/85 to Professor C.D. Poulter, Department of Chemistry, University of Utah, Salt Lake City, Utah 84112. The University of Utah is an equal opportunity/affirmative action employer. (NW1918)A

EXCITING RESEARCH OPPORTUNITY IN WEST GERMANY

CELL BIOLOGIST

With experience in light and electronmicroscopic techniques and their application to immunological and biochemical studies at the cellular and sub-cellular level. The ideal candidate should have an MD and/or Ph D, and postdoctoral experience.

In addition to the researcher's salary, this federally funded position includes salary for a Technician, start-up funds and an appropriate supply budget.

Please send CV and references to:
Dr. Rolf Kinne
Director Max-Planck-Institut fuer Systemphysiologie
Rheinlanddamm 201
4600 Dortmund 1, West Germany. (W1953)A

UNIVERSITY OF OXFORD

PROFESSORSHIP OF THE PHYSICS AND CHEMISTRY OF MINERALS

The electors intend to proceed to an election to the newly established Professorship of the Physics and Chemistry of Minerals. The stipend of the professorship is at present £20,795.

Applications (ten copies, or one from overseas), naming three referees but without testimonials, should be received not later than 27 August by **The Registrar, University Offices, Wellington Square, Oxford OX1 2JD,** from whom further particulars may be obtained. (6970)A

POSTDOCTORAL RESEARCH ASSOCIATE

in the field of Membrane Physical Biochemistry

Multinuclear NMR and fluorescence studies of the physical properties of model membranes, particularly with reference to the mechanism of action of anesthetics and alcohol The successful candidate should have a Ph.D. in chemistry or biochemistry. Possibility of interviews at Amsterdam Biochemistry Congress for finalists.

Send Curriculum Vitae and three letters of reference to: Elizabeth S. Rowe, Ph.D., Veterans Administration Medical Center, Research Service (151), 4801 Linwood Blvd., Kansas City, MO 64128; telephone 816-861-4700 ext 541. (Affiliated with the Biochemistry Department of the University of Kansas Medical Center.) (NW1913)A

UNIVERSITY OF SASKATCHEWAN

Institute of Space and Atmospheric Studies

PROFESSIONAL RESEARCH ASSOCIATE

An opening, available immediately, exists for a Professional Research Associate to work with a small research group in the general area of photochemistry of planetary atmospheres and with particular reference to oxygen chemistry The work will involve the analysis of data obtained from experiments previously flown on the Space Shuttle Candidates should have post-doctoral experience, preferably in the study of planetary atmospheres.

Those interested should write to Dr E.J. Llewellyn, Institute of Space and Atmospheric Studies, University of Saskatchewan, Saskatoon, Saskatchewan S7N OWO The salary will not be less than $26,000 per annum. (NW1907)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY AND EMBRYOLOGY

RESEARCH ASSISTANT (IA)

This postdoctoral position is available for one year in the first instance, but with the possibility of extension up to three years on an MRC grant concerned with Localisation of Neurotransmitter Receptors in the Autonomic Nervous System Scientists with experience in autoradiography, electronmicroscopy, immunohistochemistry or pharmacological or biochemical aspects of receptor biology will be particularly suited. The technical and intellectual environment for research is attractive. Salary in range £5,520 — £10,330 + £1,233 LA according to age and experience.

Applications, together with full cv and names and addresses of two referees, to Professor G Burnstock, Department of Anatomy & Embryology, University College London, Gower Street, London WC1E 6BT. (6946)A

LEUKAEMIA RESEARCH FUND CENTRE AT THE INSTITUTE OF CANCER RESEARCH:

TECHNICIAN/RESEARCH OFFICER

required to help organise a molecular biology laboratory and actively participate in its research programme into Cellular and Molecular Biology of Human Leukaemia. Experience in recombination DNA techniques essential Candidates should have a degree, HNC or equivalent qualification.

Salary will be in the range of £5,738 — £8,096 + £1,042 pa. London Weighting allowance, depending on qualifications and experience Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications in duplicate with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference 301/B/86 (6862)A

MRC

Medical Research Council

MEDICAL RESEARCH COUNCIL

Toxicology Unit

PROTEIN BIOCHEMIST/ CHEMIST

A 3 year post-doctoral appointment (usual MRC terms) is available to characterise a membrane bound protein involved in the maintenance of nerve cell axons. The initial objective is to improve existing partial purification procedures for the active-site radiolabelled enzyme to the point where sequencing etc, is practicable. Skills in protein biochemistry including affinity chromatography and liquid phase partitioning are required and acquaintance with molecular biology techniques would be an advantage

Applications to Dr. M.K. Johnson, MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF. (6964)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Biology and Industry, to

Felicity Parker, Macmillan Journals, Houndmills, Basingstoke, Hants. RG21 2XS, UK

or

Paul Siman, Bio/Technology, 65 Bleecker St., New York, NY 10012.

RESEARCH FELLOW

Position available for post-doctoral research fellow in atherosclerosis; biochemical and morphological studies of arterial wall, organ culture or aorta, prevention of atherosclerosis by early intervention, research on regression of advanced lesions. Applicants must have an M.D. or Ph.D. degree in Biomedical Science with a 2 year minimum research training in lipids and polysaccarides, atherosclerosis, and 1 year experience as research fellow. Salary: $20,350 annually, 40 hours per week.

Send résumé to: Ohio Bureau of Employment Services, Special Projects Section, 1916 Central Parkway, Cincinnati, Ohio 45214.

Reference Job Order Number 0595735. (NW1908)A

UNIVERSITY OF DUNDEE

EPARTMENT OF BIOCHEMISTRY

MRC Protein Phosphorylation Group

POSTDOCTORAL WORKER/RESEARCH ASSISTANT

pplications are invited for the bove position to work with Pro-ssor Philip Cohen, FRS on the role f protein phosphorylation in hor-one action. The position, which ecomes vacant in August 1985, is or three years in the first instance he appointment will be made at an ppropriate point on the Research nd Analogous Scale Range 1A urrently £7,520 — £12,150) de-ending on qualifications and ex-erience A background in Protein hemistry and Enzymology, or olecular Pharmacology, would be n advantage Informal enquiries an be made to Professor Cohen, elephone number Dundee 23181, xtension 4238.

Applications containing a full cur-iculum vitae and the names and ddresses of three referees should be ent to the Personnel Office, The Jniversity, Dundee, DD1 4HN as oon as possible. Please quote refer-nce EST/73/85J (6934)A

POSTDOCTORAL POSITION

Available for research on the isolation of herbicide resistant microbial genes and their subsequent expression in plant cells Expertise in recombinant DNA technology and yeast molecular biology is required.

Send C.V. and two letters of reference to: Dr G.H. RANK, DEPT. OF BIOLOGY, UNIVERSITY OF SASKATCHEWAN, SASKATOON, SASK., CANADA S7N OWO. (NW1906)A

POSTDOCTORAL PROTEIN CRYSTALLOGRAPHER

Positions are available for two crystallographers to study the structure of myosin subfragment-1. The research involves protein purification, crystal growth and data collection. Applicants with experience in any aspect of crystallography will be considered Salary commensurate with experience **Send résumé and three letters of recommendation to: Dr. Ivan Rayment, department of biochemistry, University of Arizona, Biological Sciences West, Tucson, Arizona, 85721, USA.** The University of Arizona is an equal opportunity employer (NW1900)A

ASTON UNIVERSITY

Auditory Physiology Laboratory

DEPARTMENT OF PHARMACEUTICAL SCIENCES

POSTDOCTORAL RESEARCH OFFICER

Applications are invited for a Research Officer (funded by the Wellcome Trust) to study the sites and mechanisms of toxic effects of drugs on hearing Candidates should have experience of electrophysiological recording techniques and will ideally have a PhD in some aspect of auditory physiology

The appointment will be for one year initially with the possibility of renewal Commencing salary will be within the rang £7,520 to £8,920 per annum (under review).

Informal enquiries may be directed to Dr E S Harpur, extension 4180

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), quoting Ref. 699/6, Aston University, Aston Triangle, Birmingham B4 7ET Tel 021 359 3611 Ext 4563. Closing date for receipt of applications is 19th July 1985. (6962)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

DEPARTMENTS OF MEDICINE

(MRC Clinical Immunology Research Group) and Virology

A NON-CLINICAL LECTURER

is required to join the MRC supported group in the Department of Medicine This post will complement a group working on the pathogenesis and immunology of human cytomegalovirus (Dr J G P Sissons and colleagues) — which has a close liaison with the Department of Virology (Prof B Griffin). Candidates, who should be capable of conducting independent research, should have a background in virus molecular biology and be able to contribute to work on the molecular pathogenesis of cytomegalovirus infection.

Applications (6 copies) in the form of a cv with the names of three referees should be sent, together with a supporting statement of no more than 500 words, to the Deputy Secretary, Royal Postgraduate Medical School, 150 Ducane Rd, London W12 0HS from whom further particulars may be obtained.

Closing Date: 22 July 1985. (6957)A

BASIC SCIENTIST:

Assistant Professor of Surgery, Division of Urologic Surgery at the University of Cincinnati College of Medicine. Possible secondary appointment in appropriate basic science discipline. Candidates should have a Ph.D. degree, postdoctoral experience, and demonstrated ability in research (publications in peer-reviewed journals and research grants). Areas of research might include biochemistry, cell/molecular biology, or genetics. An interest in cancer is helpful; an interest in working closely with clinicians and clinically relevant problems in urologic surgery is a must

Send letter of interest, curriculum vitae, and names of three referees by September 15, 1985 to: Dr. H. Bruce Bosmann, Chairman, CSRP Search Committee, University of Cincinnati College of Medicine, ML #555, Cincinnati, Ohio 45267-0555.

The University of Cincinnati is an equal opportunity educator and employer. (NW1915)A

UNIVERSITY OF WISCONSIN-MADISON

Mycology/Microbial Ecology

RESEARCH ASSOCIATE (postdoctoral)

postition available 1 June 1985 on the BIOTECHNOLOGY and microbial ecology of fungal pathogens (biocontrols) to watermilfoil. Research aspects include mass culture and strain improvement techniques, interaction with other control measures, and tracking marked populations in water and sediment. Funding for two years. Salary competitive and commensurate with qualifications.

Send résumé with names of 3 referees to: Dr. John H. Andrews, Department of Plant Pathology, 1630 Linden Drive, Madison, WI 53706.

An affirmative action/equal opportunity employer. (NW1914)A

GRANTS & SCHOLARSHIPS

UNIVERSITY OF LEICESTER

DEPARTMENT OF CHEMISTRY

RESEARCH SCHOLARSHIP

Applications are invited for the above from graduates in Chemistry holding a first class honours or second class honours (upper division) degree. The successful candidate will be expected to undertake research in organometallic chemistry under the supervision of Dr D L Davies and to register for a higher degree The scholarship carries a maintenance award at the same level as SERC post-graduate studentships (£2,585 — under review). The University will waive registration fees.

Further details and application forms may be obtained from Professor Peacock, Department of Chemistry, University of Leicester, University Road, Leicester LE1 7RH, to whom completed applications should be returned by 31 July 1985. (6944)H

IMPERIAL CANCER RESEARCH FUND

BURSARY FOR TRAINING IN RESEARCH

Applicants are invited for an ICRF Bursarship tenable for 3 years from October 1985 for full time students leading to a higher degree in biochemistry The bursarship offers the opportunity to join an active cell membrane research group in the Department of Biochemistry, at the Royal College of Surgeons, in collaboration with the Cell Surface Biochemistry Laboratory, ICRF

The research topic, which will be jointly supervised by Professor N Crawford and Dr M Crumpton (ICRF), involves investigating procedures for permeabilising blood cells and their use as carrier vehicles with encapsulated drugs and other agents which activates macrophages for sustained tumouricidal effects.

Applications are invited from students, no more than 25 years of age on October 1st who hold, or expect to obtain a First or Upper Second Class Honours degree in Biochemistry or Cell Biology. The award carries a grant of £4,865 with possible additional allowances.

Further details and application forms can be obtained from: Miss S M Hurley, Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PN

Please quote reference number. 85/85.

The forms should be completed and returned by 31st July 1985. (6904)H

STUDENTSHIPS

KING'S COLLEGE LONDON (KQC)

University of London

RESEARCH STUDENTSHIP

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a postgraduate research studentship to be held in the Biochemistry Department, King's College London (Strand Campus). The studentship is to be funded by The British Foundation for Age Research for the study of Age-related changes in the catabolism of crystallin fragments in lens tissue Applicants should possess (or be about to obtain) either a 1st class or an upper 2nd class honours degree in Biochemistry or a closely related subject(s).

Applications as soon as possible to Dr A R Hipkiss, Department of Biochemistry, King's College London, Strand, London, WC2R 2LS. Telephone enquiries may be made on 01-836 5454 extn 2491 (6974)F

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continued on page 18

STUDENTSHIPS

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF GENETICS
SERC CASE STUDENTSHIP

Applications are invited for an SERC CASE studentship to study the molecular biology of secondary metabolite biosynthesis by a pseudomonad. The work will include gene cloning techniques, site directed mutagenesis DNA sequencing and control of gene expression. The industrial sponsor for this award is Beechan Research.

Applications including curriculum vitae and names of two academic referees should be sent as soon as possible to Dr F C H Franklin, Department of Genetics, University of Birmingham, PO Box 363, Birmingham B15 2TT. Tel. No: 021-472 1301 Ext 2620. (6980)F

HERIOT-WATT UNIVERSITY
DEPARTMENT OF BREWING & BIOLOGICAL SCIENCES
RESEARCH STUDENTSHIP IN MICROBIAL GENETICS/ MOLECULAR BIOLOGY

A SERC Quote studentship is available in this Department tenable for three years and leading to a PhD. The project will involve the cloning and characterisation of genes for extracellular enzymes from thermophilic bacteria.

Applicants should hold a first or upper second degree in microbiology or a related discipline, and should send a curriculum vitae and names of two referees to Dr F G Priest, Department of Brewing & Biological Sciences, Heriot-Watt University, Chambers Street, Edinburgh, EH1 1HX. Closing date 4th July, 1985 (6965)F

UNIVERSITY OF SURREY
DEPARTMENT OF CHEMISTRY

Applications are invited from Chemistry graduates, or those about to graduate for the following research studentships which will become available as from 1st October, 1985.

1) A CASE studentship in conjunction with ICI Pharmaceuticaals to study
A THERMODYNAMICALLY-BASED SCALE OF HYDROGEN BOND BASICITIES
(Dr M H Abraham)

2) A CASE studentship in conjunction with the Imperial Cancer Research Fund to study
MECHANISMS OF AROMATIC HYDROXYLATIONS
(Dr J R Jones)

3) A CASE studentship in conjunction with ICI to study
THE SYNTHESIS OF POLYSULPHONES FROM ARYLSULPHONIC ACIDS AND PCl_5
(Professor J B Rose)

Interested persons should send their curriculum vitae and the names and addresses of two referees to Dr J R Jones, Chemistry Department, University of Surrey, Guildford, GU2 5XH, as soon as possible. (6978)F

QUEEN MARY COLLEGE
(University of London)
School of Biological Sciences
NERC POSTGRADUATE STUDENTSHIP

Applications are invited for a NERC Studentship to study the role of microfauna in the food webs of acid stream communities. A full curriculum vitae and the names of two referees should be sent, by 3rd July, to Dr A G Hildrew, School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS. (6958)F

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
(In collaboration with ICI Pharmaceuticals)
PhD STUDENTSHIP

In human cardiovascular pharmacology to study the humoral control of coronary artery tone in vitro using human material

Details from and applications to Dr R W Kerwin, Cardiovascular Unit, Hammersmith Hospital, 150 Ducane Rd, W12 0HS. Tel 01-743 2030 ext 690 (6956)F

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF CHILD HEALTH

Applications are invited for an MRC funded, three year
PhD STUDENTSHIP

to commence in October 1985 to investigate ketone body turnover in the neonatal period in man and experimental animals. The project will involve the use of stable-isotopically labelled substrares and gas chromotography — mass spectrometry. Candidates should have, or should expect to obtain, a First Class or Upper Second Class Honours Degree in Biochemistry or related subject.

Further details can be obtained from Professor A Aynsley-Green or Dr K Bartlett, Department of Child Health, The Medical School, The University, Framlington Place, Newcastle upon Tyne, NE2 4HH, to whom letters of application, including a curriculum vitae and the names of two academic referees, should be sent by not later than 12th July 1985. (6961)F

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place, London W2 1PG
SERC CASE STUDENTSHIP

Applications are invited for a SERC Case Studentship in collaboration with Shell Research Limited (Dr D H Hutson) for research (leading to a PhD degree) into the effect of environmental conditions on the metabolism of chemicals by fish

Applicants should have, or expect ot obtain, a first or second class honours degree in biochemistry, or chemistry, and have an interest in chemical and environmental toxicology

Applications, including the names and addresses of two referees, should be sent to the Assistant Secretary (Personnel) at the above address by 3 July 1985. For further details of the studentship contact Dr P Millburn tel no· (01) 723-1252 ext 118. (6907)F

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF INORGANIC CHEMISTRY

Applications are invited for an SERC sponsored Research Studentship leading to the degree of PhD, to investigate the synthesis of new polyoxometalates of early transition metals and their potential as ligands in organometallic chemistry. The project will involve a range of inert atmosphere, analytical, spectroscopic and electrochemical techniques The award will be tenable from October, 1985. Candidates should hold or expect to obtain a first or upper second class degree in chemistry Applications including a CV and the names and addresses of two referees should be sent as soon as possible to: Dr R J Errington, Department of Inorganic Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU from whom further information can be obtained (6926)F

UNIVERSITY OF LIVERPOOL
School of Dental Surgery
RESEARCH STUDENTSHIP

Applications are invited for a University Research Studentship for 3 years from October 1985 leading to a PhD degree Candidates should possess a first or upper second class honours degree in a biological science. The research project will be concerned either with disturbances of salivary function, involving in vivo and in vitro studies of secretory activity, or with calcification mechanisms in dental hard tissues using ultrastructural techniques Further details are available from Dr J Scott, Research Wing, Dental School (051 709 0141 extn 2973).

Applications together with the names of two referees should be received as soon as possible by the Registrar, the University, PO Box 147, Liverpool L69 3BX.

Quote Ref. RV/128/N (6968)F

Southampton THE UNIVERSITY
INSTITUTE OF CRYOGENICS
CASE RESEARCH STUDENTSHIP

Applications are invited for an SERC/British Gas CASE studentship to study instabilities in cryogenic liquids using LDA, microthermometer and flow visualisation techniques

Appy to: Professor R.G. Scurlock, Institute of Cryogenics, University of Southampton, Southampton SO9 5NH. (6928)F

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
MRC RESEARCH STUDENTSHIP and MRC RESEARCH ASSISTANTSHIP GRADE (1B)

Applicants are invited for these two vacancies in a well-established group studying the immunology of schistosomiasis, with the main aim of producing molecular vaccines Both posts are tenable for three years from September 1st, 1985

The Research Studentship will be concerned with comparative studies on the immunology of Schistosoma mansoni and S japonicum, with emphasis on the identification of "protective" parasite antigens and cloning of the corresponding antigen genes. This work will be carried out in the Wolfson Unit for Molecular Medical Microbiology and Parasitology, and the person appointed, who must hold, or hope to obtain in 1985 a First or Upper Second Class Honours degree in Molecular Biology Microbiology, or Biochemistry will be registered for a PhD degree under the supervision of Dr Q D Bickle.

The Research Assistant will be working with Drs M G Taylor and Q D Bickle on antigen purification and the production of monoclonal antibodies, and studying the mechanism of immunity in vivo. Applications are invited from those who hold, or who are about to obtain, Honours degrees in relevant biological subjects. This 3-year appointment carries a starting salary of up to £7,055 per annum, plus £1,233 per annum London Allowance, and the appointee will be encouraged to register for a higher degree.

Applications, consisting of a full curriculum vitae and naming two referees, should be sent as soon as possible to the Personnel Office London School of Hygiene and Tropical Medicine, Keppel Street London WC1E 7HT.

Further information about the research to be undertaken may be obtained by telephoning Dr Taylor or Dr Bickle on St Albans 34201.

Closing date 8 July 1985 (6975)F

FELLOWSHIPS

The Wellcome Trust

Training Fellowships for research into mental disorders

Applications are invited from medical and scientific graduates who have worked in universities, institutes or hospitals in the United Kingdom or Eire for at least the past three years, or who did so before taking up an appointment overseas, for a fellowship for research training in the field of mental disorder.

These awards, which are tenable for up to three years, are intended for clinicians with a training in psychiatry, or intending to train in psychiatry, or postdoctoral scientists (with at least one year's postdoctoral research experience) who wish to extend their research experience The research proposals should be relevant to mental health or related basic science and the applicant should intend to continue research in this field. These fellowships may be held in appropriate departments or clinical centres in Great Britain In cases where the necessary research training cannot all be obtained in Great Britain, the Trustees are prepared to consider a request that part of a fellowship be spent in a suitable centre or centres abroad.

Candidates should not normally be over 32 years of age for science graduates and 34 years for medical graduates. Applications should be made by the Head of the Department in which the candidate proposes to work. Salaries will be in the range of University Lecturer scales according to age and experience

Application forms can be obtained from: The Grants Section, The Wellcome Trust, 1 Park Square West, London, NW1 4LJ. Telephone: 01-486 4902. Applications must be returned by the 1st September 1985.

The Wellcome Trustees are concerned that research careers of outstanding young clinical and non-clinical scientists should not suffer from lack of suitable support and guidance at any stage. Exceptional candidates proposed for the above fellowships may be considered at a later date for senior fellowships which will provide additional support at a later stage in the fellow's career (not necessarily immediately after the training fellowship) to advance the fellow to a stage where he or she is ready to apply for senior research and academic positions.

(6986)E

The John Hopkins University
Department of Anesthesiology and Critical Care Medicine

POST DOCTORAL FELLOWSHIP IN RESEARCH

Applications are invited for this position available immediately to study the molecular biology of human cytochrome P450 The applicant must be creative and highly motivated to carry out independent experimentation utilizing human cytochrome P450 under study

M D or Ph D required with 1–2 years experience in molecular biology

Contact: Roy C. Levitt, M.D., The John Hopkins University, Department of Anesthesiology and Critical Care Medicine, Blalock 1410, Baltimore, Maryland 21205. Telephone (301) 955-7609. (NW1822)E

STUDENTSHIPS continued

THE ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen, AB2 9SB

SERC CASE STUDENTSHIP

DEPARTMENT OF CHEMISTRY (AGRICULTURE), GLASGOW UNIVERSITY
Rowett Research Institute, Aberdeen

Applications are invited for a studentship to investigate the nature of lignin-carbohydrate linkages in straw The project should lead to a clearer understanding of how cell-wall structure determines the digestibility not only of straw, but also grass and other forage crops, in ruminants, and how digestibility can be improved by processing Experimental work will involve the use of advanced gas and liquid chromatography, NMR, mass spectroscopy, enzyme digestion and other techniques

The student will register for a PhD degree at Glasgow University, but is expected to spend only about one year there in total, the remaining period will be spent at the Rowett Institute. In addition to a first or good second class honours degree or equivalent, the main requirement is a sound grasp of analytical techniques and an interest in their applications to biological systems. Some knowledge of animal nutrition and plant composition would be helpful but is not essential

Applicants should send a CV and the names of two referees as soon as possible to, The Secretary, Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB. Tel: 022 – 712751 Ext. 338

Closing date 18th July 1985.

(6916)F

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF HAEMATOLOGY

Applications invited for a 2 year Leukaemia Research Fund training fellowship in the joint University/NHS department of Haematology at the University of Birmingham and Queen Elizabeth Hospital The post would suit a medical graduate with MRCP qualification, and previous experience at Registrar level in Haematology Clinical and Laboratory training will be offered in the management of haematological malignancies and in Bone Marrow Transplantation A major research project will be undertaken and the successful candidate will be expected to register for a PhD or MD at an appropriate University Honorary registrar status will be sought from the Central Birmingham Health District. Salary in scale Research Fellow Clinical. Maximum starting £10,640

Applicants are invited to visit the department by arrangement with Dr B J Boughton, Senior Lecturer in Haematology (telephone 021 472 1311 ext 177) Applications (3 copies) to Assistant Registrar, Medical School, Birmingham B15 2TJ from whom further particulars are available by 14 July 1985, Quote Ref: RFC/HAE/BJB

(6932)E

CANOLFAN ASTUDIAETHAU TIROEDD CRAS COLEG PRIFYSGOL GOGLEDD CYMRU
UNIVERSITY COLLEGE OF NORTH WALES CENTRE FOR ARID ZONE STUDIES

THE YAMANI FELLOWSHIPS

His Excellency Sheikh Ahmed Zaki Yamani has generously endowed Fellowships within this Centre.

The recipients must be from Arab or Third World Countries and should contribute to the teaching and research within the Centre. Tenure may be from 3 months to 1 year.

The Centre has wide interests in the development of Agriculture and Forestry in arid and semi-arid areas and in allied scientific and environmental problems.

Support can be given for subsistence and travel costs and for experimental work.

Further details from: Professor R G Wyn Jones, Centre for Arid Zone Studies, University College of North Wales, Bangor, Gwynedd.

Applications (two copies), giving details of age, education and experience, together with the names and addresses of two referees, should be sent to the Personnel Office, University College of North Wales, Bangor, Gwynedd LL57 2DG (6942)E

THE IZAAK WALTON KILLAM

POSTDOCTORAL FELLOWSHIPS

FOR 1986-87

Value

Fellowships valued at $22,500 (1985-86, plus travel allowances, are tenable for one year at Dalhousie University in most fields of study. Application for extensions may be considered.

Qualifications

Applicants should have recently completed a Ph.D. degree at a recognized university, or expect to obtain a degree before taking up an award.

Application

Application forms may be obtained by writing to:

The Office of the Dean
Faculty of Graduate Studies
Dalhousie University
Halifax, Nova Scotia B3H 4H6

The closing date for receipt of applications is 15 December 1985. (NW1898)E

UNIVERSITY OF BIRMINGHAM

FACULTY OF SCIENCE AND ENGINEERING

DEPARTMENTS OF CHEMICAL ENGINEERING AND PLANT BIOLOGY

POSTDOCTORAL RESEARCH FELLOWSHIP AND RESEARCH ASSOCIATESHIP

Applications are invited for (a) a Postdoctoral physical/colloid chemist and (b) a graduate chemical engineer or biologist preferably with experience in biotechnology or bioengineering.

The project is a co-operative research grant funded by the SERC and is concerned with the development of novel polymers for the prevention of biofilm adhesion in process plant. The persons appointed will join groups working on biofouling (Dr T Bott, Chemical Engineering and Dr M E Callow, Plant Biology) and surface chemistry (Dr J Goodwin, Physical Chemistry, Bristol University). Co-operating bodies are International Paint plc and APV International Ltd.

Post tenable for up to three years from 1 September 1985.

Salary on the Research Fellow scale £7,520—£12,150 plus superannuation Salary on the Research Associate scale £6,600—£8,920 plus superannuation.

For further information, phone 021-472-1301 ext. 2559 quoting reference T9

No formal application form. Three copies of application, including full curriculum vitae and naming three referees to Assistant Registrar (Science and Engineering), PO Box 363, Birmingham B15 2TT by 15th July, 1985. (6931)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

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in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance Applications may be made at any time and are decided upon soon after the receipt of application

Applications for exchanges between laboratories within any one country cannot be considered Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 102240, F.R.G. (W1577)E

POSTDOCTORAL FELLOWSHIP IN MOLECULAR BIOLOGY/ BIOCHEMISTRY

Position available in Department of Microbiology at Suny Stony Brook to study Adeno-associated virus genetics, vector construction, immunology or DNA replications. **Send CV and references to Dr Nicholas Muzyczka, University of Florida, College of Medicine, Box J226 JHMHC, Gainesville, FL 32610** (NW1901)E

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DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

JUNIOR RESEARCH FELLOWSHIP

Applications are invited from graduates or students obtaining a good honours degree in Biochemistry or related subjects for a three year junior Research Fellowship, starting on 1 October 1985. The successful candidate will register for a higher degree in the University of London The project concerns the mechanisms of polyphosphoinositide metabolism and arachidonic acid release in endocrine cells. The conditions of employment and salary are similar to the SERC/MRC studentships.

A curriculum vitae and the names of two academic referees should be sent to Professor B A Cooke, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF. Telephone 01-794-0500 extension 4202 (6959)E

ASSOCIATESHIPS

UNIVERSITY OF LEICESTER

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATESHIP

Applications are invited for a postdoctoral research associateship supported by the SERC, to study phosphoryl transfer reactions including enzyme catalysed reactions Some experience of synthesis and an interest in reaction mechanism is desirable. The appointment will be for two years (start 1 October 1985). The starting salary will be in the range £7,520—£8,920 pa

Applictions with curriculum vitae and names and addresses of at least two referees should be sent, not later than 31 July, to Dr P M Cullis, Department of Chemistry, The University, Leicester LE1 7RH. (6953)O

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DEPARTMENTS OF PHARMACEUTICAL CHEMISTRY AND PHARMACOGNOSY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a SERC funded postdoctoral research assistantship to investigate "Novel phorbol esters and their interaction with protein kinase C". This multidisciplinary research post would be suitable for someone with experience in biochemistry and natural product chemistry and will be available for up to 3 years.

The project will involve the study of tumour promoting and inflammatory diterpenoid derivatives (phorbol esters) that stimulate the activity of an enzyme (protein kinase C) implicated in the control of a wide range of cellular activities.

The person appointed will work with members of staff in departments that have excellent facilities for research in natural product chemistry, biochemistry and molecular biology

Further details may be obtained from Dr Alastair Aitken (Dept of Pharmaceutical Chemistry) and Dr F J Evans (Dept pf Pharmacology) on 01 837 7651

Applications, including 2 copies of CV and names of 2 referees, to be sent to: Personnel Officer, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, to arrive within 4 weeks of the advertisement (6976)P

DURHAM UNIVERSITY

DEPARTMENT OF CHEMISTRY

Applications are invited for a postgraduate

RESEARCH ASSISTANTSHIP

tenable for 3 years from 1 October 1985 or as soon as possible thereafter to work with Dr D Parker on the chemistry of functionalised macrocycles. Applicants should have at least a 2(i) Honours Degree in Chemistry and will be expected to register for a higher degree. The project involves the development of selective lithium ionophores with a concurrent investigation of the chemistry of heterodinuclear complexes.

Applications (3 copies) naming 3 referees should be sent by 5 July to The Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (6943)P

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The next course starts in January 1986

Further information and application forms may be obtained from Dr M R Preston (MPC), Oceanography Department, Liverpool University, PO Box 147, Liverpool, L69 3BX. (6952)C

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UNIVERSITY OF ABERDEEN

DEPARTMENTS OF THERAPEUTICS AND CLINICAL PHARMACOLOGY AND PHARMACOLOGY

MSc STUDENTSHIP

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1) cryopreservation of human hepatocytes for drug toxicity studies.

2) mechanisms of drug induced toxicity using isolated proximal tubular cells.

3) polyamine metabolism in human breast cancer cells.

Further details may be obtained from Dr G M Hawksworth, Department of Therapeutics and Clinical Pharmacology, University Medical Buildings, Foresthill, Aberdeen, AB9 2ZD, (0224 681818 Ext: 2463) to whom application, including the names and addresses of two academic referees, should be sent as soon as possible. (6941)C

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Diploma in Endocrinology (Biochemistry)

Application is open for clinicians or science graudates with an endocrinology background. Applicants should, if possible, have some experience of laboratory techniques Supervisor: Professor S.R. Bloom (Endocrinology).

Further details and application forms may be obtained by writing to:

The School Office,
Royal Postgraduate Medical School,
Hammersmith Hospital,
Du Cane Road, London W12 0HS.

Completed application forms must be received before 31st July 1985 for registration on the 1985/1986 Diploma course. (6939)C

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Organizers: L.A. Herzenberg (Stanford University, USA), P. Le Bouteiller (Centre dimmunologie de Marseille-Luminy, France), Z. Mishal (C N.R.S., Vilejuif, France), M. Monsigny (Centre de biophysique moléculare, Orleans, France), J.L. Roti-Roti (Washington University of St. Louis, USA).

Lecturers will include the above plus: B. Bagwell, S. Cram, Z. Darzienkiewicz, P. Dean, M. Goldberg, J. Gray, P. Horan, C Julien, M. Loken, P. Manoni, H S Micklem, K. Muirhead, D.R. Parks, A. Truneh.

Course tuition: $500 (5 000 FF) includes laboratory fees, manual, coffee breaks, lunches and an optional Flow Cytometry Certificate.

Send C V. and tuition deposit $50 (500 FF) to the local Course Director (max 50 persons).

Dr. Claude Rosenfeld,
INSERM Unite 253,
Groupe Hospitalier Paul Brousse,
16 bis avenue Paul Vaillant Couturier,
94804 Villejuif, France.

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